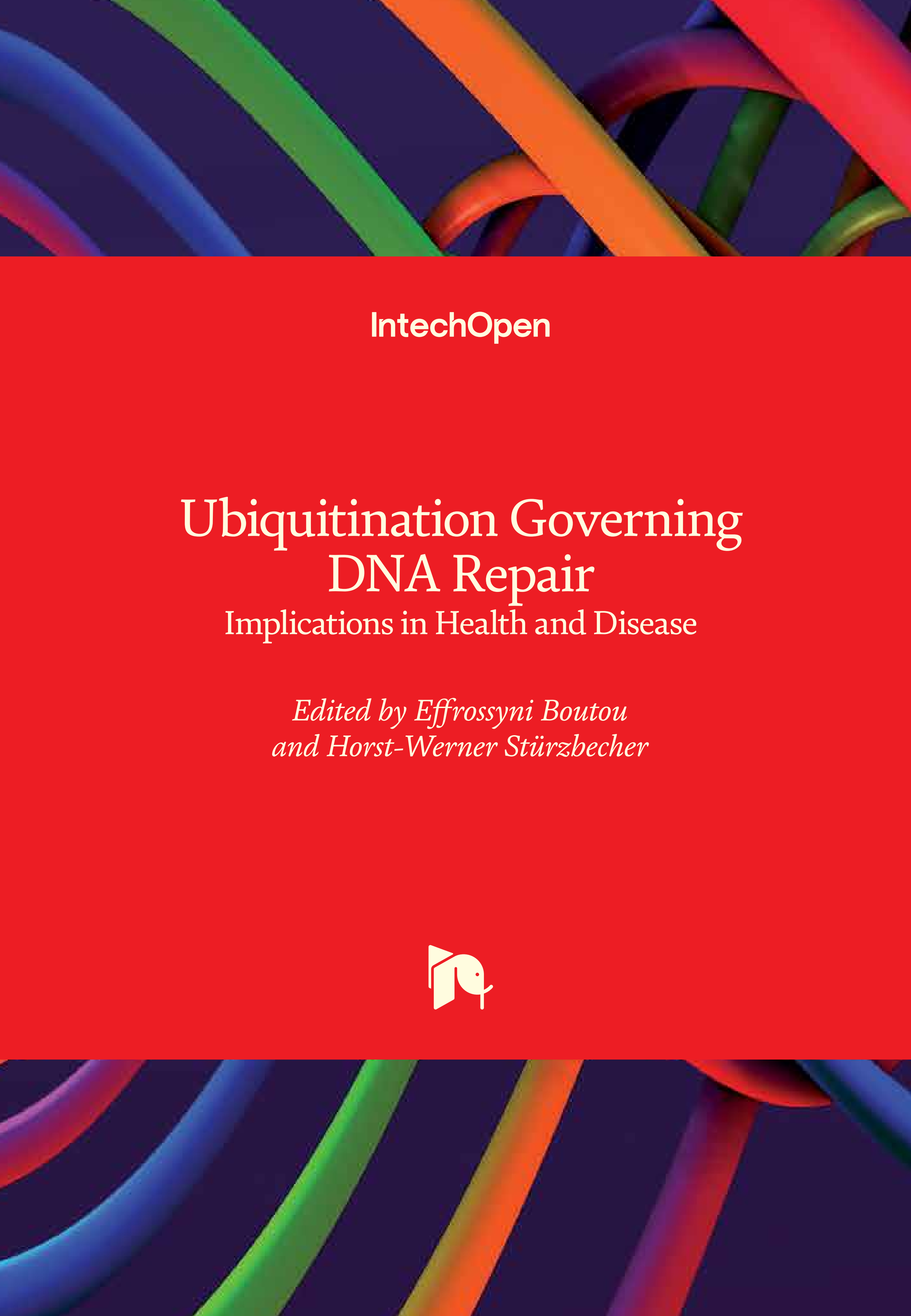

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Ubiquitination Governing DNA Repair

Implications in Health and Disease

Edited by Effrossyni Boutou
and Horst-Werner Stürzbecher

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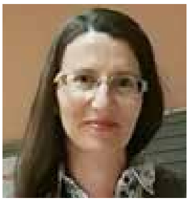
Meet the editors



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Contents

Preface

Genome integrity is maintained through a sophisticated series of mechanisms that are able to detect early, signal for, and repair DNA damage. This is termed DNA damage response (DDR) and it is crucial for survival and propagation of life. As these procedures should take place quite rapidly in order to halt cell division, or noncanonical gene expression, there is no time, in most cases, for new synthesis of factors responsible for DDR and repair. An apparent solution is recruitment by the cell of factors from other ongoing processes, reprioritizing needs, and restoring DNA damage by modifying existing molecules and their subsequent function. Signaling leading to conformational changes and affecting protein-DNA and protein-protein interactions is a very effective cellular strategy toward achieving this goal.

Likewise, phosphorylation, ubiquitination/ubiquitylation (the covalent link of one or more ubiquitously found ubiquitin molecules, by a wide variety of linear or chain creating mono- or polymer forms on mainly lysine residues of a target protein) in conjunction with the less studied SUMOylation, emerged to be critical players in regulating numerous eukaryotic cellular processes including DDR and repair pathways, cell cycle control, and cell fate decisions. Cumulative studies reveal that ubiquitination is implicated in a broad range of molecular functions that may lead to the alteration of protein stability and homeostasis, subcellular localization, or function assignment through interaction with other protein partners. It seems that different ubiquitin linkages, directing target proteins to either proteasomal degradation or conformational and thus functional modifications, cooperate to orchestrate genomic stability maintenance through a finely tuned dynamic function of both ubiquitin (ub) conjugating enzymes (E1, 2 and 3 types of ub ligases) and ub removal enzymes (Deubiquitinases, Dubs).

DDR consists of lesion type and magnitude recognition (sensing) due to either endogenous or exogenous causes, followed by signaling that activates the responsible mediators, comediators, and effectors in order to halt the cell cycle and local transcription, until efficient repair of the damage is performed and verified by the most reliable and rapid pathway available in the given cell cycle stage. This overcomes the replication stress/collapsed forks and promotes the restart of replication synthesis and this is followed, when effectively repaired, by cell cycle progression. Accordingly, repair of serious genotoxic insults, like double strand breaks (DSBs), functions through a platform capable of correcting these deleterious lesions and ensuring genome integrity. In all of these steps, protein modification by ubiquitination and SUMO consists of a crucial regulatory process that when impaired may result in aging, tumorigenesis, and other life-threatening conditions. Another important aspect to consider is genome architecture and the role of chromatin remodeling through ubiquitination, facilitating DDR and providing access to repair mechanisms, processes tightly interlinked and intercommunicating in order to handle and remove toxic DNA lesions.

This book attempts to cover current topics on ubiquitin's role in the maintenance of genome integrity through regulation of all major pathways dealing with genome restoration and cases where deregulation of ubiquitin-mediated processes is implicated in developmental disorders, cancer, and aging. More precisely, topics of the book cover the extended work on the role of ubiquitination in the initiation step of damage sensing and recognition, especially during DNA replication, the regulation of NHEJ-the preferred process for DSB repair during G1 phase of the cell cycle-the high fidelity homologous recombination (HR) repair of DSBs during the S and G2 cell cycle phases, the restoration of genotoxic lesions by base excision repair (BER), nucleotide excision repair (NER), and other types of lesion repair pathways, chromatin modification, cell cycle regulation, as well as in abnormalities like Fanconi anemia, certain cancer types, aging, and nervous system syndromes. The content also includes current findings on the role of deubiquitinases in genome stability pathways in health and disease model systems and ongoing clinical trials targeting Dubs, with optimistic results, which imply the anticipation of future perspectives in the field of diagnostics and precision therapeutics.

In conclusion, this book provides a snapshot of a comprehensive overview together with recent findings and potential implications of the ever-expanding complex regulatory mechanisms of ubiquitin-dependent DNA damage response and repair processes in health and disease. Authors include active and laboratory-based investigators energetically working in the corresponding areas. This book is intended for pre- and postgraduate students and young scientists interested in genome maintenance strategies, their intercommunication with other cellular functions, in molecular, chromatin and cellular levels and potential applications.

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Chapter 1

Ubiquitylation and SUMOylation: An Orchestrated Regulation During DNA Damage Repair

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Additional information is available at the end of the chapter

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Abstract

DNA double-strand breaks (DSBs) are cytotoxic DNA lesions that must be repaired as soon as possible because it can cause chromosomal aberrations and cell death. Homologous recombination (HR) and nonhomologous end joining (NHEJ) are the pathways that mainly repair these ruptures. HR process is finely regulated by synchronized posttranslational modifications including phosphorylation, ubiquitylation, and SUMOylation. The ubiquitin (Ub) modifications at damaged chromatin serve as recruitment platforms for DSB repair complexes by facilitating binding sites or regulating the interaction between proteins. Thus, SUMOylation has been associated with protein interaction, enzymatic activity, and chromatin mobility. Several DNA damage factors have been found to be ubiquitylated and SUMOylated including histones (H2AX) and proteins such as Mre11, Rad51, NBS1, and BRCA1. Regarding ubiquitylation-mediated regulation of DNA repair, RNF168 and RNF8 E3 ligases have turned out to be a key step in DNA damage repair regulation. Interestingly, there is evidence that the Ub signaling mechanism is ancestral, and this emphasizes its importance.

Keywords: ubiquitylation, DSB, SUMOylation, DNA repair, chromatin architecture

1. Introduction

Genome integrity is compromised by the constant attack from exogenous and endogenous DNA-damaging factors such as radiation, carcinogens, reactive radicals, and errors in DNA replication. The most deleterious DNA lesion is the double-strand breaks (DSBs) because failure to repair them results in diverse changes in DNA such as mutations or chromosomal rearrangements. Thus, to maintain genomic stability, cells have developed an elaborate DNA damage response (DDR) system to detect, signal, and repair the DNA lesions [1–3].

DSBs are repaired by two main pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ works with a fast kinetics throughout the cell cycle and joins broken DNA ends without the need of extended complementary sequences leading to an error-prone repair [4]. HR, on the other hand, takes longer and is restricted to the S and G2 phases of the cell cycle since an intact sister chromatid is required for repair based on a homologous template, and thus this process is carried out error-free [5].

HR is an evolutionary well-conserved mechanism, where nucleolytic degradation of the 5′ end in the DSB produces long 3′-single-stranded DNA (ssDNA) overhangs, and this is referred as DNA end resection [6, 7]. These dangling 3′ ends must be protected from nucleases, and the formation of tertiary structures is accomplished by **r**eplication **p**rotein **A (**RPA), which in turn is replaced by recombinase Rad51 to form the Rad51-ssDNA presynaptic filament to promote HR. Thus, DNA end resection is a key player for the Rad51-ssDNA filament formation, and it must be tightly controlled by diverse mechanisms; posttranslational modification to core components of resection machinery as well as antagonists is one of them (PMTs). These PMTs mainly phosphorylation [8] and recently ubiquitylation and SUMOylation have been shown to play an important control in many features of cellular responses to DNA damage, including the repair of DSBs [9, 10] as shown by high-throughput proteomics studies where it was observed that DSB repair is facilitated by global ubiquitylation and SUMOylation induced by DNA damage [11, 12]. This review will focus on ubiquitylation and SUMOylation participation in DSB response.

2. Ubiquitin in DSB response

Ubiquitin (Ub) is a 76 amino acid protein with seven lysine residues that can form polyubiquitin chains of eight different linkages (K6, K11, K27, K33, K48, K63, and Met1) as well as mixed and branched chains (**Figure 1**) [13]. The generation of different protein Ub chains provides structural diversity allowing proteins with specific Ub-binding domains (UBDs) to discriminate between these different structures. For example, Ub K48 and K63 polyubiquitin chains are structurally distinct and are differentially recognized by proteins containing different UBDs [14]. To date, over 200 proteins with at least 20 different types of UBDs have been identified that bind to different Ub structures in a noncovalent manner [15]. The ability of distinct protein Ub structures to specifically bind to proteins containing a particular UBD is important for generating specificity of protein-protein interactions and targeting proteins to different pathways and fates. For example, monoubiquitylation can regulate DNA repair, regulation of histone function, gene expression, and receptor endocytosis (**Figure 1**) [16].

Due to the ability of the Ub molecule to be conjugated onto diverse substrate lysine(s), protein ubiquitylation is a multifunction-oriented process using its own lysines or via its N-terminal methionine residues, to generate a diverse range of structures and therefore modify activities in protein targets [17]. Each linkage kind promotes a different protein conformation providing a certain degree of diversity, thus exposing a specific Ub-binding domain (UBD) with a particular function like favoring or inhibiting protein-protein interactions, protein localization, and/or degradation. To illustrate this, polyubiquitin chains attached to a protein in its Ub K63 linkage could mostly apply to proteins mainly distributed in the lysosome/endocytosis, DNA repair, and signal transduction (**Figure 1**). The ubiquitylation process is a bit complex; it is carried out mainly by

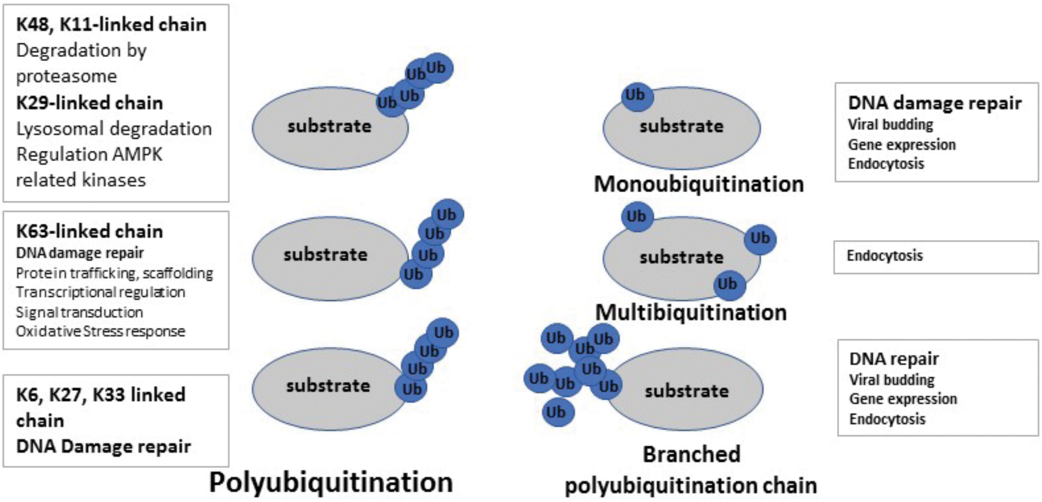

Figure 1. The ubiquitin linkage change the substrate at diverse range of structures and these modifications transform the affinity to other proteins, therefore the function is modified.

three proteins: E1 (activating enzyme), E2 (conjugating enzyme), and E3 (ligating enzymes). E1 activates ubiquitin (Ub) C-terminus by generating a thioester-linked E1~Ub conjugate which is dependent on adenosin-5′ triphosphate (ATP). Then, via a trans-thiolation reaction, the E2 active cysteine site receives the activated Ub from E1. E3 and E2 cooperate to facilitate the transfer of Ub onto a substrate lysine (K) of a protein target to form an isopeptide bond resulting in a ubiquitylated protein. E3 enzymes have been grouped in three E3 families: RING families (**r**eally **i**nteresting **n**ew **g**ene), HECT (**h**omologous to **E**6-AP **c**arboxyl **t**erminus), and hybrid RING/HECT E3 [18].

In order to promote the isopeptide formation between the lysine residue of the target protein and the glycine of the Ub C-terminus, the RING E3 ligase recruits both the E2-Ub conjugate and protein target. In contrast, HECT E3 ligases take Ub from E2-Ub conjugate on a catalytic cysteine and transfer the ubiquitin to a target lysine. On the other hand, the hybrid RING/HECT E3 ligase N-terminal RING1 domain works like the RING E3 ligases since they bind and recognize the E2-Ub conjugate, while the RING2 domain catalytic cysteine accepts a Ub molecule from E2-Ub conjugate before it is transferred to the target lysine [19]. Protein ubiquitylation is reversible through deubiquitylating enzymes (DUBs), which have the ability to cleave single Ub or polyubiquitin chains from targeted proteins.

Rad6, a postreplication repair (PRR) protein [20], was the first enzyme involved in an ubiquitylation role. Also, a mutation in ubiquitin K63R caused sensitivity to UV and DNA damage in yeast [21]. Rap80 bears a tandem ubiquitin-interacting motif (UIM) that binds to K63 linkages in vitro and is attached to Ub through K63 linkages in vivo upon DNA damage. In humans Rap80 binds to BRCA1 (**br**east **ca**ncer type **1** susceptibility) protein that has an important role during HR repair [22]. BRCA1 and Bard1 form different complexes (BRCA1-A, BRCA1-B, and BRCA1-C) with Abraxas or Bach1/FancJ or CtIP [23]. BRCA1-A interacts with regions that flank DSBs after phosphorylation, and ubiquitylation reactions promoted by the MRN complex (Mre11-Rad50-NBS1) take place. MRN complex senses the DSB, recruits (through NBS1), and activates **a**taxia **t**elangiectasia **m**utated (ATM) to initiate the DNA repair signaling response through histone H2AX phosphorylation on serine139 [24]. Thus, **Figure 1** illustrates the complex role of ubiquitin in both degradation and regulation of function on processes like DNA repair and endocytosis. The BRCA1/Rap80 complex contains other proteins such as MERIT40 and BRCC36. Interestingly, while MERIT40 facilitates BRCA1-A complex, assembly in response to DNA damage, BRCC36 is a deubiquitin enzyme with specificity on the Ub K63.

RING finger protein 8 (RNF8) is an E3 ligase that catalyzes Ub K63 linkages at DSBs in mammals. Once H2AX is phosphorylated by ATM in regions that flank DSBs, MDC1 (**m**ediator of **D**NA **d**amage **c**heckpoint 1) protein is also rapidly recruited by recognizing the phosphorylated H2AX through its BRTC domain. H2AX phosphorylation promotes RNF8 recruitment to the DSB regions by its interaction with the MDC1 terminal FHA domain. It has been proposed that RNF8 fast recruitment stimulates H1 type linker histone ubiquitylation (K63) mediated by UBC13 E2 ligase, which in turn recruits RNF168 through their UIMs, and this results in H2A ubiquitylation of H2A at K13 and K15 residues [25, 26]. These ubiquitylation modifications allow chromatin changes that facilitate the recruitment of other DSB response factors: RPA 80, 53BP1, and BRCA1 among them. Additionally, it is interesting that RNF168 extends the ubiquitylation degree on the flanking regions of DSBs, and this is required for DNA repair. This evidence highlights that ubiquitylation is a cornerstone of the DSB response, and its precise control is essential for genome stability [22].

3. SUMO in DSB response

In 1996, **s**mall **u**biquitin-related **mo**difier (SUMO) protein was discovered as a 100-amino acid-long protein. These proteins, though their sequence is not identical among them, share a common 3D structure and a C-terminal di-glycine motif that is required for its attachment to the lysine residue of the target protein via isopeptide bond [27]. SUMO could covalently be attached to target protein lysine residues by E1, E2, and E3 SUMO ligases in a similar manner to ubiquitin conjugation [28]. SUMO bears a long flexible N-terminal tail [29]. There are four different SUMO isoforms [1–4]; they are normally translated as longer precursors, consequently in order to obtain the mature forms, and they must be processed. As mentioned earlier, SUMO1 shares 48% sequence identity with SUMO2, while SUMO2 and SUMO3 present 90% sequence identity [30].

Because SUMO2 and SUMO3 isoforms are not distinguished by antibodies, they are usually referred as SUMO2/SUMO3. Further, recent data for SUMO4 indicates that this is processed to its mature form only under particular conditions [31]. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* contain a single SUMO protein Smt3 [29]. In contrast, the SUMOylation modification is carried out by one heterodimeric E1, one E2 (UBC9), and approximately 10 E3s in humans [32–34]. As for ubiquitin-like modifiers, this process is also reversible by removal of SUMO from target proteins accomplished by SUMO/sentrin-specific proteases (SENPs) [9, 35]. Two E3 SUMO ligases involved in damage DNA repair (DDR) were identified: PIAS1 and PIAS4 E3 by immunofluorescence and biochemical assays. These ligases promote BRCA1 and 53BP1 protein SUMOylation [36]. When PIAS1 and PIAS4 are removed, there is a severe impairment in Ub K63 at damage sites, thus diminishing BRCA1 and 53BP1 recruitment and causing deficient DNA repair. Therefore, in addition to ubiquitin, SUMO modifications also occur at DSBs, and these modulate the DSB response [22]. Another protein that has an important role in DDR is Rad52, and this is also SUMOylated in yeast and mammals; in *S. cerevisiae*, the RAD52 SUMOylation affects its stability and consequently the RAD52-dependent homologous recombination repair (HRR) [37]. As can be seen, SUMOylation and ubiquitylation are working together in DSB response.

4. Ubiquitin and SUMOylation of DNA end resection machinery

In response to DNA double-strand break (DSB), various elements of DNA damage response are recruited to these injured sites. The gathering of these molecules at damage sites becomes visible as foci (or **i**onizing **r**adiation **i**nduced **f**oci (IRIF)) in the nucleus, which can be observed via immunofluorescence microscopy [38].

In the initial stage of HR, the DSB ends are resected in such a way that 3′-single-strand DNA (ssDNA) overhangs are generated. This process is started by the conserved MRX (comprises by Mre11-Rad50-Xrs2) nuclease complex, which in collaboration with Sae2 in yeast, and by the MRN (including Mre11-Rad50-NBS1) complex in conjunction with CtIP (**C**-**t**erminal-binding **i**nteracting **p**rotein) in human cells; MRN/MRX complex is able to eliminate oligonucleotides from the 5′ strand, resulting in an incomplete end processing [39–42]. Additionally, the MRN/MRX complex is necessary to recruit the kinase ATM (**a**taxia **t**elangiectasia **m**utated) kinase, Exo1, Sgs1, and Dna2, to the damage site [43]. Later, resection is prolonged by the 5′-3′ exonuclease, Exo1, or by the collective activities of the Sgs1-Top3-Rmi1 (STR) complex and Dna2 (**Figure 2**) [44].

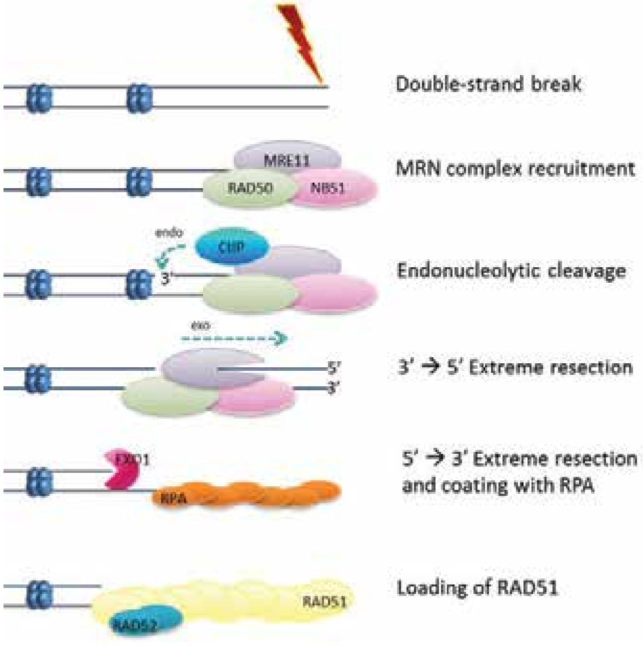

Figure 2. Scheme of DNA-end resection model. After DSB generation the MRN/X complex is recruited to the injurie site. In h. sapiens, Ctlp performs an endonucleolytic cleavage upstream from the DBS end on the 5′-terminated strand. Then, Mre11 exonuclease activity degrades DNA in a 3′-5′ direction, starting from the nick until the DSB end. The 3′ ssDNA is coated by RPA. Exonuclease EXO1 performs an extensive resection in a 5′-3′ direction. After resection, HR continues as a RAD52-mediated RPA replacement by RAD51 and further downstream steps.

After the resection process, the ssDNA overhangs are speedily coated by RPA (**r**eplication **p**rotein **A**), which is thought to eradicate secondary structures and at the same time protect the ssDNA from nuclease activities [45]. The BRCA1 protein recruits activated CtIP, PALB2, and BRCA2 (**br**east **ca**ncer **2**) to damage sites; PALB2 and BRCA2 enable RPA-RAD51 exchange on ssDNA [46, 47]. The subsequent RAD51-ssDNA filament searches for a sequence with homology, preferably on the identical sister chromatid. Afterward, the RAD51-ssDNA filaments invade the homologous sequence and anneal to the complementary ssDNA, allowing the DNA polymerases to synthesize DNA by using the undamaged DNA strand as a template. Thereby, HR repairs DSBs maintaining integrity and sequence, namely, without nucleotide deletion or alteration [38]. In response to DSBs, NBS1 interacts with components of the SCF (**S**kp1-**C**ullin1-**F**-box) E3 ligase complex and Skp2 (F-box protein) (**Figure 3**); this interaction conjugates K63-linked ubiquitin chains onto NBS1-K735 cells deficient in Skp2 which were defective in ATM activation and HR [48].

The E3 ligase RNF8 ubiquitylates NBS1 at Lys-435, mainly, and at Lys-6 that is promoted likely by E2 ligase UbcH5C. Ubiquitylation of NBS1 was detected before and after DNA damage. Studies with RNF8 mutants suggest that the interaction of RNF8 with NSB1 is mediated by the N-terminus of RNF8. RNF8 and certain RNF8 ubiquitylation activities are needed for efficient localization of NBS1 and MRN recruitment to DSB (**Figure 3**) [49].

S. cerevisiae Mre11 SUMOylation is required to interact with Ubc9 (E2) and Siz2 (an E3 related to mammalian PIAS proteins) (**Figure 3**) [11]. Also, SUMO-interacting motifs (SIMs) in Mre11 facilitate MRX complex assembly through poly-SUMO chains noncovalently recruitment [50].

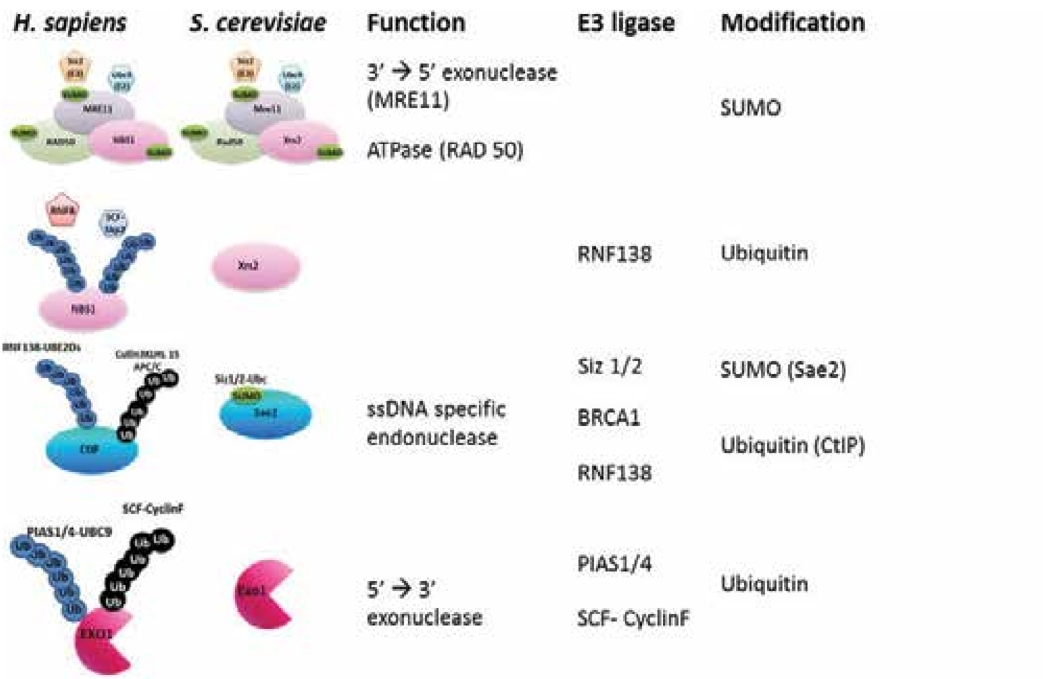

Figure 3. Illustration of some proteins involved in DNA-end resection process and their modifications. Blue dots, ubiquitin modifications involved in protein function regulation. Black dots, ubiquitin modifications involved in protein degradation. Green ovals, SUMO modification.

The heterodimeric RING-type E3 ligase BRCA1/BARD1 ubiquitylates CtIP to promote its stable retention at sites of DNA damage [51], although this physiological role still remains to be determined [52]. It has been proposed that ubiquitylation of CtIP by RNF138-UBE2D is a key event in promoting HR (**Figure 3**) [53].

During DNA end resection process, the participation of deubiquitinase (DUB) activity of USP4 (**u**biquitin-**s**pecific **p**eptidase 4) was observed. Two independent studies showed that CtIP recruitment to DSBs is regulated by interaction with USP4 and also USP4 binds to MRN complex [38, 39]. CtIP degradation via the ubiquitin-proteasome pathway is stimulated by its interaction with the CUL3 substrate adaptor Kelch-like protein 15 (KLHL15) (**Figure 3**) [42, 54].

After DNA damage, Sae2 is SUMOylated at a single conserved lysine residue (K97) mediated by Ubc9-Siz1/Siz2, and the levels of soluble Sae2 were increased [55]. An indication of Sae2 SUMOylation critical role for DNA end resection was observed in Sae2-K97R mutant cells, in which the processing and repair of DSBs were decreased [42].

It has been shown that human EXO1 is targeted for degradation by the ubiquitin-proteasome pathway. Recently, it was demonstrated that PIAS1/PIAS4-UBC9-mediated EXO1 SUMOylation (**Figure 3**) is a prerequisite for EXO1 ubiquitylation [56]. Even though the interactions between EXO1 and SENP6 de-SUMOylating enzyme [57], EXO1 with SCF-cyclin F E3 ubiquitin ligase (**Figure 3**) [12], and EXO1 with UCHL5 [58] have been studied, their participation in DNA end resection process has not been determined. PIAS1 and/or PIAS4 SUMOylates BRCA1 when it is localized at DSB sites, enhancing its ubiquitin ligase activity [36]. The MRN, Ubc9, and Siz2 allows *S. cerevisiae* Rad52 SUMOylation. This SUMOylation protects Rad52 from degradation and excludes it from nucleoli [59].

5. Chromatin remodeling

In general, any process like transcription, replication, and DNA repair requires a certain degree of chromatin access; therefore, remodeling is an important prerequisite for factors related to such processes. The participation of ubiquitylation and SUMOylation role on DNA repair on chromosome topology are very important in chromatin structure and organization.

3C (**c**hromosome **c**onformation **c**apture) is a technique where loci that are spatially closed can be formaldehyde crosslinked and identified; it was designed to determine chromatin interactions at increasing scale and resolution [60]. An upgrade of 3C is Hi-C technique, in which the only difference is that a step of biotinylation on the enzyme-restricted ends before DNA ligation has been included; this is to ensure that only ligated junction between chromosomes are purified and sequenced.

Using 3C-based technology, it has been possible to determine intrachromosomal contacts within TADs (**t**opological **a**ssociated **d**omains) that can be measured in regions of hundreds of kilobases [61]. In general, these TADs comprise long-range interactions like those found between enhancers and promoters. There are also interchromosomal contacts that are defined within same chromosome boundaries and demonstrated by the technique FISH chromosome painting [62]. These findings provide support for a nucleus architecture with layers of organization that result in a chromatin particular orchestration. Recently, it has been suggested that the chromatin organization dynamics can influence the DSB response as well as the outcome of DNA repair, which consequently will have effects on genetics stability and the production of genetic abnormalities.

These effects can be classified as bulky or large and localized.

5.1. Bulky effects

The bulky effects have been observed as long-range movement; for example, the case of localizing the VP16 activator to the nuclear periphery resulted in its relocalization to the nuclear interior, and also when RNA pol I transcription was inhibited, this caused movement of chromatin to the nucleolar periphery (**Figure 4**) [63].

Interestingly in *S. cerevisiae*, when DSBs were produced in the rDNA, these ruptures were moved to the exterior of the nucleolus [59]. Though it is not clear whether this is part of an ongoing movement or indeed due to the DSB-promoted process, nonetheless, this translocation depended on Rad52 SUMOylation which interestingly is also required for homology-directed repair (HDR). There have been other DSBs that produced chromatin mobility, and it has been shown that breaks elsewhere in the yeast genome also led to a greater mobility of chromatin that was dependent on RAD51, RAD54, MEC1, RAD9, and INO80 [64, 65].

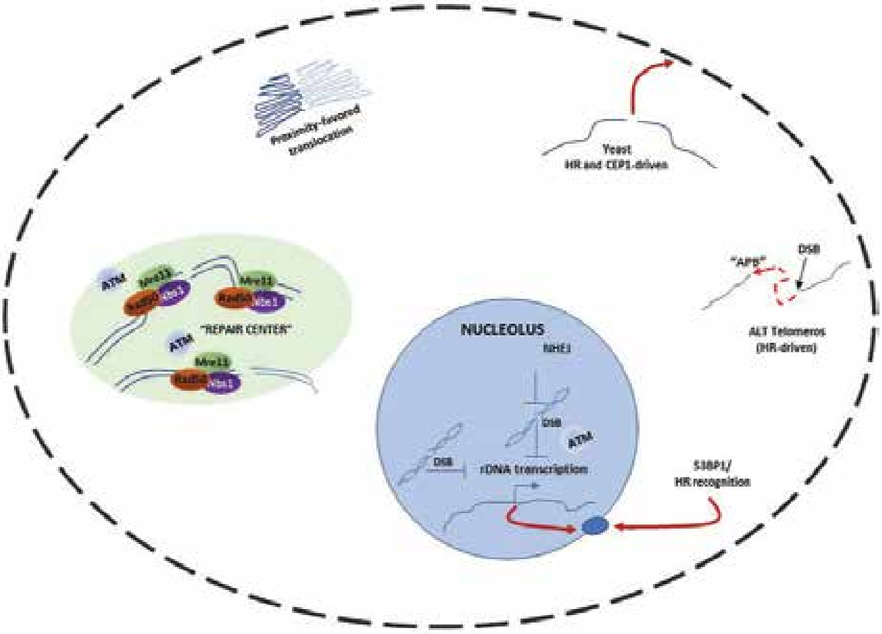

Figure 4. Chromatin remodeling during DSB response. As observed at the top, DSBs induce chromatin remodeling, as failure in DNA repair may result in translocation that occur in chromosomes in spatial proximity (TMPRSS/ETV1). Following to the right, unrepaired DSBs in yeast are recruited to SUMO mediated-nuclear periphery to be repaired. Homology repair on telomeres promotes their clustering in ALT-promyelocytic bodies (APBs). Next in same direction, unrepaired DSBs in rDNA genes in nucleoli silence transcription and this favors their relocalization to the nucleolar periphery. Often, multiple DSBs localize in repair centers and as well there are DSBs that remain in one position and depend on Ku80. Mainly where HR machinery is recruited.

It has recently been shown that INO80 also promotes chromatin movement due to DSB in telomeres, and this depends on actin polymerization [66]. It has been proposed that these movements contribute at least in part to homology searches during HR [67]. In the same line, a recent finding showed that MEC1-driven phosphorylation of the kinetochore component Cep1 induced by DSB caused centromere release from the spindle pole body explaining chromatin movement [68]. Further, it was observed that fixing telomeres to the nuclear periphery limits chromatin movement and that its physical rupture allows additional mobility. In this study, it was proposed that HR was defective and the mobility increase somehow facilitated activation of cell cycle checkpoints. Nonetheless, a wide body of evidence shows that DSBs are mobile in *S. cerevisiae* and that SUMOylation and DSB response leads to this movement, and it suggests that this movement has a positive effect on the ability to survive DNA ruptures. One of the first studies that contributed to these notions was obtained by using α-radiation to generate DSBs along a determined linear track of the nucleus [69], and the results indicated that DSBs were redistributed into clusters and that they were "repair centers" dependent on Mre11 observed in G1 phase (**Figure 4**) [70]. In the same manner, DSBs created by etoposide or gamma rays induced relocalization of damaged chromatin [71]. Consistently, repair center as observed in GFP-53BP1 foci that were induced by ionizing radiation were seen to be 1 and 2 mm apart gathered in large cluster very rapidly [72]. On the other hand, ATM loss reduced chromatin relocalization GFP-53BP1 foci when induced either by gamma rays or by charged nuclei [73] as well as with DSB generated by a nuclease [74]. In contrast, DSBs induced by UV or gamma rays were found to induce restricted mobility, however leading to a somewhat degree of chromatin decondensation [75].

In an early study, it was observed that chromatin constrain was dependent on Ku80, which suggested that NHEJ machinery rejoins fast the broken ends to limit chromatin mobility (**Figure 4**) [76].

Further, a report where transgenes were analyzed revealed long-distance movement that was dependent on MRE11 and was also associated with chromosome translocations [77]. *S. cerevisiae* generally prefers HR over NHEJ, the pathway with less error when repairing DSB since it uses a template to resolve the break. In contrast mammalian cell uses NHEJ over HR, being the most error-prone mechanism because it relies on the direct joining of broken ends [78].

DSB movement has been observed at unprotected and damaged telomeres. These DSBs are protected as they are part of the shelterin complex, thus impeding access to the DSB machinery [79]. The shelterin role has been revealed by showing that its depletion causes DSB response activation, and then telomeres are joined by NHEJ, thus inducing telomere fusions [80]. Further, 53BP1 loss reduced telomere end mobility and promoted almost complete absence of telomeric fusions [81]. Consistently with the previous data, it has been shown that this mobility is dependent on the LINC complex, which is known by connecting dynamic microtubules to the interior of the nucleus [82]. ALT (**a**lternative **l**engthening of **t**elomeres) cells employ a homology-driven mechanism to promote lengthening of telomeres [83]. When DSBs are induced in telomeres in ALT cells [84], these DSBs promoted mobility of telomere ends into clusters referred as ALT-associated PML bodies (APBs) (**Figure 4**) [85]. These movements were dependent at least partly on the HR machinery (e.g. RAD51) and also on protein involved in meiotic interhomolog recombination. Consistently, ALT telomere replication stress due to SMARCAL1 deficiency resulted in Rad51 telomere-telomere clustering and a significant telomere enlargement [86]. These findings highlight the first example of HR-mediated DSB mobility in mammalian cells and reveal that this dynamic chromatin mobility contributes to genomic stability as well as cellular immortality through telomere maintenance.

5.2. Localized movement

3C methodologies have facilitated the chromosome contacts that occur within and between chromosomes. In *S. cerevisiae*, 3C studies indicate that DSBs reduce the general frequency of local (<100 kb) interactions [87]. This reduction seems to be related to the HR-dependent DSB mobility to the nuclear periphery (**Figure 4**), as in G1-arrested cells where HR is not active. This data led to the proposal that damaged DNA is taken from the local chromatin environment to facilitate accurate DSB repair. Thus, data obtained in mouse B cells is consistent with this. Arresting cells in G1 phase to remove HR-driven repair mechanisms, DSBs within a given chromosome most frequently promoted translocation with genomic loci present in cis to these DSBs [60]. In the same line of thought, in prostate cancer cells when TMPRSS2 gene expression is stimulated by dihydrotestosterone in a TOP2-dependent manner, TOP2 catalyzed DSBs that release torsional stress which in turn inhibits transcription [88]. These ruptures have been identified, mapped, and found to be present in TMPRSS2 clinical fusions with ETS transcription factors (**Figure 4**) (e.g. ERG) [89]. Regarding nuclear organization, both TMPRSS2 and ETS transcription factor loci are often associated within the nuclear extent [90]. Therefore, chromosomal proximity can explain some translocations that are typical of genomic instability related to cancer. The relationship between transcription and DNA repair is known to modulate local chromatin structure.

6. Effect of DSB response on transcription

Many studies have described posttranslational modifications in histones that can regulate the transcription process near a DSB as part of DDR. Among them, ubiquitylation and SUMOylation modifications have been shown to silence transcription in the vicinity of DSB regions, thus allowing an efficient repair process and preventing RNA polymerase from producing aberrant transcripts. This phenomenon has been characterized in cells whose DSBs have been produced by either exogenous agents or as a part of a programed cell mechanism, like meiosis.

6.1. DSBs, transcription, and ubiquitination in somatic cells

Kruhlak et al. [91] showed for the first time a correlation between DSBs and transcription in somatic mammalian cells. In this study, they observed a decrease in transcription in nucleoli (RNA pol I) after irradiation in an ATM, Nbs1-, and DMC1-dependent manner, and consequently a prolonged and deficient repair. Later, using a reporter system that allows in single cells the visualization of repair factors recruitment, as well as local transcription, an ATM-dependent transcriptional silencing program in cis to DSBs was described. In this study, ATM prevents chromatin decondensation, thus affecting RNA polymerase II elongation at regions distal to DSBs. It was also observed that silencing, at least partially depends on RNF8 and RNF168 (E3 ubiquitin ligases), while its reversal relies on the uH2A USP16 (deubiquitylating enzyme) [92]. This study suggested that H2A ubiquitylation on areas near DSBs is important for efficient recruitment of repair factors. In contrast, deficiency of E3 ligases like RNF8 or RNF168 does not deeply impact in silencing in the context of DSB, suggesting that even though these specific ubiquitylation modifications contribute to DSB silencing, other ATM-dependent events surely cooperate in suppressing transcription [22]. TDP2 is a phosphodiesterase needed for the accurate repair of DSB caused by topoisomerase II (TOP2) abortive activity [93]. TOP2 removes hurdles on the way for efficient transcription and replication such as torsional stress from DNA, by generating intermediate cleavages and binding to the DSB 5′ terminus [94]. Normally, the cleavage and rejoining of DNA strand are transitory processes; however, this may be halted by DNA or RNA polymerases that could convert complexes into abortive DSBs which could activate the DNA repair response [95]. As shown, TDP2 ensures gene transcription from endogenous abortive TOP2 activity. Further, TDP2 has one ubiquitin-associated (UBA) domain, which is able to bind several forms of ubiquitin, thus providing potential multiple biological functions of TDP2 [96].

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References

[1] Downs JA, Nussenzweig MC, Nussenzweig A. Chromatin dynamics and the preservation of genetic information. Nature. 2007;**447**:951-958. DOI: 10.1038

[2] Ciccia A, Elledge SJ. The DNA damage response: Making it safe to play with knives. Sci - Molecular Cell. 2011;**40**:179-204. DOI: 10.1016/j.molcel.2010.09.019

[3] Lukas J, Lukas C, Bartek J. More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nature Cell Biology. 2011;**13**:1161-1169. DOI: 10.1038/nature05980

[4] Graham TGW, Walter JC, Loparo JJ. Two-stage synapsis of DNA ends during non-homologous end joining. Molecular Cell. 2016;**61**:850-858. DOI: 10.1016/j.molcel.2016.02.010

[5] Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. Cold Spring Harbor Perspectives in Biology. 2013;**5**:1-18. DOI: 10.1101/cshperspect.a012740

[6] Cejka PDNA. End resection: Nucleases team up with the right partners to initiate homologous recombination. The Journal of Biological Chemistry. 2015;**290**:22931-22938. DOI: 10.1074/jbc.R115.675942

[7] Daley JM, Niu H, Miller AS, Sung P. Biochemical mechanism of DSB end resection and its regulation. DNA Repair (Amst). 2015;**32**:66-74. DOI: 10.1016/j.dnarep.2015.04.015

[8] Tomimatsu N, Mukherjee B, Catherine Hardebeck M, Ilcheva M, Vanessa Camacho C, Louise Harris J, et al. Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. Nature Communications. 2014;**5**:3561. DOI: 10.1038/ncomms4561

[9] Jackson SP, Durocher D. Regulation of DNA damage responses by ubiquitin and SUMO. Molecular Cell. 2013;**49**:795-807. DOI: 10.1016/j.molcel.2013.01.017

[10] Schwertman P, Bekker-Jensen S, Mailand N. Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. Nature Reviews. Molecular Cell Biology. 2016;**17**:379-394. DOI: 10.1038/nrm.2016.58

[11] Psakhye I, Jentsch S. Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. Cell. 2012;**151**:807-820. DOI: 10.1016/j.cell.2012.10.021

[12] Elia AEH, Boardman AP, Wang DC, Huttlin EL, Everley RA, Dephoure N, et al. Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. Molecular Cell. 2015;**59**:867-881. DOI: 10.1016/j.molcel.2015.05.006

[13] Ikeda F, Dikic I. Atypical ubiquitin chains: New molecular signals. "Protein modifications: Beyond the usual suspects" review series. EMBO Reports. 2008;**9**:536-542. DOI: 10.1038/embor.2008.93

[14] Di Fiore PP, Polo S, Hofmann K. When ubiquitin meets ubiquitin receptors : A signalling connection. Nature Reviews. Molecular Cell Biology. 2003;**4**:491-497

[15] Haglund K, Dikic I. Ubiquitylation and cell signaling. The EMBO Journal. 2005;**24**:3353-3359. DOI: 10.1038/sj.emboj.7600808

[16] Hicke L, Schubert HL, Hill CP. Ubiquitin-binding domains. Nature Reviews. Molecular Cell Biology. 2005;**6**:610-621. DOI: 10.1042/BJ20061138

[17] Sadowski M, Suryadinata R, Tan AR, Roesley SNA, Sarcevic B. Protein monoubiquitination and polyubiquitination generate structural diversity to control distinct biological processes. IUBMB Life. 2012;**64**:136-142. DOI: 10.1002/iub.589

[18] Bergink S, Jentsch S. Principles of ubiquitin and SUMO modifications in DNA repair. Nature. 2009;**458**:461-467. DOI: 10.1038/nature07963

[19] Suryadinata R, Roesley SNA, Yang G, Sarčević B. Mechanisms of generating polyubiquitin chains of different topology. Cell. 2014;**3**:674-689. DOI: 10.3390/cells3030674

[20] Jentsch S, McGrath J, Varshavsky A. The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. Nature. 1987;**329**:131-134

[21] Spence J, Sadis S, Haas AL, Finley DA. Ubiquitin mutant with specific defects in DNA repair and multiubiquitination. Molecular and Cellular Biology. 1995;**15**:1265-1273

[22] Harding SM, Greenberg RA. Choreographing the double strand break Response: Ubiquitin and SUMO control of nuclear architecture. Frontiers in Genetics. 2016;**7**:1-12. DOI: 10.3389/fgene.2016.00103

[23] Wang G, Gao Y, Li L, Jin G, Cai Z, Chao J-I, et al. K63-linked ubiquitination in kinase activation and cancer. Frontiers in Oncology. 2012;**2**:5. DOI: 10.3389/fonc.2012.00005

[24] Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. The Journal of Biological Chemistry. 1998;**273**:5858-5868. DOI: 10.1074/jbc.273.10.5858

[25] Mattiroli F, Vissers JHA, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell. 2012;**150**:1182-1195. DOI: 10.1016/j.cell.2012.08.005

[26] Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature. 2015;**527**:389-393. DOI: 10.1038/nature15401

[27] van der Veen AG, Ploegh HL. Ubiquitin-Like Proteins. Annual Review of Biochemistry. 2012;**81**:323-357. DOI: 10.1146/annurev-biochem-093010-153308

[28] Cubeñas-Potts C, Matunis MJSUMO, Multifaceted Modifier A. Of chromatin structure and function. Developmental Cell. 2013;**24**:1-12. DOI: 10.1016/j.devcel.2012.11.020

[29] Zilio N, Ulrich HD. Functions of SUMO in the Maintenance of Genomic Stability. Springer International Publishing; 2009. pp. 41-57. DOI: 10.1007/978-90-481-2649-1

[30] Saitoh H, Hinchey J. Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. The Journal of Biological Chemistry. 2000;**275**:6252-6258. DOI: 10.1074/jbc.275.9.6252

[31] Wei W, Yang P, Pang J, Zhang S, Wang Y, Wang M-H, et al. A stress-dependent SUMO4 sumoylation of its substrate proteins. vol. 375. 2008. DOI:10.1016/j.bbrc.2008.08.028

[32] Komander D, Rape M. The ubiquitin code. Annual Review of Biochemistry. 2012;**81**:203-229. DOI: 10.1146/annurev-biochem-060310-170328

[33] Brown JS, Jackson SP. Ubiquitylation, neddylation and the DNA damage response. Open Biol. 2015;**5**:150018-150018. DOI: 10.1098/rsob.150018

[34] Stewart MD, Ritterhoff T, Klevit RE, Brzovic PS. E2 enzymes: More than just middle men. Nat Publ Gr. 2016;**26**:423-440. DOI: 10.1038/cr.2016.35

[35] Ronau JA, Beckmann JF, Hochstrasser M. Substrate specificity of the ubiquitin and ubl proteases. Cell Research. 2016;**26**:441-456. DOI: 10.1038/cr.2016.38

[36] Morris JR, Boutell C, Keppler M, Densham R, Weekes D, Alamshah A, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature. 2009;**462**:886-890. DOI: 10.1038/nature08593

[37] Sacher M, Pfander B, Hoege C, Jentsch S. Control of Rad52 recombination activity by double-strand break-induced SUMO modification. Nature Cell Biology. 2006;**8**:1284-1290. DOI: 10.1038/ncb1488

[38] Nakada S. Opposing roles of RNF8/RNF168 and deubiquitinating enzymes in ubiquitination-dependent DNA double-strand break response signaling and DNA-repair pathway choice. Journal of Radiation Research. 2016;**57**:i33-i40. DOI: 10.1093/jrr/rrw027

[39] Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature. 2008;**455**:770-774. DOI: 10.1038/nature07312

[40] Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell. 2008;**134**:981-994. DOI: 10.1016/j.cell.2008.08.037

[41] Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. Annual Review of Genetics. 2011;**45**:247-271. DOI: 10.1146/annurev-genet-110410-132435

[42] Himmels SF, Sartori AA. Controlling DNA-end resection: An emerging task for ubiquitin and SUMO. Frontiers in Genetics. 2016;**7**:1-7. DOI: 10.3389/fgene.2016.00152

[43] Shim EY, Chung W-H, Nicolette ML, Zhang Y, Davis M, Zhu Z, et al. Saccharomyces Cerevisiae Mre11/Rad50/Xrs2 and ku proteins regulate association of Exo1 and Dna2 with DNA breaks. The EMBO Journal. 2010;**29**:3370-3380. DOI: 10.1038/emboj.2010.219

[44] Mimitou EP, Symington LS. DNA end resection: Many nucleases make light work. DNA Repair (Amst). 2009;**8**:983-995. DOI: 10.1016/j.dnarep.2009.04.017

[45] Chen H, Lisby M, Symington L. RPA coordinates DNA end resection and prevents formation of DNA hairpins. Molecular Cell. 2013;**50**:589-600. DOI: 10.1016/j.molcel.2013.04.032

[46] Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature. 2009;**459**:460-463. DOI: 10.1038/nature07955

[47] Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B, et al. PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Current Biology. 2009;**19**:524-529. DOI: 10.1016/j.cub.2009.02.018

[48] Wu J, Zhang X, Zhang L, Wu C, Rezaeian AH, Chan C, et al. Skp2 E3 Ligase Integrates ATM Activation and Homologous Recombination. 2013;**46**:351-361. DOI: 10.1016/j.molcel.2012.02.018.Wu

[49] CS L, Truong LN, Aslanian A, Shi LZ, Li Y, Hwang PYH, et al. The RING finger protein RNF8 ubiquitinates Nbs1 to promote DNA double-strand break repair by homologous recombination. The Journal of Biological Chemistry. 2012;**287**:43984-43994. DOI: 10.1074/jbc.M112.421545

[50] Chen YJ, Chuang YC, Chuang CN, Cheng YH, Chang CR, Leng CH, et al. S. Cerevisiae Mre11 recruits conjugated SUMO moieties to facilitate the assembly and function of the Mre11-Rad50-Xrs2 complex. Nucleic Acids Research. 2016;**44**:2199-2213. DOI: 10.1093/nar/gkv1523

[51] Yu X, Fu S, Lai M, Baer R, Chen J, Breast B, et al. BRCA1 ubiquitinates its binding partner. CtIP. 2006;**1**:1721-1726. DOI: 10.1101/gad.1431006.CtIP

[52] Barber LJ, Boulton SJ. BRCA1 ubiquitylation of CtIP: Just the tIP of the iceberg? DNA Repair (Amst). 2006;**5**:1499-1504. DOI: 10.1016/j.dnarep.2006.08.009

[53] Schmidt CK, Galanty Y, Sczaniecka-Clift M, Coates J, Jhujh S, Demir M, et al. Systematic E2 screening reveals a UBE2D–RNF138–CtIP axis promoting DNA repair. Nature Cell Biology. 2015;**17**:1458-1470. DOI: 10.1038/ncb3260

[54] Ferretti LP, Himmels S-F, Trenner A, Walker C, von Aesch C, Eggenschwiler A, et al. Cullin3-KLHL15 ubiquitin ligase mediates CtIP protein turnover to fine-tune DNA-end resection. Nature Communications. 2016;**7**:12628. DOI: 10.1038/ncomms12628

[55] Sarangi PZX. SUMO-mediated regulation of DNA damage repair and responses. Trends in Biochemical Sciences. 2015;**40**:233-242. DOI: 10.1016/j.tibs.2015.02.006.SUMO-mediated

[56] El-shemerly M, Hess D, Pyakurel AK, Moselhy S, Ferrari S. ATR-dependent pathways control hEXO1 stability in response to stalled forks. Nucleic Acids Research. 2008;**36**:511-519. DOI: 10.1093/nar/gkm1052

[57] Bologna S, Altmannova V, Valtorta E, Koenig C, Liberali P, Gentili C, et al. Sumoylation regulates EXO1 stability and processing of DNA damage. Cell Cycle. 2015;**14**:2439-2450. DOI: 10.1080/15384101.2015.1060381

[58] Nishi R, Wijnhoven P, le Sage C, Tjeertes J, Galanty Y, Forment JV, et al. Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. Nature Cell Biology. 2014;**16**:1016-1026. DOI: 10.1038/ncb3028

[59] Torres-Rosell J, Sunjevaric I, De Piccoli G, Sacher M, Eckert-Boulet N, Reid R, et al. The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nature Cell Biology. 2007;**9**:923-931. DOI: 10.1038/ncb1619

[60] Hakim O, Misteli T. SnapShot: Chromosome conformation capture. Cell. 2012;**148**:1068-1068.e2. DOI: 10.1016/j.cell.2012.02.019

[61] Dekker J, Misteli T. Long-range chromatin interactions. Cold Spring Harbor Perspectives in Biology. 2015;**7**:a019356. DOI: 10.1101/cshperspect.a019356

[62] Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009;**326**:289-293. DOI: 10.1126/science.1181369

[63] Floutsakou I, Agrawal S, Nguyen TT, Seoighe C, Ganley ARD, McStay B. The shared genomic architecture of human nucleolar organizer regions. Genome Research. 2013;**23**(12):2003. DOI: 10.1101/gr.157941.113

[64] Dion V, Kalck V, Horigome C, Towbin BD, Gasser SM. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. Nature Cell Biology. 2012;**14**:502-509. DOI: 10.1038/ncb2465

[65] Neumann FR, Dion V, Gehlen LR, Tsai-Pflugfelder M, Schmid R, Taddei A, et al. Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. Genes & Development. 2012;**26**:369-383. DOI: 10.1101/gad.176156.111

[66] Spichal M, Brion A, Herbert S, Cournac A, Marbouty M, Zimmer C, et al. Evidence for a dual role of actin in regulating chromosome organization and dynamics in yeast. Journal of Cell Science. 2016;**129**

[67] Miné-Hattab J, Rothstein R. Increased chromosome mobility facilitates homology search during recombination. Nature Cell Biology. 2012;**14**:510-517. DOI: 10.1038/ncb2472

[68] Strecker J, Gupta GD, Zhang W, Bashkurov M, Landry M-C, Pelletier L, et al. DNA damage signalling targets the kinetochore to promote chromatin mobility. Nature Cell Biology. 2016;**18**:281-290. DOI: 10.1038/ncb3308

[69] Aten JA, Stap J, Krawczyk PM, van Oven CH, Hoebe RA, Essers J, et al. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science. 2004;**303**:92-95. DOI: 10.1126/science.1088845

[70] Stap J, Krawczyk PM, Van Oven CH, Barendsen GW, Essers J, Kanaar R, et al. Induction of linear tracks of DNA double-strand breaks by α-particle irradiation of cells. Nature Methods. 2008;**5**:261-266. DOI: 10.1038/nmeth.f.206

[71] Krawczyk PM, Borovski T, Stap J, Cijsouw T, ten Cate R, Medema JP, et al. Chromatin mobility is increased at sites of DNA double-strand breaks. Journal of Cell Science. 2012;**125**:2127-2133. DOI: 10.1242/jcs.089847

[72] Neumaier T, Swenson J, Pham C, Polyzos A, Lo AT, Yang P, et al. Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:443-448. DOI: 10.1073/pnas.1117849108

[73] Becker A, Durante M, Taucher-Scholz G, Jakob B. ATM alters the otherwise robust chromatin mobility at sites of DNA double-strand breaks (DSBs) in human cells. PLoS One. 2014;**9**:e92640. DOI: 10.1371/journal.pone.0092640

[74] Caron P, Choudjaye J, Clouaire T, Bugler B, Daburon V, Aguirrebengoa M, et al. Non-redundant functions of ATM and DNA-PKcs in response to DNA double-strand breaks. Cell Reports. 2015;**13**:1598-1609. DOI: 10.1016/j.celrep.2015.10.024

[75] Falk M, Lukasova E, Gabrielova B, Ondrej V, Kozubek S. Chromatin dynamics during DSB repair. Biochim Biophys Acta - Mol Cell Res. 2007;**1773**:1534-1545. DOI: 10.1016/j.bbamcr.2007.07.002

[76] Soutoglou E, Dorn JF, Sengupta K, Jasin M, Nussenzweig A, Ried T, et al. Positional stability of single double-strand breaks in mammalian cells. Nature Cell Biology. 2007;**9**:675-682. DOI: 10.1038/ncb1591

[77] Roukos V, Voss TC, Schmidt CK, Lee S, Wangsa D, Misteli T. Spatial dynamics of chromosome translocations in living cells. Science. 2013;**341**:660-664. DOI: 10.1126/science.1237150

[78] Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Research. 2008;**18**:134-147. DOI: 10.1038/cr.2007.111

[79] Palm W, de Lange T. How shelterin protects mammalian telomeres. Annual Review of Genetics. 2008;**42**:301-334. DOI: 10.1146/annurev.genet.41.110306.130350

[80] Doksani Y, Lange, de T. The role of double-strand break repair pathways. Cold Spring Harb Lab Press. 2014:1-16. DOI: 10.1101/cshperspect.a016576

[81] Dimitrova N, Chen Y-CM, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. Nature. 2008;**456**:524-528. DOI: 10.1038/nature07433

[82] Lottersberger F, Karssemeijer RA, Dimitrova N, de Lange T. 53BP1 and the LINC complex promote microtubule-dependent DSB mobility and DNA repair. Cell. 2015;**163**:880-893. DOI: 10.1016/j.cell.2015.09.057

[83] Dilley RL, Greenberg RA. ALTernative telomere maintenance and cancer. Trends in Cancer. 2015;**1**:145-156. DOI: 10.1016/j.trecan.2015.07.007

[84] Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, et al. Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. Nature Structural & Molecular Biology. 2013;**20**:317-325. DOI: 10.1038/nsmb.2499

[85] Cho NW, Dilley RL, Lampson MA, Greenberg RA. Interchromosomal homology searches drive directional ALT telomere movement and synapsis. Cell. 2014;**159**:108-121. DOI: 10.1016/j.cell.2014.08.030

[86] Cox KE, Maréchal A, Flynn RL. SMARCAL1 resolves replication stress at ALT telomeres. Cell Reports. 2016;**14**:1032-1040. DOI: 10.1016/j.celrep.2016.01.011

[87] Oza P, Jaspersen SL, Miele A, Dekker J, Peterson CL. Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes & Development. 2009;**23**:912-927. DOI: 10.1101/gad.1782209

[88] Gómez-Herreros F, Schuurs-Hoeijmakers JHM, McCormack M, Greally MT, Rulten S, Romero-Granados R, et al. TDP2 protects transcription from abortive topoisomerase activity and is required for normal neural function. Nature Genetics. 2014;**46**:516-521. DOI: 10.1038/ng.2929

[89] Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nature Genetics. 2010;**42**:668-675. DOI: 10.1038/ng.613

[90] Lin C, Yang L, Tanasa B, Hutt K, Ju B, Ohgi K, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell. 2009;**139**:1069-1083. DOI: 10.1016/j.cell.2009.11.030

[91] Kruhlak M, Crouch EE, Orlov M, Montaño C, Gorski SA, Nussenzweig A, et al. The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. Nature. 2007;**447**:730-734. DOI: 10.1038/nature08110

[92] Shanbhag N, Rafalska-Metcalf I. An ATM-dependent transcriptional silencing program is transmitted through chromatin in cis to DNA double strand breaks. Cell. 2010;**141**:243-247. DOI: 10.1016/j.cell.2010.04.038.An

[93] Cortes Ledesma F, El Khamisy SF, Zuma MC, Osborn K, Caldecott KW. A human 5′-tyrosyl DNA phosphodiesterase that repairs topoisomerase-mediated DNA damage. Nature. 2009;**461**:674-678. DOI: 10.1038/nature08444

[94] Nitiss JL. DNA topoisomerase II and its growing repertoire of biological functions. Nature Reviews. Cancer. 2009;**9**:327-337. DOI: 10.1038/nrc2608

[95] Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. Nature Reviews. Cancer. 2009;**9**:338-350. DOI: 10.1038/nrc2607

[96] Rao T, Gao R, Takada S, Al AM, Chen X, Walters KJ, et al. Novel TDP2-ubiquitin interactions and their importance for the repair of topoisomerase II-mediated DNA damage. Nucleic Acids Research. 2016;**44**:10201-10215. DOI: 10.1093/nar/gkw719

Chapter 2

The Five Families of DNA Repair Proteins and their Functionally Relevant Ubiquitination

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Additional information is available at the end of the chapter

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Abstract

The process of DNA repair, be it a response to replication dysfunction or genotoxic insult, is critical for the resolution of strand errors and the avoidance of DNA mismatches that could result in various molecular pathologies, including carcinogenic development. Here, we will describe the five main mechanisms by which DNA avoids mutation, namely the processes of base excision repair, mismatch repair, nucleotide excision repair, homologous recombination, and nonhomologous end joining. In particular, we will dissect the functional significance of various posttranslational modifications of the essential proteins within these pathways, including but not limited to ubiquitination, acetylation, and phosphorylation.

Keywords: base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), homologous recombination (HR), nonhomologous end joining (NHEJ), posttranslational modification

1. Introduction

The mammalian genome is under constant barrage by exogenous and endogenous insult that can beget damage and instability. Exogenous insults include exposure to UV radiation and chemical carcinogens found in the environment, while endogenous factors include ROS produced by cellular metabolism, spontaneous chemical reactions like base deamination and mistakes made during the replicative process. It is critical to the survival of the organism that each cell have the ability to resolve the damage induced by this wide variety of insults, and that the machinery responsible for responding to damage must be equally diverse.

There are five main mechanisms responsible for repairing damaged DNA, and their conservation from bacteria all the way to humans exemplifies their critical role in the maintenance of an organism's genome. These mechanisms consist of base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, and nonhomologous end joining.

2. Ubiquitination and the proteasome degradation pathway

The ubiquitin proteasome pathway (UPP) is a mechanism used for the maintenance of proper levels of cellular proteins and the destruction of old or misfolded proteins by targeting them for degradation. This targeting comes in the form of ubiquitination, the process of covalently linking a polyubiquitin chain to the protein that is recognized and bound by the 26S proteasome, which degrades the protein and releases the ubiquitin. Ubiquitin is a highly conserved 76-amino acid protein that serves as the subunit of the polyubiquitin chain. Ubiquitin is covalently linked to its target in a three-step cascade conducted by a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-ligating enzyme (E3) [1]. Apart from protein degradation, ubiquitination can also mediate protein-protein interaction.

3. Detection and repair within the DNA strand

3.1. Base excision repair

Base excision repair enzymes are responsible for correcting lesions induced by a wide variety of both endogenous and exogenous insults, including sites of base loss, nonbulky base lesions, and DNA single-strand breaks (SSBs) [2]. DNA glycosylases are responsible for the first step of base excision repair (BER) by initially detecting the damage and excising the base via hydrolyzing the N-glycosylic bond linking the DNA base to the sugar phosphate backbone. This process generates an abasic site (AP site) that AP endonuclease 1 recognizes and acts upon by cleaving the phosphodiester bond 5′ to the AP site, leaving a SSB with a 5′-sugar phosphate. A DNA repair complex composed of DNA pol β, XRCC1, and DNA ligase IIIα can recognize this SSB and remove the 5′-sugar phosphate through its AP lyase activity, and add a single nucleotide to the 3′-end through its DNA polymerase activity. The damage is finally resolved when Lig 3 seals the DNA ends together, thus completing what is referred to as short patch BER, the process by which human cells conduct the majority of their BER [3, 4].

At the moment, much of the work focusing on ubiquitination of the proteins involved in BER has been pursued by Dianov et al. [5]. This group has been able to demonstrate that, under normal conditions, BER components are targeted for destruction by the E3 ubiquitin ligase CHIP. When DNA damage occurs in cells, the BER components undergo stabilization to increase their ability to correct the damage. Specifically, pol β, XRCC1, and DNA ligase III are polyubiquitinated by CHIP and Mule when not bound to chromatin, and thus targeted for degradation [6].

DNA pol λ can also be targeted by posttranslational modifications. Pol λ contains four distinct phosphorylation sites, but phosphorylation of the Thr553 has the strongest impact on the stability of the protein. Pol λ can be phosphorylated on all of these sites by the Cdk2/cyclin A complex, but its levels of phosphorylation are reduced when it is interacting with proliferating cell nuclear antigen (PCNA) [7], a sliding clamp that associates with DNA polymerases and ensures accurate and possessive DNA synthesis [8]. Increased phosphorylation of Thr553 on pol λ positively correlates with its protein levels in the cell, likely due to the fact that phosphorylation at this site protects pol λ from ubiquitination and degradation. This stabilization occurs in the late S and G2 phase. Pol λ is closely related to pol β, and indeed both polymerases can be ubiquitinated by CHIP and Mule. It is thought that pol λ is needed in late S and G2, specifically whenever oxidative DNA damage presents at this phase and induces 8-oxo-G lesions [9, 10]. When these lesions occur, it is Mule that is responsible for regulating protein levels of pol λ. When Mule is able to ubiquitinate pol λ, this action targets both pol λ for degradation and decreases its enzymatic activity. Mule is responsible for the monoubiquitination of pol β, which can be further polyubiquitinated by CHIP and targeted for degradation [11]. While pol β is continuously expressed in unstressed cells, it is almost immediately targeted by Mule and CHIP in the absence of damage. Upon DNA damage detection, alternative reading frame (ARF) begins to accumulate and eventually inhibits Mule activity [12], allowing for pol β accumulation and activation of further BER proteins. Once the lesion(s) have been resolved ARF levels drop, Mule activity is restored, and pol β will once again be ubiquitinated and degraded. ARF is a BER protein frequently mutated in cancer cells; it functions by responding to DNA damage by directly inhibiting Mule, as well as regulating p53. The amount of ARF produce in response to DNA damage is dependent on the extent of the damage; and by inhibiting Mule activity, it allows p53 to halt replication while pol β complexes conduct repair [13]. Without ARF, both Mule and Mdm2 repress p53 activity. ARF is a 482 kDa protein belonging to the homologous to E6-AP carboxyl terminus (HECT) family of E3 ubiquitin ligases [14], named such due to their ubiquitous presence of a C-terminal HECT domain of ~350 amino acids that house their E3 catalytic activity. The HECT domain of Mule contains two subdomains connected by a flexible linker allowing these domains to undergo ubiquitin chain transfer [15]. Aside from allowing for p53 accumulation and activation of the DNA damage response in damaged cells, ARF also plays a p53-independent role in tumor suppression due to its ability to induce proliferation delay in cells lacking functional p53 and p21 [16, 17].

3.2. Nucleotide excision repair

Nucleotide excision repair is a process undertaken in both prokaryotes and eukaryotes to enzymatically remove bulky, helix-distorting base adducts from DNA. This process is the predominant method of DNA repair in mammals, especially when resolving damage induced by ultraviolet light from the sun. About 30 proteins are involved in eukaryotic nucleotide excision repair (NER), including nine major proteins identified by their mutation in humans and the development of UV-hypersensitivity as a result. Seven of these proteins, when mutated, lead to the development of Xeroderma pigmentosum syndrome (XPA to XPG) and two lead to the development of Cockayne's syndrome (CSA and CSB) [18]. Additional players in the process of NER include excision repair cross-complementing 1 (ERCC1), replication protein A (RPA), and Rad23 homologs (HR23A and HR23B) [19, 20]. The Rad23 homologs share redundancy with the function of Rad23 in yeast during the recognition of the lesion in NER. Upon initial recognition of a lesion, eukaryotic NER can continue by either the process of global genome NER (GG-NER) or transcription-coupled NER (TC-NER). GG-NER removes DNA from untranscribed regions of DNA; XPC-HR23B and UV-DDB (damaged DNA-binding protein) can recognize UV damage and recruit XPA to this resultant lesion [21]. In TC-NER, RNA polymerase II recognizes the lesion when it is a mediating transcription but finds its progress blocked by the break. This stalling of RNA pol II is recognized by CSA and CSB, which will localize to the lesion and load XPA on the site to initiate NER. After initial lesion recognition, GG-NER and TC-NER follow the same pathway to resolve the damage. XPA further recruits XPB (5′ to 3′ helicase) and XPD (3′ to 5′ helicase) to unwind the DNA at the damage site and allow for incisions on the 3′ and 5′ sides of the gap to be made by XPG and XPF-ERCC1 endonucleases, respectively [22–24].

The first association between the ubiquitin proteasome pathway (UPP) and nucleotide excision repair (NER) was due to the identification of the ubiquitin-like domain present at the N-terminus of Rad23 [25, 26] that can serve as a ubiquitin receptor, similar to the subunit of the 26S proteasome Rpn1 [27]. Both can recognize polyubiquitinated chains and transport the target proteins to the proteasome [28]. The Rad23 ubiquitin-like domain is required for sufficient NER activity, and deletion of this domain can result in UV radiation sensitivity [29]. Russell et al. demonstrated that complete inhibition of the proteasome does not affect NER, while specifically targeting 19S activity does. Further, 19S influence on NER is mediated by the Ubl domain of Rad23, suggesting to them that 19S may be acting as a molecular chaperone in the context of NER by altering the conformation of certain NER proteins [29, 30]. Rad23 avoids proteasomal degradation due to its ubiquitin-like domain via a C-terminal ubiquitin-associated (UBA) domain [31] and can impart this protection on its binding partner XPC in a mechanism that will be detailed later.

XPE-deficient cells lack the ability for UV-damaged DNA-binding component (DDB), composed of DDB1 (p127) and DDB2 (p48), to bind DNA. DDB2 and CSA are present in separate but nearly identical molecular complexes, both associated by their interaction with DDB1 [32]. Both complexes contain CUL4A and ROC1, both ubiquitin ligase subunits, as well as the constitutive photomorphogenesis 9 (COP9) signalosome (CSN). When the complex is devoid of CSN, they are able to display robust ubiquitin ligase activity. After UV exposure, CSN rapidly dissociates from the DDB2 complex and CUL4A is modified by NEDD8 (via neddylation and polyubiquitination) [33], leading to ubiquitin ligase activity from the complex. This complex ubiquitinates XPC (which is bound to HR23, the specifics of this complex detailed later), allowing both of these complexes to bind the damaged DNA. DDB2 itself is also polyubiquitinated, causing it to dissociate from the complex, and get degraded by the proteasome. Ubiquitinated XPC and HR23 remain on the DNA, where they activate the process of NER. The CSA complex is not as well characterized as the DDB2 complex. What is known is that unlike the DDB2 complex, UV-induced damage stimulates the rapid association of CSN with CSA, suppressing all ubiquitin ligase activity from the complex. A target of DDB2 complex ubiquitination is XPC, which is required for GG-NER at the damage site [34]. In undamaged cells, XPC exists in a heterotrimeric complex with either mammalian homolog of Rad23, HR23A, or HR23B. XPC is normally bound to HR23B, but in its absence HR23A is sufficient [35, 36]. This XPC complex recognizes physical aberrations in the structure of DNA rather than the lesions themselves, and is recruited after ubiquitination by the DDB2 complex.

The ubiquitination appears to be protective, as XPC is not a target of proteosomal degradation and is further stabilized through its interaction with the ubiquitin-associated (UBA) domain of the HR23 protein it is bound to [37, 38]. It is of great interest that both DDB2 and XPC are ubiquitinated and this ubiquitination yields drastically different outcomes, yet there are still parts of this mechanism that have not been defined. The specific ubiquitination sites on these two proteins have not been mapped, and the factors that specifically interact with these two proteins upon ubiquitination have yet to be defined.

Returning to the CSA complex, after UV exposure, it rapidly associates with CSN and its ubiquitin ligase activity is suppressed. This action has implications on the function of RNA polymerase II, which stalls on DNA strands during transcription when it encounters a break or adduct (any transcriptional blockade) and signals for the assembly of TC-NER machinery. Reports have indicated that UV exposure can activate CSA- and CSB-dependent polyubiquitination of RNA pol II [39], an observation that contrasts with the previously discussed reports of CSN inhibiting the CSA complex's ubiquitin ligase activity. Groisman et al. have suggested that CSN can differentially regulate the activity of DDB2 and CSA complexes, and that its interaction with CSA may not in fact be inhibitory [32]. It is also possible that there is an additional member of the CSA complex, or a separate complex is mediating ubiquitination of RNA pol II. Svejstrup et al. have argued that RNA pol II ubiquitination is conducted by a Rad26-Def complex [40]. Def1 is a protein discovered in yeast that complexes with Rad26 on chromatin, and when this protein is deleted in yeast, these cells are unable to degrade stalled DNA pol II in response to DNA lesions [40]. RNA pol II stalling has been reported to induce ubiquitination and degradation of Rpb1, the largest RNA pol II subunit, in a Def1-dependent manner [41]. When RNA pol II is polyubiquitinated after UV-induced damage (an additional E3 ligase is BRCA1/BARD1 of the homologous recombination pathway), it is either degraded or bypasses the transcriptional block, allowing mRNA synthesis to continue [42] and the damage is to be resolved later by GG-NER [43].

UV radiation has often been used to elucidate the mechanisms of NER components, as helix-distorting damage (cyclobutane pyrimidine dimers, 6-4 photoproducts) is repaired by NER [44]. These studies have also revealed the posttranslational modifications necessary for the functional relevance of these proteins. UV radiation experiments were responsible for the initial observation that genes encoding certain components of the UPP influenced the ability of cells to survive after being irradiated, and the researchers interpreted this data in a manner that highlights the proteolytic activity for the proteasome in NER [45–47]. After these initial observations, Rad23 was investigated and determined not to be targeted for ubiquitination, and Rad4 (yeast homolog of XPC) became the next potential target for ubiquitination. This focus was based on the observation that Rad4 overexpression can increase NER activity [47]. Further studies in human cells revealed that XPC also accumulated after DNA damage, and like their yeast counterparts, increased NER activity [37]. This accumulation was correlated to hHR23 in mouse cells, and it was found that Rad23 could use its UBA domains to stabilize Rad4/XPC by acting in trans [48] as well as controlling its own turnover by acting in cis [49]. The C-terminal tail of H2A is a target for posttranslational modification, with as much as 5–15% of H2A being monoubiquitinated in mammals [50]. Ubiquitinated H2A is associated with condensed DNA and gene repression, and Ring2 is the predominant

E3 ubiquitin ligase responsible for this modification [51, 52]. UV-induced DNA damage can induce monoubiquitination of H2A in close proximity to the lesions [53] in a manner very similar to its phosphorylation. Both of these histone modifications occur in UV treated, non-S-phase cells and are dependent on functional NER, and ATR signaling is required for the tail modification to occur [54, 55]. Ubiquitination of XPC, DDB2, and PCNA can still occur in NER-deficient XP-A cell lines, but H2A ubiquitination relies upon NER-sufficiency. Ubc13 and RNF8 are responsible for perpetuating sustained H2A ubiquitination so that NER can occur, but do not initially ubiquitinate H2A [56].

3.3. Mismatch repair

The DNA mismatch repair pathway is responsible for correcting mispaired nucleotides and insertion/deletion loops (IDLs) that are a consequence of replication, recombination, and repair errors [57].

The role of ubiquitination in the process of mismatch repair is relatively uncharacterized, compared with the rest of the DNA repair pathways detailed in this chapter. However, research conducted in our lab has identified that the stability of MutS protein homolog 2 (MSH2), an essential DNA mismatch repair protein, is regulated through ubiquitination by histone deacetylase 6 [58]. Ubiquitination of MutSα was first reported by Lautier et al. [59], although the enzyme responsible remained undetermined until our 2014 publication [58]. MSH2 forms two heterodimers, MSH2-MSH6 (MutSα) and MSH2-MSH3 (MutSβ). MutSα recognizes single base mismatches and 1-2 nucleotide insertions and deletions [60] while MutSβ recognizes bulky DNA adducts and larger insertions and deletions [61]. MutSα specifically recognizes DNA lesions induced by a wide variety of DNA-damaging agents (6-thioguanine, cisplatin, doxorubicin, etoposide) [62]. In the absence of MutSα, cells display resistance to these DNA-damaging agents and do not undergo apoptosis as a result of a futile repair cycle [63, 64]. Elucidation of the mechanism of MSH2 stability in cells is critical to the field of mismatch repair, as the initiation of MMR is controlled by the binding of MutSα and MutSβ to the mispair. These proteins subsequently signal the downstream effectors of MMR; MutLα (MLH1-PMS2), PCNA, and RPA, which can further lead to the recruitment of excision protein exonuclease 1 (EXO1). EXO1 excises the mismatched base, forming a gap that is filled by polymerase δ and a nick that is resolved by DNA ligase 1. When MSH2 is acetylated, it cannot be ubiquitinated, and thus is retained and is able to form MutSα and Mutsβ complexes. MSH2 turnover can be induced by HDAC6 activity, which subsequently deacetylates and ubiquinates MSH2 to target it for proteosomal degradation. This action is possible because of the E3 ubiquitin ligase activity HDAC6 possesses in its DAC1 domain (HDAC6 has two active sites: DAC1 and DAC2). HDAC6 can target MSH2 even when it is in its heterodimeric complex; MSH2 deacetylation causes it to dissociate from its stabilizing partner MSH6 [65], and as a free monomer MSH2 can be ubiquitinated [58]. MSH2 can be acetylated at four lysine residues (K845, K847, K871, and K892), and all of these sites can also be ubiquitinated. MSH2 can be protected from ubiquitination and degradation by protein kinase C (PKC), which can phosphorylate the MutSα complex [66].

Further research out of our lab has indicated that ubiquitin-specific peptidase 10 (USP10) also plays a role in MSH2 stability, but rather than targeting it for degradation like HDAC6, USP10 is responsible for stabilizing MSH2 by deubiquitinating it [67]. USP10 has recently been identified as a regulator of p53 in response to DNA damage in a tumor development context [68–70]; ATM phosphorylation of USP10 induces its translocation to the nucleus, where it stabilizes p53. However, we now know that USP10 can work in opposition to HDAC6 by interacting with the N-terminal region of MSH2, while HDAC6 interacts with the C-terminal region. Under stress conditions (IR, carcinogen treatment), USP10 phosphorylation is increased [68] suggesting enhanced translocation to the nucleus where it may increase stabilization of the MutSα complex.

MMR can respond to endogenous insult to genomic integrity as well as exogenous. Oxidative DNA damage, for example, can induce MutSα-dependent PCNA ubiquitination, a process dependent on the PCNA E3-ubiquitin ligase RAD18 [71] in a process of noncanonical MMR (ncMMR) described by Jiricny et al. [72]. Briefly, ncMMR is mostly independent of DNA replication, lacks strand directionality, and could potentially play a role in genomic instability. This type of MMR occurs outside of S-phase when the dNTP pool is limited and replicative polymerases are not present, and the activity of MutLα in this situation can result in nicks in either strand of the DNA. This noncanonical MMR activation can itself promote ubiquitination of PCNA, which is directly responsible for recruiting pol-η (an error-prone polymerase) to chromatin [72] in the absence of higher fidelity polymerases. ncMMR is currently considered a stress response to genotoxic agents that contribute to genomic instability.

4. Repair of DNA strand breakage

While base and nucleotide damage can occur both by mistakes of the replicative machinery and chemical carcinogens, more robust insults to genome stability can induce single-strand and double-strand DNA breaks. These breaks can be caused by chemical carcinogens operating by different mechanisms than the ones previously mentioned, as well as ionizing radiation.

4.1. Homologous recombination

Homologous recombination (HR) is a major DNA repair pathway in which a sister strand of DNA is used to accurately repair DSBs. DSBs generally occur in euchromatin (as heterochromatin is relatively protected in its condensed state), and must be sensed, identified, and stabilized so that repair machinery can be recruited to the site without further damage occurring. The initial sensing of these ends occurs via the joint effort of ATM, and to a lesser extent, the MRN complex. ATM is a resident protein of the nucleus, existing in its inactive dimerized form, but upon the detection of a lesion it can activate itself via autophoshorylation. ATM can recognize large-scale changes in the chromatin structure [73], RNF8- and CHFR-mediated chromatin relaxation by histone ubiquitination [74], and R-loops (RNA/DNA hybrids) at lesions blocking the transcriptional machinery [75]. Thus, begins the ATM signaling cascade, recruiting a wide variety of DNA damage response elements and break responders, as well as the proteins that modify these responders to activate or enhance their function. To open the damage site to this massive recruitment effort, ATM phosphorylates the methyltransferase MMSET to methylate the surrounding histones and promote 53BP binding [76, 77]. ATM can also phosphorylate MDC1, which leads to the recruitment of ubiquitin ligase RNF8 via its FAA domain [78], which subsequently ubiquitinates histones H2A and H2AX, and promotes the retention of the factors recruited by ATM until the damage has been fully resolved [79]. MDC1, once initially activated by ATM, can bind ATM as well as the MRN complex, thus stabilizing these critical responders at the site of damage and amplifying their continued colocalization with the breaks [80, 81]. Ubiquitinated H2A and H2AX in the presence of RNF8 can recruit a second ubiquitin ligase, RNF168, which amplifies the ubiquitination signal at these histones and ensures that BRCA1, Rap80, Rad18, and 53BP1 localize to the site of damage [82, 83].

BRCA1 is a crucial responder to DNA damage that plays roles in cell cycle checkpoints, DNA cross link repair, and replication fork stability at the sites of DNA damage. Mutations in this gene severely limit its function and force cells to repair their DSBs via the error-prone process of NHEJ, which can predispose individuals to developing breast or ovarian cancer. BRCA1 can recruit RAD51 to the sites of DSBs and is necessary for the cell to repair the damage via homologous recombination and subsequent progress through the G2/M checkpoint [84, 85]. BRCA1 can also form a complex with BRCA2, which contributes to DNA break resolution. One of the proteins that can recruit BRCA1 to the DSB site is Rap80, which directs BRCA1 to K63-linked ubiquitin chains present on postreplication repair effector and sliding clamp PCNA [86]. These ubiquitin chains are generated by RING type E3 ubiquitin ligases RNF8 and RNF168 previously recruited by ATM action [79, 87]. Depletion of RAP80 has been demonstrated to increase the frequency of HR in reporter cells, and these cells eventually developed large chromosomal rearrangements.

BRCA1 itself can also serve as an E3 ubiquitin ligase by forming an obligate RING heterodimer with binding partner BARD1 [88], and this dimerization is required for BRCA1 to exert its tumor suppressor function. BRCA1's RING domain is adjacent to a large sequence of α helices that interact with a similar α helix sequence on BARD1 [89], while the RING domain is left free to interact with E2 enzymes and exert its ubiquitin ligase activities on target proteins [90]. BRCA1-BARD1 is a type I dimeric RING E3 ubiquitin ligase, but is missing a conserved positive residue for these E3 ligases that is required for its binding activity, so this residue must be supplied by their binding partner [91]. The BRCA1-BARD1 heterodimer can target histones (H2A and H2AX), RNA polII, TFIIE, NPM1, CtIP, γ-tubulin, ER-α, and claspin [88]. BRCA1-BARD1 can also interact with 53BP1, and its ligase activity is thought to relocate 53BP1 to the periphery of the damage foci to allow for damage proteins like RPA and RAD51 to localize. E3 ligase-defective cells demonstrate reduced, but not entirely eliminated RPA and RAD51 foci in S-phase cells after being hit with a dose of IR [92]. However, in their normal S-phase counterparts, BRCA1 can counter the 53BP1-mediated stall on resection and allows HR to occur [93] by removing 53BP1 to the periphery and allowing RPA foci to form at the damage site [94]. These observations are thought to be mediated by the human homolog of the yeast SWI/SNF-like chromatin remodeler Fun30, SMARDCAD1, which is recruited by BRCA1-BARD1 to interact with BP531 and remove it from the vicinity of the break [95, 96].

53BP1 appears to serve as a regulator of end resection and DSB resolution based on its associations with factors implicated in transcriptional silencing as well as its previously discussed functions. It can control the length of the resected ends in HR, and serves to prevent aberrant resection that can lead to RAD52-mediated ssDNA annealing and subsequent chromosomal rearrangements [97]. Further evidence for the interplay between BRCA1 and 53BP1 comes from mouse studies, where researchers found that lacking BRCA1 exon two (but expressing a RING-less BRCA1) is an embryonic lethal condition that can be rescued if the deletion occurs in a 53BP−/− embryo, suggesting that murine embryos lacking RING die because of the presence of 53BP [98]. BRCA1 and BARD1 interact with cyclin-dependent kinase 9 (CDK9) via their RING finger and BRCT domains, and localize to γ-H2AX foci indicative of damage to induce the process of HR over NHEJ [99].

Neddylation is a form of posttranslational modification similar to ubiquitination that has also been implicated in the process of double-strand break repair. Neural precursor cell expressed developmentally down-regulated 8 (NEDD8) is a ubiquitin-like protein involved in regulating cell growth, viability, and development [100]. Neddylation can serve as yet another layer of regulation in the function of DNA repair in damaged cells, and targeting this process has demonstrated some efficacy in preclinical models. Given the ubiquitous nature of BRCA1 in HR, it makes sense that this protein is a target of neddylation. In order for a cell to undergo HR, it must recognize the damage and be in the correct stage of the cell cycle (in this case, S/G2) so that a sister chromatid is present for the repair machinery to use as a template. This process of choice can be mediated by BRCA1 in complex with CtIP (RBBP8) in a number of different ways. For instance, CtIP must be phosphorylated on serine residue 327 for the cell to undergo HR, otherwise repair will be conducted via the error-prone process of microhomology-mediated end joining [101]. If this complex undergoes RNF111/UBE2M-mediated neddylation, the complex is rendered unable to perform its 5′→3′ nucleolytic end resection at the DSB, and without the ssDNA overhang tails HR cannot occur [102]. The COP9 signalosome is an additional mediator of the choice between types of DSB repair mechanisms [103]. COP9, the constitutive photomorphogenesis 9 signalosome, has significant homology with the 19S lid complex of the proteome and functions by deneddylating cullin-RING ubiquitin ligases, which may subsequently coordinate CRL-mediated ubiquitination of downstream protein targets [104]. COP9 is recruited to sites of DNA damage in a neddylation-dependent mechanism, and once there mediates deep end resection of the breaks, the first step of HR.

Targeting the process of neddylation as a preclinical strategy to sensitize tumors to chemotherapy is an avenue that has just recently began to garner attention. In a model of nonsmall cell lung cancer, neddylation inhibitor MLN4924 was able to inhibit the recruitment of members of the BRCA1 complex to sites of DNA damage. Examining expression of NEDD8, BRCA1, and PARP via Kaplan–Meier survival analysis revealed that high expression of these three factors correlated with a poor overall survival [105].

4.2. Nonhomologous end joining

The first step of nonhomologous end joining is the detection of the DSB by the Ku70/80 heterodimer, a 150 kDa Ku forms a ring-like structure that surrounds a single-strand of DNA with its central channel, and threads the broken DNA ends through this channel [106]. Because this protein can only accommodate one strand of DNA, in order for DNA replication to continue after resolution of the DSBs, Ku70/Ku80 must be removed [107]. The E3 ubiquitin ligase RING finger protein 8 (RNF8) has been found to down-regulate Ku80 at sites of DNA damage. Depletion of RNF8 leads to prolonged retention of Ku80 at damage sites and impairs NHEJ [108].

DNA-PK plays a central role in NHEJ of DNA DSBs largely during the G1 phase of the cell cycle as well as in V(D)J recombination [109, 110]. A poorly characterized ringer finger protein RNF144A has been reported as an E3 ubiquitin ligase for DNA-PK catalytic subunit (DNA-PKcs). RNF144A induces ubiquitination of DNA-PKcs in vitro and in vivo and promotes its degradation. Depletion of RNF144A results in an increased level of DNA-PKcs and resistance to DNA damaging agents [111]. Overall, there is no doubt that ubiquitination – either by regulating protein degradation or protein-protein interaction- plays a critical role in all five DNA repair families. Futures studies to better understand the role of ubiquitination, ubiquitin-like modifications, and enzymes responsible for these modifications in DNA repair pathways will be warranted.

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References

[1] Weissman AM. Themes and variations on ubiquitylation, nature reviews. Molecular Cell Biology. 2001;**2**:169-178

[2] Dianov GL, Sleeth KM, Dianova II, Allinson SL. Repair of abasic sites in DNA. Mutation Research. 2003;**531**:157-163

[3] Dianov G, Price A, Lindahl T. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. Molecular and Cellular Biology. 1992;**12**:1605-1612

[4] Dianov G, Bischoff C, Piotrowski J, Bohr VA. Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. The Journal of Biological Chemistry. 1998;**273**:33811-33816

[5] Khoronenkova SV, Dianov GL. The emerging role of mule and ARF in the regulation of base excision repair. FEBS Letters. 2011;**585**:2831-2835

[6] Parsons JL, Tait PS, Finch D, Dianova II, Allinson SL, Dianov GL. CHIP-mediated degradation and DNA damage-dependent stabilization regulate base excision repair proteins. Molecular Cell. 2008;**29**:477-487

[7] Wimmer U, Ferrari E, Hunziker P, Hubscher U. Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. EMBO Reports. 2008;**9**:1027-1033

[8] Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature. 2002;**419**: 135-141

[9] van Loon B, Hubscher U. An 8-oxo-guanine repair pathway coordinated by MUTYH glycosylase and DNA polymerase lambda. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:18201-18206

[10] Maga G, Villani G, Crespan E, Wimmer U, Ferrari E, Bertocci B, Hubscher U. 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature. 2007;**447**:606-608

[11] Parsons JL, Tait PS, Finch D, Dianova II, Edelmann MJ, Khoronenkova SV, Kessler BM, Sharma RA, McKenna WG, Dianov GL. Ubiquitin ligase ARF-BP1/Mule modulates base excision repair. The EMBO Journal. 2009;**28**:3207-3215

[12] Lee C, Smith BA, Bandyopadhyay K, Gjerset RA. DNA damage disrupts the p14ARF-B23(nucleophosmin) interaction and triggers a transient subnuclear redistribution of p14ARF. Cancer Research. 2005;**65**:9834-9842

[13] Khan S, Guevara C, Fujii G, Parry D. p14ARF is a component of the p53 response following ionizing irradiation of normal human fibroblasts. Oncogene. 2004;**23**:6040-6046

[14] Chen D, Kon N, Li M, Zhang W, Qin J, Gu W. ARF-BP1/mule is a critical mediator of the ARF tumor suppressor. Cell. 2005;**121**:1071-1083

[15] Pandya RK, Partridge JR, Love KR, Schwartz TU, Ploegh HL. A structural element within the HUWE1 HECT domain modulates self-ubiquitination and substrate ubiquitination activities. The Journal of Biological Chemistry. 2010;**285**:5664-5673

[16] Eymin B, Leduc C, Coll JL, Brambilla E, Gazzeri S. p14ARF induces G2 arrest and apoptosis independently of p53 leading to regression of tumours established in nude mice. Oncogene. 2003;**22**:1822-1835

[17] Normand G, Hemmati P.G, Verdoodt B, von Haefen C, Wendt J, Guner D, May E, Dorken B, Daniel PT. p14ARF induces G2 cell cycle arrest in p53- and p21-deficient cells by down-regulating p34cdc2 kinase activity. The Journal of Biological Chemistry. 2005;**280**:7118-7130

[18] Berneburg M, Lehmann AR. Xeroderma pigmentosum and related disorders: Defects in DNA repair and transcription. Advances in Genetics. 2001;**43**:71-102

[19] Wood RD. Nucleotide excision repair in mammalian cells. The Journal of Biological Chemistry. 1997;**272**:23465-23468

[20] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001; **411**:366-374

[21] Volker M, Mone MJ, Karmakar P, van Hoffen A, Schul W, Vermeulen W, Hoeijmakers JH, van Driel R, van Zeeland AA, Mullenders LH. Sequential assembly of the nucleotide excision repair factors in vivo. Molecular Cell. 2001;**8**:213-224

[22] Coin F, Oksenych V, Egly JM. Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair. Molecular Cell. 2007;**26**:245-256

[23] Hanawalt PC. Subpathways of nucleotide excision repair and their regulation. Oncogene. 2002;**21**:8949-8956

[24] Sijbers AM, de Laat WL, Ariza R.R, Biggerstaff M, Wei YF, Moggs JG, Carter KC, Shell BK, Evans E, de Jong MC, Rademakers S, de Rooij J, Jaspers NG, Hoeijmakers JH, Wood RD. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell. 1996;**86**:811-822

[25] Watkins JF, Sung P, Prakash L, Prakash S. The *Saccharomyces cerevisiae* DNA repair gene RAD23 encodes a nuclear protein containing a ubiquitin-like domain required for biological function. Molecular and Cellular Biology. 1993;**13**:7757-7765

[26] Madura K, Varshavsky A. Degradation of G alpha by the N-end rule pathway. Science (New York, N.Y.). 1994;**265**:1454-1458

[27] Lambertson D, Chen L, Madura K. Pleiotropic defects caused by loss of the proteasome-interacting factors Rad23 and Rpn10 of *Saccharomyces cerevisiae*. Genetics. 1999;**153**:69-79

[28] Elsasser S, Chandler-Militello D, Muller B, Hanna J, Finley D. Rad23 and Rpn10 serve as alternative ubiquitin receptors for the proteasome. The Journal of Biological Chemistry. 2004;**279**:26817-26822

[29] Russell SJ, Reed SH, Huang W, Friedberg EC, Johnston SA. The 19S regulatory complex of the proteasome functions independently of proteolysis in nucleotide excision repair. Molecular Cell. 1999;**3**:687-695

[30] Gillette TG, Huang W, Russell SJ, Reed SH, Johnston SA, Friedberg EC. The 19S complex of the proteasome regulates nucleotide excision repair in yeast. Genes & Development. 2001;**15**:1528-1539

[31] Elsasser S, Gali RR, Schwickart M, Larsen CN, Leggett DS, Muller B, Feng MT, Tubing F, Dittmar GA, Finley D. Proteasome subunit Rpn1 binds ubiquitin-like protein domains. Nature Cell Biology. 2002;**4**:725-730

[32] Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K, Nakatani Y. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. Cell. 2003;**113**:357-367

[33] Cope GA, Suh GS, Aravind L, Schwarz SE, Zipursky SL, Koonin EV, Deshaies RJ. Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science (New York, N.Y.). 2002;**298**:608-611

[34] Sugasawa K, Okuda Y, Saijo M, Nishi R, Matsuda N, Chu G, Mori T, Iwai S, Tanaka K, Tanaka K, Hanaoka F. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Cell. 2005;**121**:387-400

[35] Masutani C, Sugasawa K, Yanagisawa J, Sonoyama T, Ui M, Enomoto T, Takio K, Tanaka K, van der Spek PJ, Bootsma D, et al. Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. The EMBO Journal. 1994;**13**:1831-1843

[36] Shivji MK, Eker AP, Wood RD. DNA repair defect in xeroderma pigmentosum group C and complementing factor from HeLa cells. The Journal of Biological Chemistry. 1994;**269**:22749-22757

[37] Ng JM, Vermeulen W, van der Horst GT, Bergink S, Sugasawa K, Vrieling H, Hoeijmakers JH. A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. Genes & Development. 2003; **17**:1630-1645

[38] Okuda Y, Nishi R, Ng JM, Vermeulen W, van der Horst GT, Mori T, Hoeijmakers JH, Hanaoka F, Sugasawa K. Relative levels of the two mammalian Rad23 homologs determine composition and stability of the xeroderma pigmentosum group C protein complex. DNA Repair. 2004;**3**:1285-1295

[39] Bregman DB, Halaban R, van Gool AJ, Henning KA, Friedberg EC, Warren SL. UV-induced ubiquitination of RNA polymerase II: A novel modification deficient in Cockayne syndrome cells. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**:11586-11590

[40] Woudstra EC, Gilbert C, Fellows J, Jansen L, Brouwer J, Erdjument-Bromage H, Tempst P, Svejstrup JQ. A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature. 2002;**415**:929-933

[41] Huibregtse JM, Yang JC, Beaudenon SL. The large subunit of RNA polymerase II is a substrate of the Rsp5 ubiquitin-protein ligase. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:3656-3661

[42] Kleiman FE, Wu-Baer F, Fonseca D, Kaneko S, Baer R, Manley JL. BRCA1/BARD1 inhibition of mRNA 3′ processing involves targeted degradation of RNA polymerase II. Genes & Development. 2005;**19**:1227-1237

[43] Svejstrup JQ. Rescue of arrested RNA polymerase II complexes. Journal of Cell Science. 2003;**116**:447-451

[44] de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. Genes & Development. 1999;**13**:768-785

[45] Sweder K, Madura K. Regulation of repair by the 26S proteasome. Journal of Biomedicine & Biotechnology. 2002;**2**:94-105

[46] Lommel L, Ortolan T, Chen L, Madura K, Sweder KS. Proteolysis of a nucleotide excision repair protein by the 26S proteasome. Current Genetics. 2002;**42**:9-20

[47] Lommel L, Chen L, Madura K, Sweder K. The 26S proteasome negatively regulates the level of overall genomic nucleotide excision repair. Nucleic Acids Research. 2000;**28**: 4839-4845

[48] Chen L, Shinde U, Ortolan TG, Madura K. Ubiquitin-associated (UBA) domains in Rad23 bind ubiquitin and promote inhibition of multi-ubiquitin chain assembly. EMBO Reports. 2001;**2**:933-938

[49] Heessen S, Masucci MG, Dantuma NP. The UBA2 domain functions as an intrinsic stabilization signal that protects Rad23 from proteasomal degradation. Molecular Cell. 2005;**18**:225-235

[50] Zhang Y. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes & Development. 2003;**17**:2733-2740

[51] de Napoles M, Mermoud JE, Wakao R, Tang YA, Endoh M, Appanah R, Nesterova TB, Silva J, Otte AP, Vidal M, Koseki H, Brockdorff N. Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Developmental Cell. 2004;**7**:663-676

[52] Levinger L, Varshavsky A. Selective arrangement of ubiquitinated and D1 protein-containing nucleosomes within the drosophila genome. Cell. 1982;**28**:375-385

[53] Bergink S, Salomons FA, Hoogstraten D, Groothuis TA, de Waard H, Wu J, Yuan L, Citterio E, Houtsmuller AB, Neefjes J, Hoeijmakers JH, Vermeulen W, Dantuma NP. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. Genes & Development. 2006;**20**:1343-1352

[54] O'Driscoll M, Ruiz-Perez VL, Woods CG, Jeggo PA, Goodship JA. A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. Nature Genetics. 2003;**33**:497-501

[55] Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner WM, Petrini JH, Haber JE, Lichten M. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Current Biology: CB. 2004;**14**:1703-1711

[56] Marteijn JA, Bekker-Jensen S, Mailand N, Lans H, Schwertman P, Gourdin AM, Dantuma NP, Lukas J, Vermeulen W. Nucleotide excision repair-induced H2A ubiquitination is dependent on MDC1 and RNF8 and reveals a universal DNA damage response. The Journal of Cell Biology. 2009;**186**:835-847

[57] Schroering AG, Edelbrock MA, Richards TJ, Williams KJ. The cell cycle and DNA mismatch repair. Experimental Cell Research. 2007;**313**:292-304

[58] Zhang M, Xiang S, Joo HY, Wang L, Williams KA, Liu W, Hu C, Tong D, Haakenson J, Wang C, Zhang S, Pavlovicz RE, Jones A, Schmidt KH, Tang J, Dong H, Shan B, Fang B, Radhakrishnan R, Glazer PM, Matthias P, Koomen J, Seto E, Bepler G, Nicosia SV, Chen J, Li C, Gu L, Li GM, Bai W, Wang H, Zhang X. HDAC6 deacetylates and ubiquitinates MSH2 to maintain proper levels of MutSalpha. Molecular Cell. 2014;**55**:31-46

[59] Hernandez-Pigeon H, Laurent G, Humbert O, Salles B, Lautier D. Degradation of mismatch repair hMutSalpha heterodimer by the ubiquitin-proteasome pathway. FEBS Letters. 2004;**562**:40-44

[60] Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science (New York, N.Y.). 1995; **268**:1909-1912

[61] Genschel J, Littman SJ, Drummond JT, Modrich P. Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. The Journal of Biological Chemistry. 1998;**273**:19895-19901

[62] Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 1998;**4**:1-6

[63] Li GM. The role of mismatch repair in DNA damage-induced apoptosis. Oncology Research. 1999;**11**:393-400

[64] Li GM. Mechanisms and functions of DNA mismatch repair. Cell Research. 2008;**18**:85-98

[65] Chang DK, Ricciardiello L, Goel A, Chang CL, Boland CR. Steady-state regulation of the human DNA mismatch repair system. The Journal of Biological Chemistry. 2000;**275**: 18424-18431

[66] Humbert O, Hermine T, Hernandez H, Bouget T, Selves J, Laurent G, Salles B, Lautier D. Implication of protein kinase C in the regulation of DNA mismatch repair protein expression and function. The Journal of Biological Chemistry. 2002;**277**:18061-18068

[67] Zhang M, Hu C, Tong D, Xiang S, Williams K, Bai W, Li GM, Bepler G, Zhang X. Ubiquitin-specific peptidase 10 (USP10) Deubiquitinates and stabilizes MutS homolog 2 (MSH2) to regulate cellular sensitivity to DNA damage. The Journal of Biological Chemistry. 2016;**291**:10783-10791

[68] Yuan J, Luo K, Zhang L, Cheville JC, Lou Z. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell. 2010;**140**:384-396

[69] Jochemsen AG, Shiloh Y. USP10: Friend and foe. Cell. 2010;**140**:308-310

[70] Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, Cai Y, Norberg HV, Zhang T, Furuya T, Jin M, Zhu Z, Wang H, Yu J, Li Y, Hao Y, Choi A, Ke H, Ma D, Yuan J. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011; **147**:223-234

[71] Zlatanou A, Despras E, Braz-Petta T, Boubakour-Azzouz I, Pouvelle C, Stewart GS, Nakajima S, Yasui A, Ishchenko AA, Kannouche PL. The hMsh2-hMsh6 complex acts in concert with monoubiquitinated PCNA and pol eta in response to oxidative DNA damage in human cells. Molecular Cell. 2011;**43**:649-662

[72] Pena-Diaz J, Bregenhorn S, Ghodgaonkar M, Follonier C, Artola-Boran M, Castor D, Lopes M, Sartori AA, Jiricny J. Noncanonical mismatch repair as a source of genomic instability in human cells. Molecular Cell. 2012;**47**:669-680

[73] Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003;**421**:499-506

[74] Wu J, Chen Y, Lu LY, Wu Y, Paulsen MT, Ljungman M, Ferguson DO, Yu X. Chfr and RNF8 synergistically regulate ATM activation. Nature Structural & Molecular Biology. 2011;**18**:761-768

[75] Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vrouwe MG, Demmers JA, van IJcKen WF, Grosveld FG, Medema RH, Hoeijmakers JH, Mullenders LH, Vermeulen W, Marteijn JA. The core spliceosome as target and effector of non-canonical ATM signalling. Nature. 2015; **523**:53-58

[76] Huyen Y, Zgheib O, Ditullio RA Jr, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature. 2004;**432**:406-411

[77] Pei H, Zhang L, Luo K, Qin Y, Chesi M, Fei F, Bergsagel PL, Wang L, You Z, Lou Z. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature. 2011;**470**:124-128

[78] Bekker-Jensen S, Mailand N. The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. FEBS Letters. 2011;**585**:2914-2919

[79] Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**:887-900

[80] Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, Alt FW, Chen J. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Molecular Cell. 2006;**21**:187-200

[81] Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer Research. 2004;**64**:2390-2396

[82] Wilson MD, Benlekbir S, Fradet-Turcotte A, Sherker A, Julien JP, McEwan A, Noordermeer SM, Sicheri F, Rubinstein JL, Durocher D. The structural basis of modified nucleosome recognition by 53BP1. Nature. 2016;**536**:100-103

[83] Fradet-Turcotte A, Canny MD, Escribano-Diaz C, Orthwein A, Leung CC, Huang H, Landry MC, Kitevski-LeBlanc J, Noordermeer SM, Sicheri F, Durocher D. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature. 2013;**499**:50-54

[84] Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Molecular Cell. 1999;**3**:389-395

[85] Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. Molecular Cell. 1999;**4**:511-518

[86] Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science (New York, N.Y.). 2007;**316**:1194-1198

[87] Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell. 2007;**131**:901-914

[88] Wu W, Koike A, Takeshita T, Ohta T. The ubiquitin E3 ligase activity of BRCA1 and its biological functions. Cell Division. 2008;**3**:1

[89] Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE. Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nature Structural Biology. 2001;**8**:833-837

[90] Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**:11364-11369

[91] Taherbhoy AM, Huang OW, Cochran AG. BMI1-RING1B is an autoinhibited RING E3 ubiquitin ligase. Nature Communications. 2015;**6**:7621

[92] Densham RM, Morris JR. The BRCA1 ubiquitin ligase function sets a new trend for remodelling in DNA repair. Nucleus (Austin, Tex.). 2017;**8**:116-125

[93] Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, Xu X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;**141**:243-254

[94] Chapman JR, Sossick AJ, Boulton SJ, Jackson SP. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. Journal of Cell Science. 2012;**125**:3529-3534

[95] Costelloe T, Louge R, Tomimatsu N, Mukherjee B, Martini E, Khadaroo B, Dubois K, Wiegant WW, Thierry A, Burma S, van Attikum H, Llorente B. The yeast Fun30 and human SMARCAD1 chromatin remodellers promote DNA end resection. Nature. 2012;**489**:581-584

[96] Chen X, Cui D, Papusha A, Zhang X, Chu CD, Tang J, Chen K, Pan X, Ira G. The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. Nature. 2012;**489**:576-580

[97] Ochs F, Somyajit K, Altmeyer M, Rask MB, Lukas J, Lukas C. 53BP1 fosters fidelity of homology-directed DNA repair. Nature Structural & Molecular Biology. 2016;**23**:714-721

[98] Li M, Cole F, Patel DS, Misenko SM, Her J, Malhowski A, Alhamza A, Zheng H, Baer R, Ludwig T, Jasin M, Nussenzweig A, Serrano L, Bunting SF. 53BP1 ablation rescues genomic instability in mice expressing 'RING-less' BRCA1. EMBO Reports. 2016;**17**: 1532-1541

[99] Nepomuceno TC, Fernandes VC, Gomes TT, Carvalho RS, Suarez-Kurtz G, Monteiro AN, Carvalho MA. BRCA1 recruitment to damaged DNA sites is dependent on CDK9. Cell cycle (Georgetown, Tex.). 2017;**16**:665-672

[100] Xirodimas DP. Novel substrates and functions for the ubiquitin-like molecule NEDD8. Biochemical Society Transactions. 2008;**36**:802-806

[101] Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature. 2009;**459**:460-463

[102] Jimeno S, Fernandez-Avila MJ, Cruz-Garcia A, Cepeda-Garcia C, Gomez-Cabello D, Huertas P. Neddylation inhibits CtIP-mediated resection and regulates DNA double strand break repair pathway choice. Nucleic Acids Research. 2015;**43**:987-999

[103] Meir M, Galanty Y, Kashani L, Blank M, Khosravi R, Fernandez-Avila MJ, Cruz-Garcia A, Star A, Shochot L, Thomas Y, Garrett LJ, Chamovitz DA, Bodine DM, Kurz T, Huertas P, Ziv Y, Shiloh Y. The COP9 signalosome is vital for timely repair of DNA double-strand breaks. Nucleic Acids Research. 2015;**43**:4517-4530

[104] Lee MH, Zhao R, Phan L, Yeung SC. Roles of COP9 signalosome in cancer. Cell cycle (Georgetown, Tex.). 2011;**10**:3057-3066

[105] Guo ZP, Hu YC, Xie Y, Jin F, Song ZQ, Liu XD, Ma T, Zhou PK. MLN4924 suppresses the BRCA1 complex and synergizes with PARP inhibition in NSCLC cells. Biochemical and Biophysical Research Communications. 2017;**483**:223-229

[106] Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature. 2001;**412**:607-614

[107] Postow L. Destroying the ring: Freeing DNA from Ku with ubiquitin. FEBS Letters. 2011;**585**:2876-2882

[108] Feng L, Chen J. The E3 ligase RNF8 regulates KU80 removal and NHEJ repair. Nature Structural & Molecular Biology. 2012;**19**:201-206

[109] Davis AJ, Chen BP, Chen DJ. DNA-PK: A dynamic enzyme in a versatile DSB repair pathway. DNA Repair. 2014;**17**:21-29

[110] Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nature Cell Biology. 2014;**16**:2-9

[111] Ho SR, Mahanic CS, Lee YJ, Lin WC. RNF144A, an E3 ubiquitin ligase for DNA-PKcs, promotes apoptosis during DNA damage. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**:E2646-2655

Chapter 3

Regulation of the Base Excision Repair Pathway by Ubiquitination

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Additional information is available at the end of the chapter

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Abstract

Genome integrity is under constant threat from cellular reactive oxygen species generated by endogenous and exogenous mutagens. The base excision repair (BER) pathway consequently plays a crucial role in the repair of DNA base damage, sites of base loss and DNA single strand breaks that can cause genome instability and ultimately the development of human diseases, including premature ageing, neurodegenerative disorders and cancer. Proteins within the base excision repair pathway are increasingly being found to be regulated and controlled by post-translational modifications, and indeed ubiquitination performs a key role in the maintenance of repair protein levels but may also impact on protein activity and cellular localisation. This process is therefore important in maintaining an efficient cellular DNA damage response, and if not accurately controlled, can cause DNA damage accumulation and promote mutagenesis and genomic instability. In this chapter, we will present up-to-date information on the evidence of ubiquitination of base excision repair proteins, the enzymes involved and the molecular and cellular consequences of this process.

Keywords: DNA repair, base excision repair, DNA damage, ubiquitin, ubiquitination

1. Introduction

Every human cell per day is thought to generate greater than 10,000 DNA base lesions and single strand breaks (SSBs) due to the instability of the DNA molecule [1]. These are largely created by cellular reactive oxygen species that are generated by hydrolysis, oxidative metabolism and environmental factors, including ionising radiation (IR). Typical sites of damage include sites of base loss (abasic sites), oxidised DNA bases (e.g. 8-oxoguanine and thymine glycol) and SSBs. If this DNA damage is left unrepaired, it can cause mutagenesis and genome instability which are contributors to the development of human diseases, including premature ageing, neurodegenerative disorders and cancer. The base excision repair (BER) pathway was first identified in the 1970s by Tomas Lindahl (co-recipient of the 2015 Nobel Prize in Chemistry), who discovered the existence of a uracil DNA *N*-glycosylase that is able to excise uracil residues from DNA [2]. Lindahl then suggested that in order for repair to be completed, an endonuclease, a DNA polymerase and a DNA ligase would be required. This marked the establishment that a specific repair pathway exists in human cells to repair DNA base damage, and now nearly a half a century later, the major enzymes and mechanisms involved in BER are well known.

As Lindahl had shown, the first step of BER is recognition of the specific damaged DNA base by a DNA glycosylase. In fact 11 DNA glycosylases are now known to exist with each removing particular types of DNA base damage [3, 4]. Indeed there are three uracil DNA glycosylase enzymes that recognise uracil lesions (namely uracil DNA glycosylase, UNG; single-strand-selective monofunctional uracil DNA glycosylase, SMUG1; and thymine DNA glycosylase, TDG), one enzyme that recognises alkylated bases (*N*-methylpurine DNA glycosylase, MPG), two mismatch-specific glycosylases (methyl-CpG binding domain protein 4, MBD4 and MutY homologue, MUTYH) and five glycosylases that recognise oxidised bases (8-oxoguanine DNA glycosylase 1, OGG1; endonuclease III-like protein 1, NTH1; endonuclease VIII-like proteins 1, 2 and 3; NEIL1, NEIL2 and NEIL3). In general, DNA glycosylases utilise a base-flipping mechanism whereby the base is flipped 180° from the sugar phosphate backbone breaking the hydrogen bonds between the bases in the process, and placing the damaged base into the active site of the DNA glycosylase. However, there are two types of glycosylase enzyme as one type will only remove the damaged base (monofunctional enzyme) whereas another type will remove the base but also cleave the DNA backbone (bifunctional enzyme). The monofunctional DNA glycosylases (UNG, SMUG1, TDG, MPG, MUTYH and MBD4) that remove the damaged base will create an abasic site by cleaving the *N*-glycosidic bond linking the base to the phosphodiester backbone. The abasic site is then recognised by AP endonuclease 1 (APE1) that cleaves 5′- to the lesion, creating a one nucleotide gap containing a 3′-hydroxyl group on one end, and a 5′-deoxyribose phosphate (5′-dRP) group on the other [5, 6]. The 5′-dRP group is subsequently removed by the lyase activity of DNA polymerase β (Pol β) that also simultaneously inserts the correct, undamaged nucleotide [7, 8]. The remaining nick in the DNA backbone is then sealed by DNA ligase IIIα (Lig IIIα) that is in a stable complex with X-ray cross-complementing protein 1 (XRCC1), to restore the original DNA sequence (**Figure 1**) [9, 10]. In contrast, bifunctional DNA glycosylases create a single nucleotide gap that is flanked by different ends, depending on the glycosylase employed. OGG1 and NTH1 are known to catalyse β-elimination which creates a 5′-phosphate and a 3′-α,β-unsaturated aldehyde, however, these are thought to be low efficiency activities and therefore with the high cellular abundance of APE1, it is thought that APE1 can actually circumvent this product and cleave the abasic site itself [11]. The bifunctional DNA glycosylases NEIL1, NEIL2 and NEIL3 catalyse β,δ-elimination which create a phosphate moiety on both the 5′- and 3′-end of the single nucleotide gap. Since the 3′-phosphate is not the required end for Pol β activity, this requires removal by polynucleotide kinase phosphatase (PNKP) (**Figure 1**) [12]. Despite the dependence on either APE1 or PNKP, following bifunctional DNA glycosylase activity the end product is the same as monofunctional DNA glycosylase activity in that both Pol β and XRCC1-Lig IIIα are required to complete the repair process.

The pathway described above is commonly referred to as the short patch BER pathway, through which the majority of DNA repair events proceed [13]. However under certain conditions, particularly when the DNA ends are resistant to processing (e.g. if the 5′-dRP becomes reduced and thus cannot be cleaved by Pol β), then long-patch BER is employed (**Figure 1**). Following the addition of the first nucleotide by Pol β [14], there is a polymerase switch to DNA polymerases δ/ε (Pol δ/ε), which are principally involved in DNA replication. These polymerases typically add two to eight more nucleotides into the repair gap thus generating a 5′-flap structure. This structure is a substrate for flap endonuclease-1 (FEN-1), which acts in a proliferating cell nuclear antigen (PCNA)-dependent manner and then finally DNA ligase I (Lig I) completes the repair

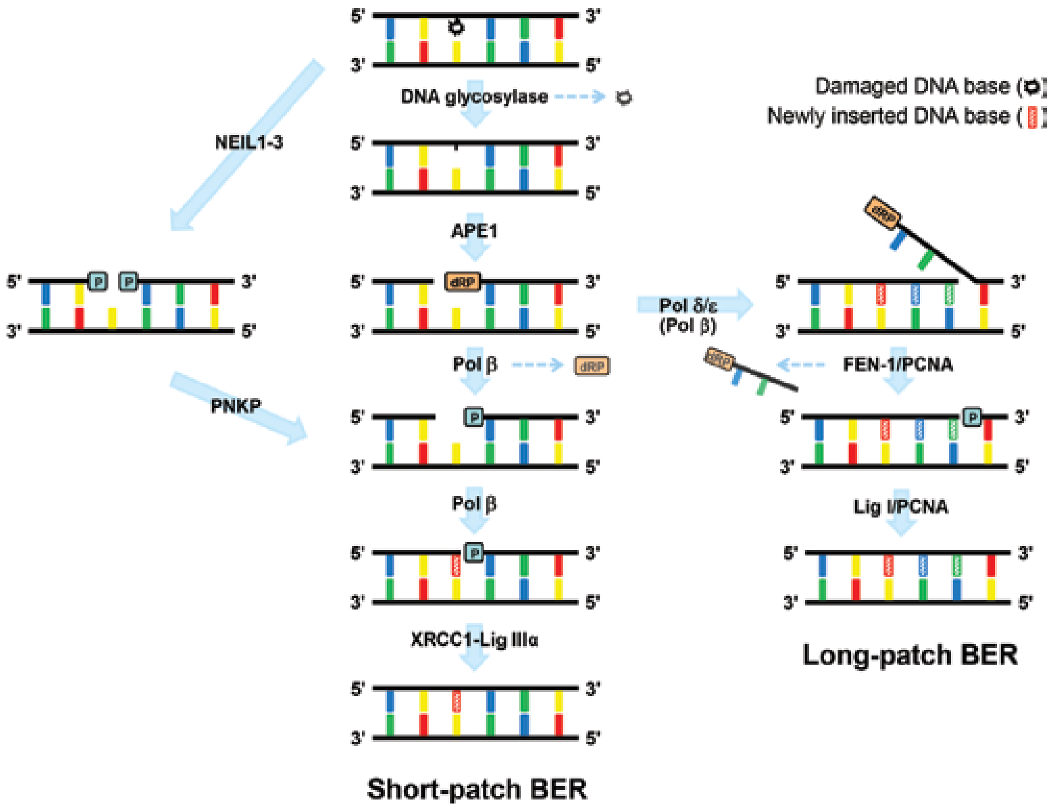

Figure 1. The mechanism of repair of DNA base damage by the BER pathway. The damaged DNA base is recognised and excised by damage-specific DNA glycosylases that catalyse cleavage of the N-glycosidic bond, creating an abasic site. APE1 recognises the abasic site and cleaves the DNA backbone, generating a single strand break containing a 5′-dRP moiety. The 5′-dRP is subsequently removed by the dRP lyase activity of Pol β that furthermore inserts a new undamaged nucleotide into the repair gap (central branch). If BER is initiated by the NEIL DNA glycosylases (NEIL1, NEIL2 and NEIL3), these enzymes generate a single nucleotide gap containing 3′- and 5′-phosphate ends through β,δ-elimination activity. The 3′-phosphate is subsequently removed by PNKP prior to the activity of Pol β that fills in the repair gap (left branch). Finally, the Lig IIIa-XRCC1 complex completes short patch BER by sealing the remaining nick in the phosphodiester backbone. If the 5′-dRP moiety is resistant to Pol β dRP lyase activity, a polymerase switch to Pol δ/ε occurs, which then stimulate the addition of two to eight more nucleotides into the single nucleotide gap. This generates a 5′-flap structure which is recognised and excised by FEN-1, in a PCNA-dependent manner (right branch). To complete long patch BER DNA ligase I, also in association with PCNA, seals the remaining nick in the DNA backbone.

process by sealing the remaining nick. It is important to note that with the constant, significant amount of DNA base damage and SSBs being induced in cells, that BER is a constitutively active process.

2. Regulation of BER proteins through ubiquitination

Since BER is the major cellular mechanism for the repair of DNA base damage and SSBs, and thus for the maintenance of genome stability, it is important that this process is maintained and controlled. The most efficient way of achieving this, particularly in responding to fluctuations in the cellular DNA damage environment, is via controlling cellular protein activity, localisation and overall protein levels. Indeed there is a growing list of the various protein post-translational modifications (PTMs) of BER proteins that have been reported to achieve this [15]. However a role for ubiquitination in controlling BER protein levels, and thus cellular BER activity, has been highlighted particularly in the last decade. Polyubiquitination of BER proteins catalysed by specific E3 ubiquitin ligases has been shown to largely control cellular protein levels via degradation by the 26S proteasome, but additionally monoubiquitination has been observed in some instances that can act by compartmentalising BER proteins or controlling BER protein activity. There are also instances of crosstalk between ubiquitination and other PTMs in controlling cellular BER. Below we aim to summarise all of the available evidence highlighting the enzymes and mechanisms involved in the control of BER proteins through ubiquitination.

2.1. Ubiquitination of DNA glycosylases

2.1.1. Uracil DNA glycosylases: UNG, SMUG1, MBD4, TDG

Of the four members of the uracil DNA glycosylases, only UNG, SMUG1 and TDG have been shown to be ubiquitinated by specific E3 ubiquitin ligases. Binding of the human immunodeficiency virus type 1 (HIV-1) accessory protein Vpr to UNG and SMUG1 was shown to induce their ubiquitination-dependent proteasomal degradation following expression in 293T cells. This was thought to be promoted through the E3 ubiquitin ligase scaffold proteins, Cullin 1 (Cul1) and Cullin 4 (Cul4), as Vpr interacts with these ligases along with UNG and SMUG1 following overexpression and immunoprecipitation from 293T cells [16]. Vpr was subsequently shown to bind to damage-specific DNA-binding protein 1 (DDB1), which is another component of Cul4A E3 ubiquitin ligases, that mediates the degradation of UNG in 293T cells [17]. This is thought to be a specific mechanism that allows the HIV virus to regulate the levels of abasic sites in viral reverse transcripts and thus promotes viral replication. Therefore whether UNG and SMUG1 are targeted for ubiquitination during normal cellular processing and for BER is not yet known. The third member of the uracil DNA glycosylase family, TDG, is largely known for being regulated by the small ubiquitin-like modifier (SUMO). TDG was shown to be modified by SUMO-1 and SUMO-2/3 on lysine 330 following immunoprecipitation from HeLa cells, and this reduces the abasic site affinity of TDG [18]. TDG SUMOylation induces a conformational change in the protein which overcomes product inhibition and is thus a mechanism for increasing enzymatic turnover [19, 20]. More recently, and similar to UNG and

SMUG1, TDG has been shown to be a target for ubiquitination-dependent degradation by a Cul4-DDB1 associated E3 ubiquitin ligase complex [21, 22]. Specifically, TDG degradation was catalysed by Cul4A-DDB1 in association with the RING finger protein ROC1/RBX1 and Cdt2 (collectively called CRL4^{Cdt2}), in a PCNA-dependent manner. This was discovered both in a *Xenopus* extract system during DNA repair but also in HeLa cells during S phase of the cell cycle where TDG interacts with PCNA, and is thought to provide a mechanism for the regulated control of TDG protein levels. The fourth uracil DNA glycosylase member, MBD4 which actually removes mismatches opposite guanine, has not been reported to be ubiquitinated. However MBD4 has been shown to interact with both the E3 ubiquitin ligase, ubiquitin-like with PHD and RING finger domains 1 (UHRF1) and with the deubiquitinating enzyme, ubiquitin specific protease 7 (USP7) following overexpression of the protein in HEK293T cells [23]. Given these interactions, MBD4 could potentially be a target for regulation by ubiquitination.

2.1.2. *Helix-hairpin-helix (HhH) DNA glycosylases: OGG1, NTH1, MUTYH*

The HhH DNA glycosylases are named after the DNA-binding motif which is present in all three members of the family and are OGG1, NTH1 and MUTYH. OGG1 is the major DNA glycosylase targeting 8-oxoguanine DNA base damage and only one report has suggested that it is a target for ubiquitination. Specifically OGG1 was found to be degraded following mild hyperthermia by the E3 ubiquitin ligase C-terminus of Hsc70-interacting protein (CHIP) [24]. CHIP is well known to be involved in protein quality control, by targeting damaged or misfolded proteins for ubiquitination-dependent degradation via interaction with the molecular chaperones Hsc70 and Hsp90 [25], and as will become clear later in this chapter, can target multiple BER proteins for degradation via the proteasome. Degradation of OGG1 by CHIP through heat inactivation in HeLa cells was shown to cause a reduction in the efficiency of repair of oxidised DNA base damage and cell growth inhibition following treatment with a photosensitiser. NTH1 is the second member of the HhH DNA glycosylases that excises oxidised pyrimidines from DNA, including 5-hydroxycytosine and thymine glycol, although there are no current reports that it is directly targeted for ubiquitination. However there is evidence that MUTYH, the third member of the family that specifically removes adenine residues incorrectly incorporated opposite 8-oxoguanine residues during DNA transcription or replication, is ubiquitinated both *in vitro* and *in vivo*. Ubiquitination between residues 475 and 500 within the MUTYH protein was shown to be catalysed by the Mcl1 ubiquitin ligase E3/ARF binding protein 1 (Mule/ARF-BP1) that subsequently stimulates proteasomal degradation [26]. This was evidenced *in vitro* using recombinant proteins, and also *in vivo* ubiquitination of MUTYH decreased in HEK293T cells following Mule siRNA and led to increased protein stability. A ubiquitination-deficient mutant of MUTYH lacking five critical lysine residues (477, 478, 495, 506 and 507) that were mutated to arginines, was found to be more stable in HEK293T cells than the wild type protein and preferentially bound to chromatin [26]. Mule overexpression in A2780 cells led to an increased mutation frequency following potassium bromate treatment that was predicted to be a consequence of the lack of MUTYH through Mule-dependent degradation. Mule has also been demonstrated to regulate the protein levels of Pol β and Pol λ (see below), and therefore appears to be a critical regulator of BER at both the base excision and gap filling stages. How the activity of Mule is co-ordinated towards these specific proteins and stages is not currently clear.

2.1.3. Endonuclease VIII-like glycosylases: NEIL1, NEIL2, NEIL3

NEIL1 and NEIL2 both have a broad range substrate specificity that largely covers those of the HhH enzymes OGG1 and NTH1. However, these DNA glycosylases appear more active on single stranded DNA and bubble structures and so may be more important during replication and transcription where these structures are formed [27, 28]. There is also evidence that NEIL1 is active at complex DNA damage sites, where two or more DNA base lesions are formed in close proximity [29, 30], and on telomeric DNA [31]. NEIL3 substrate activity has also proven to be elusive but can similarly act on telomeric DNA [31, 32] and more recent data has described a role for NEIL3 in unhooking of DNA interstrand crosslinks [33]. The only evidence of ubiquitination-dependent regulation of the NEIL DNA glycosylases is through very recent data involving NEIL1. Using an *in vitro* ubiquitination system in combination with fractionated HeLa cell extracts, NEIL1 was demonstrated to be targeted by two E3 ubiquitin ligases, namely Mule and tripartite motif 26 (TRIM26) [34]. Both enzymes appear to promote ubiquitination of NEIL1 on the same C-terminal lysine residues (319, 333, 356, 357, 361, 374 and 376) within the protein, and an siRNA knockdown of either Mule or TRIM26 in U2OS cells caused an increase in cellular NEIL1 protein levels demonstrating that they both target the protein for ubiquitination-dependent degradation. Interestingly in response to IR-induced DNA damage, there was an accumulation of NEIL1 protein which occurred in a Mule-dependent, but not a TRIM26-dependent, manner. This demonstrated a requirement for both Mule and TRIM26 in controlling the cellular steady state levels of NEIL1, in addition to those required in response to DNA damage.

2.1.4. N-methyl purine glycosylase (MPG)

MPG is a DNA glycosylase that excises alkylated DNA base damage, including 3-methyladenine and 7-methylguanine. There is no current evidence that this enzyme is regulated directly by ubiquitination, although MPG has been reported to interact with the E3 ubiquitin ligases UHRF1 and UHRF2 following overexpression in HEK293 cells, and interacts with UHRF1 in a number of cancer cell lines, including MCF7, HeLa and H1299 [35].

2.2. Ubiquitination of DNA strand break binders/processors

2.2.1. Poly(ADP-ribose) polymerase 1 (PARP-1)

PARP-1 functions in binding to SSBs created during BER, where it mediates poly(ADP-ribosyl)ation of itself and other proteins involved in the repair process and thus promotes the assembly of repair protein complexes, chromatin remodelling and its own eventual dissociation from the DNA. Inhibitors targeting PARP-1 activity have recently been approved for the treatment of BRCA-deficient cancers, through which they cause synthetic lethality. This therapeutic exploitation provides an added incentive to enhance our understanding of the regulation of cellular PARP-1, particularly through ubiquitination.

The first report to show that PARP-1 is ubiquitinated was in mouse embryonic fibroblasts following treatment with the proteasome inhibitor ALLN [36]. PARP-1 ubiquitination was further examined *in vitro* and shown to involve specifically lysine 48-linked chains, suggesting that this would likely target the enzyme for proteasomal degradation. PARP-1 modification by SUMOylation, specifically SUMO-2 at lysine 203 and 486 induced by the PIASy SUMO E3 ligase, has been demonstrated following overexpression of the enzymes in HeLa cells in response to heat shock stress, which also rendered PARP-1 as a target for ubiquitination by the poly-SUMO-specific E3 ubiquitin ligase ring finger protein 4 (RNF4) [37]. PARP-1 ubiquitination resulted in degradation of the protein, and provides evidence of crosstalk between two PTMs thought to be involved in regulating PARP-1 transcriptional activation, rather than playing a role during BER. Another incidence of crosstalk has been revealed between PARP-1 ubiquitination and poly(ADP-ribosyl)ation. The E3 ubiquitin ligase ring finger protein 146 (RNF146), also known as Iduna, ubiquitinates PARP-1 *in vitro* and in MCF7 cells overexpressing Iduna leading to its proteasomal degradation providing that PARP-1 itself is poly(ADP-ribosyl)ated [38]. This phenomenon was more pronounced following N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-induced alkylated base damage, suggesting a DNA damage inducible response. Furthermore, an shRNA-induced knockdown of Iduna caused an accumulation of abasic sites following MNNG treatment, and an accumulation of SSBs following IR in MCF7 cells. This is difficult to comprehend given the abundance of cellular APE1 and its ability to cleave any abasic sites following MNNG treatment, and that PARP-1 should still be able to dissociate from IR-induced SSBs following poly(ADP-ribosyl)ation and allow for subsequent repair protein recruitment. However since Iduna has also been shown to polyubiquitinate other BER proteins, including XRCC1 and Lig III, the effects of Iduna on the celllular DNA damage response are not clear cut.

A third E3 ubiquitin ligase has been identified for PARP-1, namely the checkpoint with forkhead-associated and RING finger domain protein (CHFR), which was shown to polyubiquitinate PARP-1 *in vitro* and following overexpression of CHFR in HEK293T and HCT116 cells *in vivo* [39]. CHFR regulates the mitotic checkpoint and following mitotic stress PARP-1 was shown to undergo poly(ADP-ribosyl)ation, and similarly to the case for Iduna, this facilitated polyubiquitination-dependent degradation of the protein. CHFR-knockout mouse embryonic fibroblasts displayed elevated PARP-1 levels and did not undergo cell cycle arrest in response to mitotic stress. An additional report similarly described poly(ADP-ribosyl)ated PARP-1 as a CHFR target [40]. CHFR was recruited to laser-induced DNA damage sites in a poly(ADP-ribosyl)ation-dependent manner in U2OS cells as revealed by PARP inhibition and PARP-1 siRNA. Interestingly, lysine 48-linked ubiquitin chains were conjugated to poly(ADP-ribosyl)ated, but not unmodified, PARP-1 by CHFR *in vitro* in the presence of the E2 conjugating enzyme UbcH5C, but lysine-63 linked chains were added in the presence of Ubc13/Uev1a. Both lysine 48 and 63-linked chains were then found attached to PARP-1 following irradiation of CHRF-overexpressing HCT116 cells. Potentially more important than its eventual degradation, CHFR-mediated ubiquitination prompted the displacement of PARP-1 from chromatin, and CHFR-knockout mouse embryonic fibroblasts were demonstrated to display delayed SSB repair kinetics and increased sensitivity to IR.

As multiple E3 ubiquitin ligases have been implicated as effectors of PARP-1 ubiquitination, more research is required to determine which of these are crucially involved in the regulation of steady state PARP-1 levels and which function specifically during BER. It is apparent that

PARP-1 regulation is multifaceted, with the added complexity of crosstalk between ubiquitination and other PTMs such as SUMOylation and poly(ADP-ribosyl)ation, therefore it may be some time before the intricacies of this regulation are elucidated.

2.2.2. *AP endonuclease I (APE1)*

APE1 is the major enzyme targeting abasic sites for incision in human cells, and both an overabundance and lack of this protein can cause genome instability, so the protein levels must be tightly regulated. APE1 was first shown to be monoubiquitinated within the N-terminus of the protein in HCT116 cells by overexpression of the E3 ubiquitin ligase mouse double minute homologue 2 (MDM2), the major enzyme regulating the p53 tumour suppressor protein [41]. Depletion of MDM2 consequently increased APE1 protein levels, thought to be as a result of reduced ubiquitination-dependent degradation. The same authors then reported that phosphorylation of APE1 at threonine 233 by cyclin-dependent kinase 5 (Cdk5)-enhanced MDM2-dependent ubiquitination of APE1 [42]. Indeed, a phosphomimetic mutant (T233E) of APE1 exhibited augmented ubiquitination following expression in HCT116, SW480 and A549 cells. However MDM2 knockout mouse embryonic fibroblasts expressing the phosphomimetic mutant of APE1 still displayed significant APE1 ubiquitination, demonstrating the existence of other E3 ubiquitin ligases for the protein. In fact utilising an *in vitro* ubiquitination assay incorporating APE1 as a substrate and fractionated proteins from HeLa whole cell lysates has revealed that the major E3 ubiquitin ligase targeting APE1 for ubiquitination was ubiquitin protein ligase E3 component N-recognin 3 (UBR3) [43]. *In vitro* ubiquitination of APE1 by UBR3 was localised within the N-terminus on multiple lysine residues (6, 7, 24, 25, 27, 31, 32 and 35). UBR3 knockout mouse embryonic fibroblasts displayed significantly higher APE1 protein levels, suggesting that ubiquitination targeted APE1 for proteasomal degradation, and consequently led to an increase in endogenously formed DNA double strand breaks and genomic instability. A third E3 ubiquitin ligase has recently been identified for APE1, namely the Parkinson's disease-associated protein, Parkin [44]. Overexpression of Parkin was found to ubiquitinate APE1 in A549 cells, and in an engineered mouse embryonic fibroblast cell line containing low APE1 protein overexpression of both Parkin and APE1 caused a decrease in protein stability. However, inducible expression of Parkin in 293-PaPi cells did not alter endogenous APE1 protein levels and a combination of Parkin and hydrogen peroxide treatment only caused an ~30% reduction in the protein, suggesting that Parkin may only have a minor role in APE1 regulation.

2.2.3. *Polynucleotide kinase phosphatase (PNKP)*

PNKP acts to remove the 3′-phosphate group remaining from the actions of NEIL1–3 during BER, but also displays kinase activity for 5′-DNA ends and thus plays a role in the repair of SSBs and double strand breaks. A crosstalk between phosphorylation and ubiquitination has been revealed to be important in the regulation of PNKP protein levels. Phosphorylation catalysed by the ataxia telangiectasia mutated (ATM) protein kinase on serines 114 and 126 of PNKP was shown to stabilise the protein in response to oxidative stress in HCT116 cells, which was mediated through inhibition of ubiquitination, and which was required for efficient SSB repair [45]. An *in vitro* ubiquitination assay, using PNKP as a substrate in combination with fractionated proteins from HeLa whole cell lysates, revealed that Cul4A-DDB1 in association with the WD-40 repeat protein serine-threonine kinase receptor-associated protein (STRAP) was the major E3 ubiquitin ligase complex that was targeting PNKP for ubiquitination, specifically on lysines 414, 417 and 484. A phosphomimetic mutant (serine 114 and 126 to glutamic acid) was more stable than the wild type protein following expression in HCT116 cells, and the protein itself was resistant to *in vitro* ubiquitination by Cul4A-DDB1-STRAP. Mouse embryonic fibroblasts from STRAP knockout cells also had significantly elevated PNKP protein levels due to reduced ubiquitination-dependent degradation, and displayed increased resistance to oxidative stress. In addition, an interaction between PNKP and the deubiquitination enzyme ataxin-3 has been demonstrated *in vivo*, and which promotes the 3′-phosphatase activity of PNKP *in vitro* [46]. However whether this enzyme contributes to regulating PNKP protein levels, particularly in opposition to Cul4A-DDB1-STRAP-mediated ubiquitination, has yet to be investigated.

2.2.4. Flap endonuclease-1 (FEN-1)

FEN-1 acts to remove the flap structures created by Pol δ/ε during long-patch BER. Ubiquitinated FEN-1 has been observed at the end of a sequence of PTMs initiated in late S phase of the cell cycle [47]. It was observed in HeLa cells that phosphorylation of FEN-1 at serine 187, promotes SUMOylation at lysine 168 with SUMO-3, which in turn stimulates polyubiquitination at lysine 354 by the E3 ligase pre-mRNA processing factor 19 (PRP19) to stimulate proteasomal degradation. This was largely discovered through overexpression of individual components within the pathway in HeLa cells, rather than examining endogenous proteins. Furthermore, PRP19 was only characterised in ubiquitinating FEN-1 *in vitro* using partially purified HeLa cell extracts and a complete suppression of ubiquitination was not observed following immunodepletion of PRP19, suggesting the existence of alternative E3 ubiquitin ligases for FEN-1. Nevertheless, this sequence of events beginning in late S phase is thought to regulate FEN-1 protein levels at the end of DNA replication, rather than being required for long-patch BER. Therefore further work is required to determine whether this, or an alternate mechanism for FEN-1 ubiquitination, plays a role in the regulation of this protein during BER.

2.3. Ubiquitination of DNA polymerases

2.3.1. DNA polymerase β (Pol β)

Pol β is the principal polymerase employed within the BER pathway, and primarily acts to insert the correct nucleotide into the repair gap, but also acts as a dRP lyase activity. The stability of Pol β was found to be significantly reduced in XRCC1 deficient EM-C11 cells and in HeLa cells following XRCC1 siRNA treatment, suggesting that Pol β and XRCC1-Lig IIIα form a stable complex that protects Pol β from degradation [48]. The major E3 ubiquitin ligase for Pol β was then revealed through the utilisation of *in vitro* ubiquitination assays in combination with fractionated HeLa cell extracts to be CHIP. Ubiquitination was localised to the 8 kDa N-terminal domain, which contains the dRP lyase activity, and CHIP depletion by siRNA in HeLa cells led to increased protein levels of Pol β whereas overexpression of CHIP reduced cellular Pol β. Interestingly, this investigation highlighted that CHIP also appeared to be involved in the ubiquitination-dependent degradation of XRCC1 and Lig IIIα, in addition to Pol β. This study was followed by the identification of a second E3 ubiquitin ligase that specifically catalysed monoubiquitination of Pol β [49]. Monoubiquitination was shown to occur in the same 8 kDa N-terminal region as that targeted by CHIP, but was catalysed by Mule. The precise ubiquitinated residues were identified as lysine 41, 61 and 81, and a lysine to arginine mutant Pol β protein was more stable than the wild type protein following expression in HeLa cells. Monoubiquitinated Pol β was observed exclusively within the cytoplasm in HeLa cells and was therefore deemed a specific target for ubiquitination-dependent degradation mediated by CHIP. Indeed, an siRNA knockdown of Mule decreased the levels of monoubiquitinated Pol β, increased total cellular Pol β levels and caused an increase in the rate of repair of SSBs and alkali-labile sites induced by hydrogen peroxide. Conversely a knockdown of ARF, which inhibits the activity of Mule, caused an increase in monoubiquitinated Pol β and a decrease in the rate of repair of hydrogen peroxide-induced SSBs and alkali-labile sites. A later study agreed that Pol β stability was dependent on XRCC1 and was regulated by ubiquitination-dependent degradation, but reported that this ubiquitination occurred on lysines 206 and 244 and was not reliant on Mule or CHIP [50]. However, the experimental system employed was very artificial, utilising an unusual cell line containing deletions of, amongst others, the ARF protein and modified to stably overexpress Pol β rather than examining endogenous protein levels. The identification of the deubiquitination enzyme that is able to reverse Mule- and CHIP-dependent ubiquitination of Pol β, and in fact the only such enzyme characterised in regulating BER, has been identified as ubiquitin specific protease 47 (USP47). USP47 was purified and identified from fractionated HeLa cell extracts in combination with an *in vitro* deubiquitination assay using ubiquitinated Pol β as a substrate, and was demonstrated to be capable of removing both CHIP-dependent polyubiquitin chains and Mule-dependent monoubiquitin moieties from Pol β [51]. An siRNA knockdown of USP47 in HeLa cells resulted in an increase in Mule-dependent monoubiquitinated Pol β, a reduction in cytoplasmic and therefore overall Pol β protein levels, and ultimately led to reduced efficiency of repair of SSBs and alkali-labile sites created through oxidative and alkylated DNA base damage. This study led to a complete picture of how Pol β protein levels are regulated in the cellular response to DNA damage, which involves an interplay between Mule, CHIP, ARF and USP47 activities that control a specific cytoplasmic pool of Pol β that acts as a source of protein that can be utilised in the nucleus in the event of any increase in DNA damage. The above studies together establish that Pol β protein levels are tightly regulated by the promotion or reversal of ubiquitination-dependent proteasomal degradation.

2.3.2. DNA polymerase λ (Pol λ)

Although Pol β is the chief polymerase in the BER pathway, Pol λ is thought to have a back-up role, specifically in the bypass of 8-oxoguanine lesions and thus avoiding the tendency for the misincorporation of the wrong adenine base opposite the lesion. Initial evidence that Pol λ is regulated by ubiquitination was demonstrated by the observation that a Cdk2/cyclin

A phosphorylation defective mutant of Pol λ at threonine 553, was less stable than the wild type protein following expression in either 293T or U2OS cells, and that this was mediated via increased ubiquitination [52]. Phosphorylation of Pol λ was observed most notably in late S and G2 phases of the cell cycle and was thought to stabilise the protein via inhibition of ubiquitination and to allow Pol λ to repair any DNA damage incurred at this stage. The major E3 ubiquitin ligase responsible for Pol λ ubiquitination was subsequently identified using the protein as a substrate in *in vitro* ubiquitination assays containing fractionated HeLa whole cell lysates and shown to be Mule [53]. Mule was found to ubiquitinate Pol λ on lysines 27 and 273 *in vitro*, and an siRNA-mediated depletion of Mule in HEK293T cells significantly increased Pol λ protein levels. Cdk2/cyclin A-dependent phosphorylation of Pol λ was found to inhibit Mule-dependent ubiquitination, and promote binding of the protein to chromatin via interaction with MutYH. The E3 ubiquitin ligase CHIP has also been shown to ubiquitinate Pol λ *in vitro*, although there is no evidence that CHIP plays a role in the regulation of cellular Pol λ *in vivo* [54]. Since Pol λ and Pol β are both regulated by Mule, this suggests that Mule plays a vital role in controlling the polymerase stage of BER, in addition to the base excision stage described above, and thus is a central player in coordinating the cellular DNA damage response.

2.3.3. DNA polymerase δ/ε (Pol δ/ε)

Pol δ and ε participate in long-patch BER by adding multiple complimentary nucleotides into the repair gap vacated by Pol β, thus creating a 5′-flap structure which is a substrate for FEN-1. Pol δ is synthesised in human cells as a heterotetramer of subunits p125, p68, p50 and p12. Using an *in vitro* ubiquitination assay with fractionated HeLa cell lysates, the p12 subunit has been shown to be a target for ubiquitination by the E3 ubiquitin ligase RNF8 [55]. An siRNA knockdown of RNF8 in A549 cells led to an increased stability of p12, particularly following UVC irradiation but also following MNNG treatment. However the precise contribution of this mechanism to BER efficiency is currently unknown. Additionally there is no evidence suggesting that Pol ε is regulated by ubiquitination.

2.4. Ubiquitination of DNA ligases

2.4.1. X-ray cross-complementing protein 1 and DNA ligase IIIα (XRCC1-Lig IIIα)

Lig IIIα functions in a stable complex with the scaffold protein XRCC1 to seal the nick in the DNA backbone to complete the BER process. Lig IIIα itself been shown to undergo ubiquitination in two separate reports. In the first, CHIP was demonstrated as an E3 ubiquitin ligase for Lig IIIα *in vitro,* but also an siRNA knockdown of CHIP in HeLa cells caused an accumulation of Lig IIIα protein *in vivo* as a consequence of a lack of ubiquitination-dependent degradation [48]. A second E3 ubiquitin ligase for Lig IIIα *in vitro* has been identified as Iduna/RNF146, which ubiquitinates the protein but only when modified by poly(ADP-ribosyl)ation [38]. However in this study, Iduna was shown to interact with and ubiquitinate a number of DNA repair proteins, including both Lig IIIα and XRCC1 but also PARP1 (as discussed previously) which was dependent on protein poly(ADP-ribosyl)ation. Therefore the particular significance of Iduna-mediated ubiquitination of Lig IIIα and XRCC1 specifically on BER regulation remains unclear. XRCC1 has been shown to be phosphorylated *in vitro* and *in vivo* by casein kinase 2 (CK2) and this prevents the ubiquitination-dependent degradation of the protein. This was demonstrated by reduced stability of XRCC1 following CK2 siRNA in HeLa cells and that a CK2 phosphorylation deficient mutant of XRCC1 expressed in EM9 cells was less stable than the wild type protein as a direct consequence of increased ubiquitination [56]. A separate study also demonstrated ubiquitination of XRCC1, although conversely a phosphorylation deficient mutant of XRCC1 expressed in U2OS cells appeared not to be stabilised following proteasomal inhibition [57]. Similar to Lig IIIα, XRCC1 has been found to be ubiquitinated *in vitro* by the E3 ubiquitin ligase CHIP, and an siRNA-mediated depletion of CHIP in HeLa cells resulted in an increase in XRCC1 protein levels owing to a reduction in ubiquitin-dependent protein degradation [48]. Overexpression of CHIP in HeLa cells was also found to cause a reduced stability of XRCC1 protein. These findings were supported by a separate study that demonstrated that CHIP-dependent ubiquitination of XRCC1 occurs, but only when the protein is not bound to Pol β or heat shock protein 90 (HSP90) [50]. Regarding the site of ubiquitination within XRCC1, this has been identified within the BRCA1 C-terminus (BRCT II) motif after it was demonstrated that truncated XRCC1 lacking this domain was considerably more stable than the full length protein when expressed in either HeLa or EM-C11 cells [48]. This ubiquitination site within the BRCT II motif of XRCC1 was also verified in an independent study [57].

2.4.2. DNA ligase I (Lig I)

Lig I is employed during long-patch BER, but is also involved in DNA replication. The only reported evidence of Lig I ubiquitination is through the Cul4A-DDB1 E3 ubiquitin ligase complex [58]. Overexpression and immunoprecipitation of Lig I from 293T cells revealed that lysine 376, and possibly lysine 79 and 192, were potential ubiquitination sites and that a lysine to arginine mutant of Lig I at four sites (79, 192, 226 and 376) was more stable than the wild type protein to degradation through serum starvation. Lig I was then demonstrated to interact with and to be ubiquitinated *in vitro* by a Cul4A-DDB1 complex with the associated factor DCAF7. An siRNA knockdown of DCAF7 in GM00847 cells was shown to supress the degradation of Lig I following serum starvation. This study would suggest that Lig I protein levels are controlled during DNA replication, however the impact of this mechanism for the efficiency of long-patch BER, is still unknown.

2.5. Summary and future outlook

An increasing amount of evidence is strengthening the fact that BER is carefully regulated and controlled by ubiquitination. This largely appears to be a mechanism for controlling cellular BER protein levels via the 26S proteasome and therefore plays an important role in supressing DNA damage accumulation and coordinating an efficient cellular DNA damage response. In this Chapter we have presented evidence that the majority of BER proteins have been shown *in vitro* and *in vivo* to be targeted for ubiquitination by specific E3 ubiquitin ligases. However there are other proteins (e.g. the DNA glycosylases MBD4, MTH1, NEIL2 and NEIL3) which have not yet been demonstrated to be ubiquitinated directly (**Table 1**). It is interesting to note

E3 ubiquitin ligase	BER protein	Reference
CHIP	Lig III	[48]
	OGG1	[24]
	Pol β	[48]
	Pol λ	[54]
	XRCC1	[48]
Cul1	SMUG1	[16]
	UNG	[16]
Cul4	Lig I	[58]
	PNKP	[45]
	SMUG1	[16]
	TDG	[21, 22]
	UNG	[16]
Iduna/RNF146	Lig III	[38]
	PARP-1	[38]
	XRCC1	[38]
MDM2	APE1	[41]
Mule	MUTYH	[26]
	NEIL1	[34]
	Pol β	[49]
	Pol λ	[53]
RNF8	Pol δ	[55]
TRIM26	NEIL1	[34]
UBR3	APE1	[43]
Unknown	MBD4	
	NEIL2	
	NEIL3	
	NTH1	
	Pol ε	

Table 1. Summary of the known E3 ubiquitin ligases targeting BER proteins for ubiquitination.

that some of the identified E3 ubiquitin ligases appear to target more than one BER protein for ubiquitination. For example, Mule can ubiquitinate both the DNA glycosylases NEIL1 and MUYTH, and the DNA polymerases Pol β and Pol λ for ubiquitination-dependent degradation which would suggest that this E3 ubiquitin ligase, and others targeting multiple BER proteins, can control BER at several different points in the repair pathway. The Cullins, Cul1 and

Cul4, also appear to regulate several BER members although this is unsurprising given that they represent the largest family of E3 ubiquitin ligases with several hundred members. In fact the most important element of these complexes is the adaptor proteins that provide specificity of ubiquitination to their target protein. Indeed Cdt2, DCAF7 and STRAP have already been identified as adaptors of the Cul4A-DDB1 complexes that target TDG, Lig I and PNKP, respectively for ubiquitination. Nevertheless, we currently do not have a clear understanding of how these ubiquitination events are controlled and co-ordinated to ensure an efficient BER response to DNA damage. In particular there is insufficient knowledge on how the identified E3 ubiquitin ligase enzymes are activated and directed to their specific enzyme targets. This could be achieved by either compartmentalisation of the enzymes or targets within the cell, or by activation of ubiquitination enzymatic activity by PTMs. Secondly, with ubiquitination being a reversible process, the identities of deubiquitination enzymes that work in concert with E3 ubiquitin ligases in the regulated control of BER proteins have not yet been fully elucidated. In fact the only evidence for this is by USP47, which has been demonstrated to be actively involved in the deubiquitination of Pol β. Thirdly, in addition to ubiquitination, it is clear that BER proteins are also regulated by other PTMs, including acetylation, methylation, phosphorylation and SUMOylation. There is some evidence of crosstalk between these modifications and ubiquitination in regulating BER protein levels and activities, particularly for PARP-1, FEN-1 and PNKP, although this is not yet fully understood. However these regulatory "switches" are undoubtedly an efficient way of controlling the cellular BER response to DNA damage. Ultimately further research is necessary in order to fully identify and understand the specific E3 ubiquitin ligase and deubiquitination enzymes for individual BER proteins, and the precise mechanisms that are co-ordinated in association with other PTMs, which act to ensure an efficient repair process.

In addition to discovering the molecular details for regulating cellular BER, research into the associations of these and the development of human diseases, including premature ageing, neurodegenerative diseases and cancer is essential. It is understood that BER protein levels are frequently misregulated in these diseases although whether defective ubiquitination is contributory to this effect is largely unknown and understudied. This information may also uncover novel therapeutic strategies for the treatment of specific diseases. Indeed the BER pathway is known to be an attractive therapeutic target, which is exemplified by the success of PARP inhibitors in the treatment of BRCA-deficient breast cancers which block BER and cause synthetic lethality due to the inability of these cells to process DNA double strand breaks. It is therefore entirely possible that the discovery of E3 ubiquitin ligases or deubiquitination enzymes targeting BER proteins may provide novel cellular targets for drugs or small molecule inhibitors which can be used in combination with radiotherapy and/or chemotherapy for treatment of diseases, such as cancer.

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References

[1] Lindahl T. Instability and decay of the primary structure of DNA. Nature. 1993;**362**:709-715

[2] Lindahl T. An N-glycosidase from *Escherichia coli* that releases free uracil from DNA containing deaminated cytosine residues. Proceedings of the National Academy of Sciences of the United States of America. 1974;**71**(9):3649-3653

[3] Jacobs AL, Schar P. DNA glycosylases: In DNA repair and beyond. Chromosoma. 2012; **121**(1):1-20. DOI: 10.1007/s00412-011-0347-4

[4] Wallace SS. DNA glycosylases search for and remove oxidized DNA bases. Environmental and Molecular Mutagenesis. 2013;**54**(9):691-704. DOI: 10.1002/em.21820

[5] Demple B, Herman T, Chen DS. Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: Definition of a family of DNA repair enzymes. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**(24):11450-11454

[6] Robson CN, Hickson ID. Isolation of cDNA clones encoding a human apurinic/apyrimidinic endonuclease that corrects DNA repair and mutagenesis defects in *E. coli xth* (exonuclease III) mutants. Nucleic Acids Research. 1991;**19**:5519-5523

[7] Matsumoto Y, Kim K. Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. Science. 1995;**269**(5224):699-702

[8] Sobol RW et al. Requirement of mammalian DNA polymerase-beta in base-excision repair. Nature. 1996;**379**(6561):183-186

[9] Cappelli E et al. Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. The Journal of Biological Chemistry. 1997;**272**(38):23970-23975

[10] Nash RA et al. XRCC1 protein interacts with one of two distinct forms of DNA ligase III. Biochemistry. 1997;**36**(17):5207-5211

[11] Vidal AE et al. Mechanism of stimulation of the DNA glycosylase activity of hOGG1 by the major human AP endonuclease: Bypass of the AP lyase activity step. Nucleic Acids Research. 2001;**29**:1285-1292

[12] Wiederhold L et al. AP endonuclease-independent DNA base excision repair in human cells. Molecular Cell. 2004;**15**(2):209-220

[13] Dianov G, Price A, Lindahl T. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. Molecular and Cellular Biology. 1992;**12**:1605-1612

[14] Podlutsky AJ et al. Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. The EMBO Journal. 2001;**20**(6):1477-1482

[15] Carter RJ, Parsons JL. Base excision repair, a pathway regulated by posttranslational modifications. Molecular and Cellular Biology. 2016;**36**(10):1426-1437. DOI: 10.1128/MCB.00030-16

[16] Schrofelbauer B et al. Human immunodeficiency virus type 1 Vpr induces the degradation of the UNG and SMUG uracil-DNA glycosylases. Journal of Virology. 2005;**79**(17):10978-10987. DOI: 10.1128/jvi.79.17.10978-10987.2005

[17] Schrofelbauer B, Hakata Y, Landau NR. HIV-1 Vpr function is mediated by interaction with the damage-specific DNA-binding protein DDB1. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(10):4130-4135. DOI: 10.1073/pnas.0610167104

[18] Hardeland U et al. Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. The EMBO Journal. 2002;**21**(6):1456-1464

[19] Steinacher R, Schar P. Functionality of human thymine DNA glycosylase requires SUMO-regulated changes in protein conformation. Current Biology. 2005;**15**(7):616-623. DOI: 10.1016/j.cub.2005.02.054

[20] Smet-Nocca C et al. SUMO-1 regulates the conformational dynamics of thymine-DNA Glycosylase regulatory domain and competes with its DNA binding activity. BMC Biochemistry. 2011;**12**:4. DOI: 10.1186/1471-2091-12-4

[21] Slenn TJ et al. Thymine DNA glycosylase is a CRL4Cdt2 substrate. The Journal of Biological Chemistry. 2014;**289**(33):23043-23055. DOI: 10.1074/jbc.M114.574194

[22] Shibata E, Dar A, Dutta A. CRL4Cdt2 E3 ubiquitin ligase and proliferating cell nuclear antigen (PCNA) cooperate to degrade thymine DNA glycosylase in S phase. The Journal of Biological Chemistry. 2014;**289**(33):23056-23064. DOI: 10.1074/jbc.M114.574210

[23] Meng H, Harrison DJ, Meehan RR. MBD4 interacts with and recruits USP7 to heterochromatic foci. Journal of Cellular Biochemistry. 2015;**116**(3):476-485. DOI: 10.1002/jcb.25001

[24] Fantini D et al. Rapid inactivation and proteasome-mediated degradation of OGG1 contribute to the synergistic effect of hyperthermia on genotoxic treatments. DNA Repair (Amsterdam). 2013;**12**(3):227-237. DOI: 10.1016/j.dnarep.2012.12.006

[25] McDonough H, Patterson C. CHIP: A link between the chaperone and proteasome systems. Cell Stress & Chaperones. 2003;**8**(4):303-308

[26] Dorn J et al. Regulation of human MutYH DNA glycosylase by the E3 ubiquitin ligase mule. The Journal of Biological Chemistry. 2014;**289**(10):7049-7058. DOI: 10.1074/jbc.M113.536094

[27] Dou H, Mitra S, Hazra TK. Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. The Journal of Biological Chemistry. 2003;**278**(50):49679-49684

[28] Dou H et al. Interaction of the human DNA glycosylase NEIL1 with proliferating cell nuclear antigen. The potential for replication-associated repair of oxidized bases in mammalian genomes. The Journal of Biological Chemistry. 2008;**283**(6):3130-3140. DOI: 10.1074/jbc.M709186200

[29] Parsons JL et al. NEIL1 is the major DNA glycosylase that processes 5-hydroxyuracil in the proximity of a DNA single-strand break. Biochemistry. 2007;**46**(13):4158-4163

[30] Parsons JL, Zharkov DO, Dianov GL. NEIL1 excises 3′ end proximal oxidative DNA lesions resistant to cleavage by NTH1 and OGG1. Nucleic Acids Research. 2005;**33**(15): 4849-4856

[31] Zhou J et al. Neil3 and NEIL1 DNA glycosylases remove oxidative damages from quadruplex DNA and exhibit preferences for lesions in the telomeric sequence context. The Journal of Biological Chemistry. 2013;**288**(38):27263-27272. DOI: 10.1074/jbc.M113.479055

[32] Zhou J et al. The NEIL glycosylases remove oxidized guanine lesions from telomeric and promoter quadruplex DNA structures. Nucleic Acids Research. 2015;**43**(8):4039-4054. DOI: 10.1093/nar/gkv252

[33] Semlow DR et al. Replication-dependent unhooking of DNA interstrand cross-links by the NEIL3 glycosylase. Cell. 2016;**167**(2):498-511 e14. DOI: 10.1016/j.cell.2016.09.008

[34] Edmonds MJ et al. Ubiquitylation-dependent regulation of NEIL1 by Mule and TRIM26 is required for the cellular DNA damage response. Nucleic Acids Research. 2017;**45**(2):726-738. DOI: 10.1093/nar/gkw959

[35] Liang C et al. Identification of UHRF1/2 as new N-methylpurine DNA glycosylase-interacting proteins. Biochemical and Biophysical Research Communications. 2013;**433** (4):415-419. DOI: 10.1016/j.bbrc.2013.02.126

[36] Wang T et al. Polyubiquitylation of PARP-1 through ubiquitin K48 is modulated by activated DNA, NAD$^+$, and dipeptides. Journal of Cellular Biochemistry. 2008;**104**(1):318-328. DOI: 10.1002/jcb.21624

[37] Martin N et al. PARP-1 transcriptional activity is regulated by sumoylation upon heat shock. The EMBO Journal. 2009;**28**(22):3534-3548. DOI: 10.1038/emboj.2009.279

[38] Kang HC et al. Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(34):14103-14108. DOI: 10.1073/pnas.1108799108

[39] Kashima L et al. CHFR protein regulates mitotic checkpoint by targeting PARP-1 protein for ubiquitination and degradation. The Journal of Biological Chemistry. 2012;**287**(16): 12975-12984. DOI: 10.1074/jbc.M111.321828

[40] Liu C et al. CHFR is important for the first wave of ubiquitination at DNA damage sites. Nucleic Acids Research. 2013;**41**(3):1698-1710. DOI: 10.1093/nar/gks1278

[41] Busso CS, Iwakuma T, Izumi T. Ubiquitination of mammalian AP endonuclease (APE1) regulated by the p53-MDM2 signaling pathway. Oncogene. 2009;**28**(13):1616-1625. DOI: 10.1038/onc.2009.5

[42] Busso CS, Wedgeworth CM, Izumi T. Ubiquitination of human AP-endonuclease 1 (APE1) enhanced by T233E substitution and by CDK5. Nucleic Acids Research. 2011;**39**(18):8017-8028. DOI: 10.1093/nar/gkr401

[43] Meisenberg C et al. Ubiquitin ligase UBR3 regulates cellular levels of the essential DNA repair protein APE1 and is required for genome stability. Nucleic Acids Research. 2012;**40**(2):701-711. DOI: 10.1093/nar/gkr744

[44] Scott TL et al. Polyubiquitination of apurinic/apyrimidinic endonuclease 1 by Parkin. Molecular Carcinogenesis. 2017;**56**(2):325-336. DOI: 10.1002/mc.22495

[45] Parsons JL et al. Phosphorylation of PNKP by ATM prevents its proteasomal degradation and enhances resistance to oxidative stress. Nucleic Acids Research. 2012;**40**(22):11404-11415. DOI: 10.1093/nar/gks909

[46] Chatterjee A et al. The role of the mammalian DNA end-processing enzyme polynucleotide kinase 3′-phosphatase in spinocerebellar ataxia type 3 pathogenesis. PLoS Genetics. 2015;**11**(1):e1004749. DOI: 10.1371/journal.pgen.1004749

[47] Guo Z et al. Sequential posttranslational modifications program FEN1 degradation during cell-cycle progression. Molecular Cell. 2012;**47**(3):444-456. DOI: 10.1016/j.molcel.2012.05.042

[48] Parsons JL et al. CHIP-mediated degradation and DNA damage-dependent stabilization regulate base excision repair proteins. Molecular Cell. 2008;**29**(4):477-487. DOI: 10.1016/j.molcel.2007.12.027

[49] Parsons JL et al. Ubiquitin ligase ARF-BP1/Mule modulates base excision repair. The EMBO Journal. 2009;**28**(20):3207-3215. DOI: 10.1038/emboj.2009.243

[50] Fang Q et al. HSP90 regulates DNA repair via the interaction between XRCC1 and DNA polymerase beta. Nature Communications. 2014;**5**:5513. DOI: 10.1038/ncomms6513

[51] Parsons JL et al. USP47 is a deubiquitylating enzyme that regulates base excision repair by controlling steady-state levels of DNA polymerase beta. Molecular Cell. 2011;**41**(5):609-615. DOI: 10.1016/j.molcel.2011.02.016

[52] Wimmer U et al. Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. EMBO Reports. 2008;**9**(10):1027-1033. DOI: 10.1038/embor.2008.148

[53] Markkanen E et al. Regulation of oxidative DNA damage repair by DNA polymerase lambda and MutYH by cross-talk of phosphorylation and ubiquitination. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(2):437-442. DOI: 10.1073/pnas.1110449109

[54] Markkanen E et al. Ubiquitylation of DNA polymerase lambda. FEBS Letters. 2011; **585**(18):2826-2830. DOI: 10.1016/j.febslet.2011.03.069

[55] Zhang S et al. Identification of RNF8 as a ubiquitin ligase involved in targeting the p12 subunit of DNA polymerase delta for degradation in response to DNA damage. The Journal of Biological Chemistry. 2013;**288**(5):2941-2950. DOI: 10.1074/jbc.M112.423392

[56] Parsons JL et al. XRCC1 phosphorylation by CK2 is required for its stability and efficient DNA repair. DNA Repair (Amsterdam). 2010;**9**(7):835-841. DOI: 10.1016/j.dnarep.2010.04.008

[57] Wei L et al. Damage response of XRCC1 at sites of DNA single strand breaks is regulated by phosphorylation and ubiquitylation after degradation of poly(ADP-ribose). Journal of Cell Science. 2013;**126**(Pt 19):4414-4423. DOI: 10.1242/jcs.128272

[58] Peng Z et al. Human DNA ligase I interacts with and is targeted for degradation by the DCAF7 specificity factor of the Cul4-DDB1 ubiquitin ligase complex. The Journal of Biological Chemistry. 2016;**291**(42):21893-21902. DOI: 10.1074/jbc.M116.746198

Chapter 4

The Role of Deubiquitinases in DNA Double-Strand Break Repair

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Additional information is available at the end of the chapter

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Abstract

DNA double-strand break (DSB) is a type of the most critical DNA lesions, and if not repaired promptly, it can result in cell death or a wide variety of genetic alterations including genome instability, large- or small-scale deletions, chromosome loss, loss of heterozygosity, and translocations. DSBs are repaired by double-strand break repair (DSBR), including nonhomologous end-joining (NHEJ) and homologous recombination (HR) pathway, and defects in these pathways cause genome instability and promote tumorigenesis. Accumulating evidence has demonstrated that the superfamily of deubiquitinases (DUBs) can regulate the action and stability of DNA repair enzymes involving in DSBR via modifying ubiquitination levels, a reversible posttranslational modification pathway. In this review, we will discuss ubiquitination/deubiquitination modification involving in DSBR genes, the role of DUBs in DSBR and corresponding mechanisms, and the potential effects of this modification on human diseases.

Keywords: double-strand break repair, deubiquitinase, ubiquitination, deubiquitination, double-strand break

1. Introduction

DNA double-strand break (DSB) is a fatal alteration in the chemical structure of DNA; if it has not been repaired in time, it may destroy the stability of genome and lead to a series of human diseases. Usually, they result from a variety of causes including abnormal metabolic process, ionizing radiation, ultraviolet radiation, and active oxygen damage factors [1, 2]. In organism, DNA double-strand break repair (DSBR), a complex reaction system consisting of nonhomologous end-joining (NHEJ) and homologous recombination (HR) pathway, can repair DSBs [3, 4]. Accumulating evidence has demonstrated that ubiquitination and deubiquitination modification play a vital role in controlling the capacity of DSBR via regulating the action and stability of DNA repair enzymes involving in DSBR pathway. In the past decades, there has been great advance in the role of deubiquitinases (DUBs) in DNA damage repair. Here, we reviewed ubiquitination/deubiquitination modification of DSBR genes, the role of DUBs in DSBR and corresponding mechanisms, and the potential effects of this modification on human diseases.

2. Ubiquitination and deubiquitination

Ubiquitin is an important single-chain polypeptide consisting of 76 amino acid residues and ubiquitously exists in almost all eukaryotic cells and tissues [5, 6]. This polypeptide is characterized by highly conserved protein from yeast to human [6] and is invariant in higher plants and differs by only three residues from animals [7]. Structurally, ubiquitin polypeptide chain appears to be a highly compact β-grasp fold with an α-helix in the cavity formed by a five-strand mixed β-sheet and a marked hydrophobic core formed between the β-sheet and the α-helix [8]. A flexible six-residue tail in the C-terminal of ubiquitin protrudes from β-grasp fold and is requested for forming the bond between ubiquitin and its substrate [9].

Ubiquitination is defined as the process that ubiquitin attaches to its target proteins via catalysis of enzymes. This process is a reversible posttranslational modification that can regulate various processes including cell proliferation, apoptosis, transcription, protein stability and translocation, and DNA damage repair [10–12]. Ubiquitination process is an ATP-dependent enzymatic cascade reaction [13, 14]. During cascades reaction, C-terminal of ubiquitin is first adenylated by ubiquitin-activating enzyme (E1) via forming a bond between the adenosine monophosphate (AMP) and the C-terminal glycine carboxyl group of ubiquitin, and subsequently, the E1 cysteine side chain directly binds to C-terminal and results in the formation of a thiol-ester linkage. Then, the activated ubiquitin is presented to the active cysteine in a ubiquitin-conjugating enzyme (E2). The E2 delivers the ubiquitin to its substrate cooperating with ubiquitin ligases (E3), which plays a role in substrate recognition. Finally, the C-terminal glycine of the ubiquitin binds to a lysine residue of the substrate with an isopeptide bond. After multiple cycles of cascade reaction, substrate will bind one or more polyubiquitin chains that are formed between the lysine side of one ubiquitin and the C-terminal carboxyl group of another ubiquitin [13, 14]. The 26S proteasome can specifically recognize these target proteins with ubiquitination modification and lead them into ubiquitin-proteasome pathway (UPP) for inducting protein degradation, a key role of ubiquitination. However, UPP is not the only role of ubiquitination. Ubiquitination can also regulate protein activity and the interaction among proteins [13, 14].

Deubiquitination is the reverse process of ubiquitination and regulated by deubiquitinases (DUBs). DUBs, also known as deubiquitinating enzymes, can cleave the bonds between substrate and polyubiquitin chains and improve the stability of substrate. They can also remove single ubiquitin molecule from polyubiquitin chains. Until now, approximately 561 DUBs have been identified in the human genome [15], and most of them are cysteine proteases. According to the difference in their structure and function, DUBs are divided into six classes: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease protein domain proteases (MJDs), JAMM/MPN domain-associated metallopeptidases (JAMMs), and the monocyte chemotactic protein-induced protein (MCPIP) family. These enzymes can stabilize protein and play a crucial role in the life process [13, 14].

3. DSBs and DSBR pathways

3.1. DSBs and DSB response

DSBs are vital DNA damages caused by a variety of physiological or pathological factors. V(D)J recombination has been identified as the only physiological reason inducing DSBs that result from the recombination of variable (V), diversity (D), and joining (J) gene segments. It often appears in the early development process of the vertebrate immune system. Evidence has shown that diverse immunoglobulins and T-cell receptors are generated due to this special recombination pathway. During V(D)J recombination, DNA strands are cut by RAG-1 and RAG-2 protein between the recombination signal sequences (RSS) heptamer and the flanking sequence and result in the formation of DSBs [16, 17], whereas the ends of the broken strands are subsequently processed and connected through NHEJ pathway [18].

For pathological factors, reactive oxygen species (ROSs) resulting from cellular oxidation are one main source of pathological DSBs. Studies have shown that about one percent of the oxygen that we breathe is converted into oxidative free radicals and ultimately can cause DSBs in different degrees [19]. Pathological DSBs can also arise from DNA replication across a nick that is caused by exogenous or endogenous sources. Such ionizing radiation as X-rays and gamma rays may produce free radicals and induce the formation of DSBs [20]. This type of DSBs only occurs in the S phase and is repaired through HR pathway. Additionally, one unusual cause producing DSBs is the topoisomerase II poisons that can lead to DSBs formation, apoptotic cell death, and genomic instability via stabilizing the DNA topoisomerase II cleavable complexes [21]. Another unusual cause is physical stress on the DNA duplex, which may be from the mitotic spindle on chromosomal fusions or telomere failures [22].

Studies have shown that DSBs can induce DNA damage response, and such E3 ligases as ring finger protein (RNF8) subsequently accumulate around the lesions. After that, RNF8-recruiting RNF168 promotes histone H2A Lys13,15 mono-ubiquitination (H2AK13, 15ub). Therefore, the accumulation of DNA-repair regular factors, such as receptor-association protein (RAP80) and TP53 binding protein (53BP1), is allowed [23–26]. Finally, the ataxia telangiectasia mutated (ATM) and ATM/rad3-related (ATR) kinases, a central regulator of DSB response, are activated and induce the activation of Chk1 and Chk2 kinases and TP53 protein. The activated Chk1 and Chk2 kinases arrest cell cycle to obtain sufficient time for DNA repair, while activate TP53 induces cell death [27, 28].

3.2. DSBR pathways

Merely one DSB that triggers apoptosis or destroys a critical gene is enough to lead a cell to death [29], whereas losing ability to repair DSBs can also lead to genome rearrangement and cellular transformation [30]. In organism, the two primary pathways to correct DSBs are known as HR pathway and NHEJ pathway. For NHEJ pathway, it can repair DSBs with nonhomological damaged ends and is the primary DSBR pathway in mammalian cells. This pathway consists of classical-NHEJ (C-NHEJ) and alternative-NHEJ (A-NHEJ). In C-NHEJ, Ku heterodimer (Ku70 and Ku80 subunits) recognizes and binds to the ends of a DSB to prevent the free ends from degradation. Subsequently, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited and then binds to Ku heterodimer to recruit XRCC4 and DNA ligase 4 (LIG4). XRCC4 and LIG4 form a complex with XLF to ligate the broken ends [31, 32]. Until now, although the detailed mechanism of NHEJ is poorly understood, a recent study has partly revealed the mechanism about how the complex of XRCC4, LIG4, and XLF connects the fragments of broken DNA [33]. It has shown that XRCC4-XLF complex first bridges the two DNA molecules generated by DSBs, and the bridge can slide along the DNA. Then, the ends of broken DNA are rapidly reconnected. Evidence from molecular epidemiological and genetical studies displays that low or losing capacity of NEEJ pathway is positively associated with the deficiency of immune reaction [34, 35]. For example, about 15% of human severe combined immune deficiency (SCID) has been observed to feature low NHEJ capacity caused by null mutations of Artemis gene [34, 35]. Patients carrying the mutations in the DNA ligase IV gene that is crucial in NHEJ pathway presented some NBS-like features; however, cancers were not observed on these patients [36].

For HR pathway, it was first illuminated in *Escherichia coli* and *Saccharomyces cerevisiae* [37], and the similar mechanisms of the key reaction in HR pathway are observed in bacteria, yeast and human cells. An intact double-strand DNA that has highly homologous sequence of the damaged molecule is needed to act as the template to direct repair [38]. HR pathway includes three main steps: termini procession, strand invasion and branch migration, and Holliday junction formation. The ends of DSB are first processed by a nuclease, such as Mre11-Rad50-NBS1 (MRN) complex, and produce a single-stranded region with a 3′ overhang. Replication protein A (RPA) subsequently binds to the single-strand region for stabilizing and protecting this single-strand status [39, 40]. The core procedure of HR pathway is RAD51-depended strand invasion and branch migration. RAD51 displaces the RPA from single-strand DNA to form a nucleoprotein filament and then directs the later to recognize homologous duplex DNA [41]. DNA strand exchange generates a Holliday junction between the homologous damaged and undamaged DNAs under the condition of cooperating RAD51 with RAD52, RAD54, and RAD55/57 protein. Finally, the MUS81/MMS4 can resolve Holliday junction to stop the process of HR pathway [3].

Except for above-mentioned directly regulated proteins, DSB response factors (including ATM/ATR and BRCA1/BRCA2) can indirectly regulate the capacity of HR pathway [42–46]. The defects of ATM may alter kinetics of radiation-induced RAD51 formation and the hallmark of RAD51 activation [42]. ATM/ATR can also mediate the phosphorylation of PALB2 to promote the formation of RAD51 nucleofilaments [43]. However, roles of ATM and ATR in

HR pathway are still poorly understood. BRCA1 is a protein with 1863 amine acids encoded by breast cancer susceptibility gene and can target DSB lesion through its N-terminal RING domain binding to BRCA1-associated RING domain 1 (BARD1) [44]. BRCA1 can also promote HR pathway via cooperating with RAD51 and forming the complex of BRCA1-PALB2-BRCA2-RAD51 (BRCC) [44]. Surprisingly, BRCA1 can also prevent HR pathway by its incorporating into the complex of BRCA1-Abraxas-RAP80-MERIT40 (BRCA1-A). This may be because BRCA1-A can limit DNA end-resection or sequester BRCA1 away from HR sites by binding to RNF8/RNF168-ubiquitylated chromatin [45, 46]. Studies have shown that low or lost capacity of HR pathway resulting from these causes may cause a series of cancer-prone diseases, including ataxia telangiectasia (AT), Nijmegen breakage syndrome (NBS), Bloom syndrome, Werner syndrome, and Fanconi anemia, reviewed by Thompson and Schild [47].

4. Deubiquitinases regulating DSBR

4.1. USPs

USPs, the largest subfamily of DUBs with approximately 100 members and the most divers structures, belong to cysteine protease family (clan CA, family C19) and were first identified in *Saccharomyces cerevisiae* [48]. The size of USPs is ranging between 330 and 3500 amino acids, with 800 and 1000 residues. These DUBs have three functional domains: a catalytic domain, a protein-protein interaction domain, and localization domain [48]. In the catalytic domain, USPs are marked with two short and well-conserved sequences, also called as the N-terminal Cys-box and the C-terminal His-box. These sequences are essential for catalytic activity of USPs [48, 49], while other domains provide the information of binding to their target protein. Interestingly, almost all the UBP deubiquitinases display a conserved three-domain architecture, comprising Fingers, Palm, and Thumb, and their C terminus are settled in the active site between the Palm and the Thumb, except for CYLD that has an obviously truncated Fingers subdomain [50, 51]. A later study has shown that the core catalytic domain of USPs contains six conserved boxes, and that boxes 1 and 2, boxes 3 and 4, and boxes 5 and 6 formed Thumb subdomain, Fingers subdomain, and Palm subdomain, respectively [52]. USPs have been found to involve in many diseases, such as cancer, inflammation and viral diseases [53]. At least 15 of USPs, including USP1, USP3, USP4, USP6, USP7, USP10, USP11, USP15, USP20, USP26, USP29, USP37, USP42, USP44, and USP51, can regulate DSBR.

USP1 contains 785 amino acids, and its catalytic domain is one of the largest among all USPs. Although two insertions between boxes 2 and 3 and between boxes 5 and 6 have been identified to locate away from the ubiquitin binding site of USP1, it is still not clear whether these insertions can reach the active site [52]. As USP1 has been reported to overexpress in osteosarcoma and non–small cell lung cancer, inhibitors of USP1 are supposed to have anticancer potential [54, 55]. Interestingly, USP1 can be stabilized by USP1-associated factor 1 (UAF1) that can increase the catalytic activity of USP1 [56]. This indicates that USP1 need to form a complex with UAF1 to carry out its functions. A recent study has further proved that three cell clones, USP1$^{-/-}$, UAF1$^{-/-/-}$, and USP1$^{-/-}$ UAF1$^{-/-/-}$ double-knockout cells, showed hypersensitivity to both camptothecin and poly (ADP-ribose) polymerase (PARP), suggesting that the USP1/UAF1 complex can promote HR capacity. Moreover, the USP1/UAF1 complex promoting HR capacity is at least in part associated with the suppression of NHEJ, although corresponding mechanisms still need to be further researched [57].

USP3 is a nuclear protein that presents in the chromatin fraction and is also a chromatin-associated DUB [58]. In 1999, Sloper-Mold *et al.* firstly identified and analyzed USP3 and found that a human USP3 gene probe detected two different mRNA transcripts that were expressed at low levels in all examined tissues [59]. USP3 is required for the deubiquitination of H2A and H2B to revert corresponding mono-ubiquitination. It has been displayed that USP3 can also regulate the cellular levels of ubiquitinated H2A and H2B (uH2A and uH2B), as H2A and H2B are the two major mono-ubiquitinated chromosomal protein [13, 58]. In addition, uH2A and uH2B have been revealed to associate with transcriptional regulation, where USP3 potentially plays a vital role [14]. Furthermore, the results from a study on mice with the deficiency of USP3 have shown that these mice can develop tumor spontaneously, and cells with the deficiency of USP3 fail to preserve chromosomal integrity [60]. For DSBR pathway, USP3 plays a key role in regulate DSB response. Transient USP3 silencing will cause spontaneous DNA damage, and DNA damage response will be enhanced at the same time [60]. The ubiquitination of histone H2A and γH2AX initiated by RNF168 and RNF8 in DSB response generates a cascade reaction and results in the accumulation of DSBR enzymes, whereas USP3 can oppose RNF168 and RNF8 via deubiquitination modification for the ubiquitinated H2A and γH2AX. Moreover, ectopic expression of USP3 can also block the accumulation of downstream repair enzymes such as BRCA1 and 53BP1 [61].

Except for USP3, several other USPs (including USP6, USP51, USP29, and USP44) can also deubiquitinate H2A [26, 62, 63]. Among these USPs, USP51 acts as a DUB for histone H2B mono-ubiquitination (H2Bub1), and the depletion of USP51 will suppress DSB reaction and tumor growth [64].

USP4, also named as ubiquitous nuclear protein (UNP), was initially found to promote carcinogenesis of lung and act as an oncogene [65, 66]. The following studies showed that USP4 is overexpressed in several types of human cancers such as hepatocellular carcinoma and plays a crucial role in the progression of tumorigenesis [67, 68]. Growing evidence has exhibited that USP4 affecting tumorigenesis may be correlated with abnormal DSBR capacity [67]. During DSBR pathway, USP4 may display its regulation functions on DSBR in several different processes, including DSB response and HR capacity. It has been identified to act as an important TP53 regulator that can decrease TP53 by deubiquitinating and stabilizing ARF-BP1, a ubiquitin ligase for p53 degradation [67]. During HR pathway, USP4 is required for CtIP recruitment to DNA damage site. It also regulates the resection of DNA DSBs via interacting with CtIP and the MRE11-RAD50-NBS1 (MRN) complex. The depletion of USP4 may abolish DNA end resection [69]. In addition, USP4 is ubiquitinated on multiple sites, and auto-deubiquitination of USP4 can promote CtIP recruitment and affect HR capacity [70].

USP11 and USP15 are two paralogs of USP4, and all of them share a common functional domain consisting of two ubiquitin-like (UBL) and a motif with ubiquitin-specific protease (DUSP) activity [71, 72]. USP11 is identified as a component of HR pathway, but the molecular mechanism is not clear [73], while USP15 is a DUB for murine double minute-2 (Mdm2), one of the E3 ligases that play a major role in regulating TP53 [74]. Thus, cell apoptosis induced by TP53 in DSB response may be inhibited by USP15 via deubiquitinating and stabilizing Mdm2. Except for USP15, USP26 can also deubiquitinate Mdm2 and play the same role as USP15 regulating TP53 [75]. Furthermore, USP26 and USP37 have been shown to inhibit the formation of BRCA1-A and promote the formation of BRCC. This function may involve in HR pathway [76]. However, further studies are needed to elucidate how USP26 and USP37 regulate HR pathway.

USP7, also called herpesvirus-associated ubiquitin-specific protease (HAUSP), is identified to act as a factor that promotes viral lytic growth, because it is associated with a herpesvirus protein ICP0 that is crucial for the viral lytic cycle [77, 78]. Substrates of USP7 are widespread, and a large part of them are tumor suppressors or oncogenes, such as TP53, PTEN, Chk1, Mdm2, and FOXO [79]. USP7 can regulate these tumor suppressors and play a key role in DSB response [80–82]. For example, USP7 directly deubiquitinates Chk1 *in vivo* and *in vitro* [83]; however, its family brother USP20 can only indirectly enhance the activity of ATR-Chk1 signaling by deubiquitinating Claspin [80]. Interestingly, deubiquitination of TP53 by USP7 prevents TP53 from degradation, whereas deubiquitination of Mdm2 by USP7 increases ubiquitination of TP53 and promotes the degradation of TP53 [81, 82]. This implies that the regulation of TP53 by USP7 is very complicated. Although USP7 displays its deubiquitination potential for both TP53 and Mdm2 that are substrates each other, this regulation potential is affected by different modificative status [81, 82]. Studies have shown that TP53 and Mdm2 bind to the same domain of USP7, but the binding capacity of Mdm2 is stronger except for phosphorylated status of Mdm2 induced by DNA damages [81, 82]. Additionally, USP10, USP29, and USP42 can deubiquitinate TP53, as well as USP7 [84–86]. However, they do not have the ability of deubiquitinating Mdm2. Thus, different USPs may exhibit different regulative potential for DSBR pathway via affecting different DNA repair factors such as Chk1, TP53, Mdm2, and so on [80–82] (**Table 1**).

4.2. OTUs

OTUs are divided into three subclasses: Otubians, A20-like OTUs, and other OTUs [91]. Otubians consist of OTUB 1 and OTUB 2 that are the first two proteins identified to display the DUB activity *in vitro* [92]. Structurally, OTUs are partly similar to USPs, exception for the incomplete catalytic triad [93, 94]. OTUs functionally involve in the regulation of diverse progresses, such as virus-triggered interferon induction, T cell anergy, and deubiquitination of p53 [87, 95, 96]. Interestingly, OTUB1 is a Lys48-specific DUB that can cleave ubiquitin from branched-polyubiquitin chains but not from ubiquitinated substrates. This DUB can bind to UBC13 (a cognate E2 enzyme for RNF168) and enhance DSB response potential via suppressing RNF168-dependent polyubiquitination but not via its catalytic ability [88]. OTUB1 also has the potential for directly deubiquitinating and stabilizing TP53 protein, which results in the decrease of cell death because of the increasing TP53 function [87]. Moreover, p53 is also the substrate of another OTU, OUTD5 [89]. OUTD5 has been shown to cleave the polyubiquitin chain from an essential type I interferon adaptor protein TRAF3 to interrupt the type I interferon signaling cascade [97]. As a DUB for p53, it can form a direct complex with p53 and is

DUB	Substrates	Process	Reference
USP1	Unclear	Promote HR and partly suppress NHEJ	[57]
USP3	H2A, γH2AX	Suppress DNA DSB response	[61]
USP4	ARF-BP1, USP4	Suppress p53-dependent apoptosis in DSB response	[67, 70]
USP6	H2A	Suppress DNA DSB response	[26]
USP7	Chk1, p53, Mdm2	Promote p53-dependent apoptosis in DSB response	[81–83]
USP10	p53	Promote p53-dependent apoptosis in DSB response	[84]
USP11	unclear	Promote HR	[73]
USP15	Mdm2	Suppress p53-dependent apoptosis in DSB response	[74]
USP20	Claspin	Promote DNA DSB response	[80]
USP26	Mdm2	Suppress p53-dependent apoptosis in DSB response and promote HR	[75, 76]
USP29	H2A, p53	Suppress DNA DSB response and promote p53-dependent apoptosis in DSB response	[63, 85]
USP37	Unclear	Promote HR	[76]
USP42	p53	Promote p53-dependent apoptosis in DSB response or promote DSB response	[86]
USP44	H2A	Suppress DNA DSB response	[63]
USP51	H2A, H2B	Suppress DNA DSB response	[62, 64]
OTUB1	p53	Promote p53-dependent apoptosis in DSB response not via its catalytic ability	[87, 88]
OTUD5	p53	Promote p53-dependent apoptosis in DSB response	[89]
POH1	K63	Promote HR but not via deubiquitinating K63	[90]

Table 1. DUBs regulate DNA DSBR.

required for the p53-dependent apoptosis in response to DSB. Recently, increasing evidence has exhibited that the dysregulation of this DUB may involve in the development of several types of cancer, such as lung, colorectal, and colon cancer [98–100]. Taken together, the regulation of OTUs may result in the defects of DSBR and ultimately promote damaged-cell carcinogenesis.

4.3. JAMM/MPN domain-associated metallopeptidases (JAMMs)

JAMMs, the important members of metalloproteinase (MMP), contain JAMM/MPN domain-associated metallopeptidases sequences. These sequences include three conserved residues (two His and one Asp) that make up of catalytic center with two zinc ions [101]. The 26S proteasome-associated PAD1 homolog 1 (POH1) is a representative member of JAMMs and plays a key role in DSBR pathway. POH1 has been shown to be required for HR, which was supposed to associate with its ability to restrict 53BP1 through cleaving ubiquitin from the polyubiquitin chains of K63 protein. However, the result from another study showed that POH1-regulating HR process was independent of 53BP1 [90]. Thus, further studies are needed to elucidate detailed regulative mechanisms.

5. Summary and future directions

DSBR is a crucial DNA repair pathway and requests a series of DNA repair enzymes, whose activation is usually controlled via the post-translational modification regulation. In the regulation of DSBR capacity, DUBs play a vital role via deubiquitinating key proteins involving in DSBR pathway and/or enhance DSB response. However, there are several issues to be noted. First, although DUBs are a large posttranslational modification factor, only small part of them have functionally been identified. Second, despite DUBs that regulate DSBR capacity via increasing the stability and activation of DSBR enzymes, the detailed mechanisms are still unclear. Finally, some other signal pathways may affect DSBR, and it is not clear whether DUBs regulate these signal pathways. Thus, further studies are needed to solve more detailed molecular mechanisms of DUBs regulating DSBR.

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Abbreviations

AMP	adenosine monophosphate
DSB	DNA double-strand break
DSBR	double-strand break repair
DUB	deubiquitinase
HR	homologous recombination
MCPIP	the monocyte chemotactic protein-induced protein

MJD	Machado-Joseph disease protein domain protease
NHEJ	non-homologous end-joining
OUT	ovarian tumor protease
ROS	reactive oxygen species
RSS	recombination signal sequence
UCH	ubiquitin carboxy-terminal hydrolase
UPP	ubiquitin-proteasome pathway
USP	ubiquitin-specific protease

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References

[1] Ciccia A, Elledge SJ. The DNA damage response: Making it safe to play with knives. Molecular Cell. 2010;**40**:179-204. DOI: 10.1016/j.molcel.2010.09.019

[2] Lagerwerf S, Vrouwe MG, Overmeer RM, Fousteri MI, Mullenders LH. DNA damage response and transcription. DNA Repair (Amst). 2011;**10**:743-750. DOI: 10.1016/j.dnarep.2011.04.024

[3] Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annual Review of Biochemistry. 2004;**73**:39-85. DOI: 10.1146/annurev.biochem.73.011303.073723

[4] O'Driscoll M, Jeggo PA. The role of double-strand break repair - insights from human genetics. Nature Reviews. Genetics. 2006;**7**:45-54. DOI: 10.1038/nrg1746

[5] Goldstein G, Scheid M, Hammerling U, Schlesinger DH, Niall HD, Boyse EA. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proceedings of the National Academy of Sciences of the United States of America. 1975;**72**:11-15. DOI: 10.8424/PMID.1078892

[6] Wilkinson KD, Audhya TK. Stimulation of ATP-dependent proteolysis requires ubiquitin with the COOH-terminal sequence Arg-Gly-Gly. The Journal of Biological Chemistry. 1981;**256**:9235-9241. DOI: 10.9258/PMID.6267067

[7] Callis J, Carpenter T, Sun CW, Vierstra RD. Structure and evolution of genes encoding polyubiquitin and ubiquitin-like proteins in Arabidopsis Thaliana ecotype Columbia. Genetics. 1995;**139**:921-939. DOI: 10.6731/PMID.7713442

[8] Vijay-Kumar S, Bugg CE, Cook WJ. Structure of ubiquitin refined at 1.8 a resolution. Journal of Molecular Biology. 1987;**194**:531-544. DOI: 10.1016/0022-2836(87)90679-6

[9] Smalle J, Vierstra RD. The ubiquitin 26S proteasome proteolytic pathway. Annual Review of Plant Biology. 2004;**55**:555-590. DOI: 10.1146/annurev.arplant.55.031903.141801

[10] Koepp DM, Harper JW, Elledge SJ. How the cyclin became a cyclin: Regulated proteolysis in the cell cycle. Cell. 1999;**97**:431-434. DOI: 10.1016/S0092-8674(00)80753-9

[11] Hofmann RM, Pickart CM. Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. Cell. 1999;**96**:645-653. DOI: 10.1016/S0092-8674(00)80575-9

[12] Ghosh S, May MJ, Kopp EB, NF-kappa B. Rel proteins: Evolutionarily conserved mediators of immune responses. Annual Review of Immunology. 1998;**16**:225-260. DOI: 10.1146/annurev.immunol.16.1.225

[13] Zhang Y. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes & Development. 2003;**17**:2733-2740. DOI: 10.1101/gad.1156403

[14] Shilatifard A. Chromatin modifications by methylation and ubiquitination: Implications in the regulation of gene expression. Annual Review of Biochemistry. 2006;**75**:243-269. DOI: 10.1146/annurev.biochem.75.103004.142422

[15] Puente XS, Lopez-Otin C. A genomic analysis of rat proteases and protease inhibitors. Genome Research. 2004;**14**:609-622. DOI: 10.1101/gr.1946304

[16] McBlane JF, van Gent DC, Ramsden DA, Romeo C, Cuomo CA, Gellert M, Oettinger MA. Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. Cell. 1995;**83**:387-395. DOI: 10.1016/0092-8674(95)90116-7

[17] Gellert M. V(D)J recombination: RAG proteins, repair factors, and regulation. Annual Review of Biochemistry. 2002;**71**:101-132. DOI: 10.1146/annurev.biochem.71.090501.150203

[18] Grawunder U, Harfst E. How to make ends meet in V(D)J recombination. Current Opinion in Immunology. 2001;**13**:186-194. DOI: 10.1016/S0952-7915(00)00203-X

[19] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiological Reviews. 1979;**59**:527-605. DOI: 10.9333/PMID.7713442

[20] Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human nonhomologous DNA end-joining. Nature Reviews. Molecular Cell Biology. 2003;**4**:712-720. DOI: 10.1038/nrm1202

[21] Kellner U, Sehested M, Jensen PB, Gieseler F, Rudolph P. Culprit and victim -- DNA topoisomerase II. The Lancet Oncology. 2002;**3**:235-243. DOI: 10.1016/S1470-2045(02)00715-5

[22] Murnane JP. Telomeres and chromosome instability. DNA Repair (Amst). 2006;**5**:1082-1092. DOI: 10.1016/j.dnarep.2006.05.030

[23] Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, Pelletier L, Jackson SP, Durocher D. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science. 2007;**318**:1637-1640. DOI: 10.1126/science.1150034

[24] Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell. 2007;**131**:901-914. DOI: 10.1016/j.cell.2007.09.041

[25] Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**:887-900. DOI: 10.1016/j.cell.2007.09.040

[26] Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, Ellenberg J, Panier S, Durocher D, Bartek J, Lukas J, Lukas C. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446. DOI: 10.1016/j.cell.2008.12.041

[27] Reinhardt HC, Yaffe MB. Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2, and MK2. Current Opinion in Cell Biology. 2009;**21**:245-255. DOI: 10.1016/j.ceb.2009.01.018

[28] Takai H, Naka K, Okada Y, Watanabe M, Harada N, Saito S, Anderson CW, Appella E, Nakanishi M, Suzuki H, Nagashima K, Sawa H, Ikeda K, Motoyama N. Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. The EMBO Journal. 2002;**21**:5195-5205. DOI: 10.1093/emboj/cdf506

[29] Rich T, Allen RL, Wyllie AH. Defying death after DNA damage. Nature. 2000;**407**:777-783. DOI: 10.1038/35037717

[30] Ferguson DO, Alt FW. DNA double strand break repair and chromosomal translocation: Lessons from animal models. Oncogene. 2001;**20**:5572-5579. DOI: 10.1038/sj.onc.1204767

[31] Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. Cell. 1993;**72**:131-142. DOI: 10.1016/0092-8674(93)90057-W

[32] Ramsden DA, Gellert M. Ku protein stimulates DNA end joining by mammalian DNA ligases: A direct role for Ku in repair of DNA double-strand breaks. The EMBO Journal. 1998;**17**:609-614. DOI: 10.1093/emboj/17.2.609

[33] Brouwer I, Sitters G, Candelli A, Heerema SJ, Heller I, de Melo AJ, Zhang H, Normanno D, Modesti M, Peterman EJ, Wuite GJ. Sliding sleeves of XRCC4-XLF bridge DNA and connect fragments of broken DNA. Nature. 2016;**535**:566-569. DOI: 10.1038/nature18643

[34] Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, Tezcan I, Sanal O, Bertrand Y, Philippe N, Fischer A, de Villartay JP. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell. 2001;**105**:177-186. DOI: 10.1016/S0092-8674(01)00309-9

[35] Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 2002;**108**:781-794. DOI: 10.1016/S0092-8674(02)00671-2

[36] O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B, Hirsch B, Gennery A, Palmer SE, Seidel J, Gatti RA, Varon R, Oettinger MA, Neitzel H, Jeggo PA, Concannon P. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Molecular Cell. 2001;**8**:1175-1185. DOI: 10.1016/S1097-2765(01)00408-7

[37] Game JCDNA. Double-strand breaks and the RAD50-RAD57 genes in saccharomyces. Seminars in Cancer Biology. 1993;**4**:73-83. DOI: 10.579X/PMID.8513150

[38] Johnson RD, Jasin M. Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. The EMBO Journal. 2000;**19**:3398-3407. DOI: 10.1093/emboj/19.13.3398

[39] Iftode C, Daniely Y, Borowiec JA. Replication protein a (RPA): The eukaryotic SSB. Critical Reviews in Biochemistry and Molecular Biology. 1999;**34**:141-180. DOI: 10.1080/10409239991209255

[40] Jackson D, Dhar K, Wahl JK, Wold MS, Borgstahl GE. Analysis of the human replication protein a:Rad52 complex: Evidence for crosstalk between RPA32, RPA70, Rad52 and DNA. Journal of Molecular Biology. 2002;**321**:133-148. DOI: 10.1016/S0022-2836(02)00541-7

[41] Gasior SL, Olivares H, Ear U, Hari DM, Weichselbaum R, Bishop DK. Assembly of RecA-like recombinases: Distinct roles for mediator proteins in mitosis and meiosis. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:8411-8418. DOI: 10.1073/pnas.121046198

[42] Morrison C, Sonoda E, Takao N, Shinohara A, Yamamoto K, Takeda S. The controlling role of ATM in homologous recombinational repair of DNA damage. The EMBO Journal. 2000;**19**:463-471. DOI: 10.1093/emboj/19.3.463

[43] Ahlskog JK, Larsen BD, Achanta K, Sorensen CS. ATM/ATR-mediated phosphorylation of PALB2 promotes RAD51 function. EMBO Reports. 2016;**17**:671-681. DOI: 10.15252/embr.201541455

[44] Li M, Yu X. Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. Cancer Cell. 2013;**23**:693-704. DOI: 10.1016/j.ccr.2013.03.025

[45] Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science. 2007;**316**:1202-1205. DOI: 10.1126/science.1139621

[46] Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. The Journal of Biological Chemistry. 2011;**286**:13669-13680. DOI: 10.1074/jbc.M110.213728

[47] Thompson LH, Schild D. Recombinational DNA repair and human disease. Mutation Research. 2002;**509**:49-78. DOI: 10.1016/S0027-5107(02)00224-5

[48] Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R. A genomic and functional inventory of deubiquitinating enzymes. Cell. 2005;**123**:773-786. DOI: 10.1016/j.cell.2005.11.007

[49] Tobias JW, Varshavsky A. Cloning and functional analysis of the ubiquitin-specific protease gene UBP1 of Saccharomyces Cerevisiae. The Journal of Biological Chemistry. 1991;**266**:12021-12028. DOI: 10.9258/PMID.2050695

[50] Hu M, Li P, Li M, Li W, Yao T, Wu JW, Gu W, Cohen RE, Shi Y. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. Cell. 2002;**111**:1041-1054. DOI: S0092-8674(02)01199-6

[51] Komander D, Lord CJ, Scheel H, Swift S, Hofmann K, Ashworth A, Barford D. The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. Molecular Cell. 2008;**29**:451-464. DOI: 10.1016/j.molcel.2007.12.018

[52] Ye Y, Scheel H, Hofmann K, Komander D. Dissection of USP catalytic domains reveals five common insertion points. Molecular BioSystems. 2009;**5**:1797-1808. DOI: 10.1039/b907669g

[53] Daviet L, Colland F. Targeting ubiquitin specific proteases for drug discovery. Biochimie. 2008;**90**:270-283. DOI: 10.1016/j.biochi.2007.09.013

[54] Williams SA, Maecker HL, French DM, Liu J, Gregg A, Silverstein LB, Cao TC, Carano RA, Dixit VM. USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. Cell. 2011;**146**:918-930. DOI: 10.1016/j.cell.2011.07.040

[55] Garcia-Santisteban I, Peters GJ, Giovannetti E, Rodriguez JA. USP1 deubiquitinase: Cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. Molecular Cancer. 2013;**12**:91. DOI: 10.1186/1476-4598-12-91

[56] Cohn MA, Kowal P, Yang K, Haas W, Huang TT, Gygi SP, D'Andrea AD. A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. Molecular Cell. 2007;**28**:786-797. DOI: 10.1016/j.molcel.2007.09.031

[57] Murai J, Yang K, Dejsuphong D, Hirota K, Takeda S, D'Andrea AD. The USP1/UAF1 complex promotes double-strand break repair through homologous recombination. Molecular and Cellular Biology. 2011;**31**:2462-2469. DOI: 10.1128/MCB.05058-11

[58] Nicassio F, Corrado N, Vissers JH, Areces LB, Bergink S, Marteijn JA, Geverts B, Houtsmuller AB, Vermeulen W, Di Fiore PP, Citterio E. Human USP3 is a chromatin modifier required for S phase progression and genome stability. Current Biology. 2007;**17**:1972-1977. DOI: 10.1016/j.cub.2007.10.034

[59] Sloper-Mould KE, Eyre HJ, Wang XW, Sutherland GR, Baker RT. Characterization and chromosomal localization of USP3, a novel human ubiquitin-specific protease. The Journal of Biological Chemistry. 1999;**274**:26878-26884. DOI: 10.1074/jbc.274.38.26878

[60] Lancini C, van den Berk PC, Vissers JH, Gargiulo G, Song JY, Hulsman D, Serresi M, Tanger E, Blom M, Vens C, van Lohuizen M, Jacobs H, Citterio E. Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells. The Journal of Experimental Medicine. 2014;**211**:1759-1777. DOI: 10.1084/jem.20131436

[61] Sharma N, Zhu Q, Wani G, He J, Wang QE, Wani AA. USP3 counteracts RNF168 via deubiquitinating H2A and gammaH2AX at lysine 13 and 15. Cell Cycle. 2014;**13**:106-114. DOI: 10.4161/cc.26814

[62] Wang Z, Zhang H, Liu J, Cheruiyot A, Lee JH, Ordog T, Lou Z, You Z, Zhang Z. USP51 deubiquitylates H2AK13,15ub and regulates DNA damage response. Genes & Development. 2016;**30**:946-959. DOI: 10.1101/gad.271841.115

[63] Mosbech A, Lukas C, Bekker-Jensen S, Mailand N. The deubiquitylating enzyme USP44 counteracts the DNA double-strand break response mediated by the RNF8 and RNF168 ubiquitin ligases. The Journal of Biological Chemistry. 2013;**288**:16579-16587. DOI: 10.1074/jbc.M113.459917

[64] Atanassov BS, Mohan RD, Lan X, Kuang X, Lu Y, Lin K, McIvor E, Li W, Zhang Y, Florens L, Byrum SD, Mackintosh SG, Calhoun-Davis T, Koutelou E, Wang L, Tang DG, Tackett AJ, Washburn MP, Workman JL, Dent SY. ATXN7L3 and ENY2 coordinate activity of multiple H2B Deubiquitinases important for cellular proliferation and tumor growth. Molecular Cell. 2016;**62**:558-571. DOI: 10.1016/j.molcel.2016.03.030

[65] Gray DA, Inazawa J, Gupta K, Wong A, Ueda R, Takahashi T. Elevated expression of Unph, a proto-oncogene at 3p21.3, in human lung tumors. Oncogene. 1995;**10**:2179-2183. DOI: 10.9232/PMID.7784062

[66] Gilchrist CA, Baker RT. Characterization of the ubiquitin-specific protease activity of the mouse/human Unp/Unph oncoprotein. Biochimica et Biophysica Acta. 2000;**1481**: 297-309. DOI: 10.3002/PMID.11018721

[67] Zhang X, Berger FG, Yang J, Lu X. USP4 inhibits p53 through deubiquitinating and stabilizing ARF-BP1. The EMBO Journal. 2011;**30**:2177-2189. DOI: 10.1038/emboj.2011.125

[68] Heo MJ, Kim YM, Koo JH, Yang YM, An J, Lee SK, Lee SJ, Kim KM, Park JW, Kim SG. microRNA-148a dysregulation discriminates poor prognosis of hepatocellular carcinoma in association with USP4 overexpression. Oncotarget. 2014;**5**:2792-2806. DOI: 10.18632/oncotarget.1920

[69] Liu H, Zhang H, Wang X, Tian Q, Hu Z, Peng C, Jiang P, Wang T, Guo W, Chen Y, Li X, Zhang P, Pei H. The Deubiquitylating enzyme USP4 cooperates with CtIP in DNA double-strand break end resection. Cell Reports. 2015;**13**:93-107. DOI: 10.1016/j.celrep.2015.08.056

[70] Wijnhoven P, Konietzny R, Blackford AN, Travers J, Kessler BM, Nishi R, Jackson SP. USP4 auto-Deubiquitylation promotes homologous recombination. Molecular Cell. 2015;**60**:362-373. DOI: 10.1016/j.molcel.2015.09.019

[71] Zhu X, Menard R, Sulea T. High incidence of ubiquitin-like domains in human ubiquitin-specific proteases. Proteins. 2007;**69**:1-7. DOI: 10.1002/prot.21546

[72] Vlasschaert C, Xia X, Coulombe J, Gray DA. Evolution of the highly networked deubiquitinating enzymes USP4, USP15, and USP11. BMC Evolutionary Biology. 2015;**15**:230. DOI: 10.1186/s12862-015-0511-1

[73] Wiltshire TD, Lovejoy CA, Wang T, Xia F, O'Connor MJ, Cortez D. Sensitivity to poly(ADP-ribose) polymerase (PARP) inhibition identifies ubiquitin-specific peptidase 11 (USP11) as a regulator of DNA double-strand break repair. The Journal of Biological Chemistry. 2010;**285**:14565-14571. DOI: 10.1074/jbc.M110.104745

[74] Zou Q, Jin J, Hu H, Li HS, Romano S, Xiao Y, Nakaya M, Zhou X, Cheng X, Yang P, Lozano G, Zhu C, Watowich SS, Ullrich SE, Sun SC. USP15 stabilizes MDM2 to mediate cancer-cell survival and inhibit antitumor T cell responses. Nature Immunology. 2014;**15**:562-570. DOI: 10.1038/ni.2885

[75] Lahav-Baratz S, Kravtsova-Ivantsiv Y, Golan S, Ciechanover A. The testis-specific USP26 is a deubiquitinating enzyme of the ubiquitin ligase Mdm2. Biochemical and Biophysical Research Communications. 2017;**482**:106-111. DOI: 10.1016/j.bbrc.2016.10.135

[76] Typas D, Luijsterburg MS, Wiegant WW, Diakatou M, Helfricht A, Thijssen PE, van den Broek B, Mullenders LH, van Attikum H. The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucleic Acids Research. 2015;**43**:6919-6933. DOI: 10.1093/nar/gkv613

[77] Vucic D, Dixit VM, Wertz IE. Ubiquitylation in apoptosis: A post-translational modification at the edge of life and death. Nature Reviews. Molecular Cell Biology. 2011;**12**:439-452. DOI: 10.1038/nrm3143

[78] Faustrup H, Bekker-Jensen S, Bartek J, Lukas J, Mailand N. USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. The Journal of Cell Biology. 2009;**184**:13-19. DOI: 10.1083/jcb.200807137

[79] Sacco JJ, Coulson JM, Clague MJ, Urbe S. Emerging roles of deubiquitinases in cancer-associated pathways. IUBMB Life. 2010;**62**:140-157. DOI: 10.1002/iub.300

[80] Yuan J, Luo K, Deng M, Li Y, Yin P, Gao B, Fang Y, Wu P, Liu T, Lou Z. HERC2-USP20 axis regulates DNA damage checkpoint through Claspin. Nucleic Acids Research. 2014;**42**:13110-13121. DOI: 10.1093/nar/gku1034

[81] Meulmeester E, Pereg Y, Shiloh Y, Jochemsen AG. ATM-mediated phosphorylations inhibit Mdmx/Mdm2 stabilization by HAUSP in favor of p53 activation. Cell Cycle. 2005;**4**:1166-1170. DOI: 10.4161/cc.4.9.1981

[82] Hu M, Gu L, Li M, Jeffrey PD, Gu W, Shi Y. Structural basis of competitive recognition of p53 and MDM2 by HAUSP/USP7: Implications for the regulation of the p53-MDM2 pathway. PLoS Biology. 2006;**4**:e27. DOI: 10.1371/journal.pbio.0040027

[83] Alonso-de Vega I, Martin Y, Smits VA. USP7 controls Chk1 protein stability by direct deubiquitination. Cell Cycle. 2014;**13**:3921-3926. DOI: 10.4161/15384101.2014.973324

[84] Yuan J, Luo K, Zhang L, Cheville JC, Lou Z. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell. 2010;**140**:384-396. DOI: 10.1016/j.cell.2009.12.032

[85] Liu J, Chung HJ, Vogt M, Jin Y, Malide D, He L, Dundr M, Levens D. JTV1 co-activates FBP to induce USP29 transcription and stabilize p53 in response to oxidative stress. The EMBO Journal. 2011;**30**:846-858. DOI: 10.1038/emboj.2011.11

[86] Hock AK, Vigneron AM, Carter S, Ludwig RL, Vousden KH. Regulation of p53 stability and function by the deubiquitinating enzyme USP42. The EMBO Journal. 2011;**30**:4921-4930. DOI: 10.1038/emboj.2011.419

[87] Sun XX, Dai MS. Deubiquitinating enzyme regulation of the p53 pathway: A lesson from Otub1. World Journal of Biological Chemistry. 2014;**5**:75-84. DOI: 10.4331/wjbc.v5.i2.75

[88] Nakada S, Tai I, Panier S, Al-Hakim A, Iemura S, Juang YC, O'Donnell L, Kumakubo A, Munro M, Sicheri F, Gingras AC, Natsume T, Suda T, Durocher D. Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. Nature. 2010;**466**:941-946. DOI: 10.1038/nature09297

[89] Luo J, Lu Z, Lu X, Chen L, Cao J, Zhang S, Ling Y, Zhou X. OTUD5 regulates p53 stability by deubiquitinating p53. PLoS One. 2013;**8**:e77682. DOI: 10.1371/journal.pone.0077682

[90] Butler LR, Densham RM, Jia J, Garvin AJ, Stone HR, Shah V, Weekes D, Festy F, Beesley J, Morris JR. The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response. The EMBO Journal. 2012;**31**:3918-3934. DOI: 10.1038/emboj.2012.232

[91] Komander D, Clague MJ, Urbe S. Breaking the chains: Structure and function of the deubiquitinases. Nature Reviews. Molecular Cell Biology. 2009;**10**:550-563. DOI: 10.1038/nrm2731

[92] Balakirev MY, Tcherniuk SO, Jaquinod M, Chroboczek J. Otubains: A new family of cysteine proteases in the ubiquitin pathway. EMBO Reports. 2003;**4**:517-522. DOI: 10.1038/sj.embor.embor824

[93] Nanao MH, Tcherniuk SO, Chroboczek J, Dideberg O, Dessen A, Balakirev MY. Crystal structure of human otubain 2. EMBO Reports. 2004;**5**:783-788. DOI: 10.1038/sj.embor.7400201

[94] Wang T, Yin L, Cooper EM, Lai MY, Dickey S, Pickart CM, Fushman D, Wilkinson KD, Cohen RE, Wolberger C. Evidence for bidentate substrate binding as the basis for the K48 linkage specificity of otubain 1. Journal of Molecular Biology. 2009;**386**:1011-1023. DOI: 10.1016/j.jmb.2008.12.085

[95] Li S, Zheng H, Mao AP, Zhong B, Li Y, Liu Y, Gao Y, Ran Y, Tien P, Shu HB. Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. The Journal of Biological Chemistry. 2010;**285**:4291-4297. DOI: 10.1074/jbc.M109.074971

[96] Soares L, Seroogy C, Skrenta H, Anandasabapathy N, Lovelace P, Chung CD, Engleman E, Fathman CG. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. Nature Immunology. 2004;**5**:45-54. DOI: 10.1038/ni1017

[97] Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, O'Rourke KM, Eby M, Pietras E, Cheng G, Bazan JF, Zhang Z, Arnott D, Dixit VM. DUBA: A deubiquitinase that regulates type I interferon production. Science. 2007;**318**:1628-1632. DOI: 10.1126/science.1145918

[98] Baietti MF, Simicek M, Abbasi Asbagh L, Radaelli E, Lievens S, Crowther J, Steklov M, Aushev VN, Martinez Garcia D, Tavernier J, Sablina AA. OTUB1 triggers lung cancer development by inhibiting RAS monoubiquitination. EMBO Molecular Medicine. 2016;**8**:288-303. DOI: 10.15252/emmm.201505972

[99] Zhou Y, Wu J, Fu X, Du W, Zhou L, Meng X, Yu H, Lin J, Ye W, Liu J, Peng H, Liu RY, Pan C, Huang W. OTUB1 promotes metastasis and serves as a marker of poor prognosis in colorectal cancer. Molecular Cancer. 2014;**13**:258. DOI: 10.1186/1476-4598-13-258

[100] Liu X, Jiang WN, Wang JG, Chen H. Colon Cancer bears overexpression of OTUB1. Pathology, Research and Practice. 2014;**210**:770-773. DOI: 10.1016/j.prp.2014.05.008

[101] Tran HJ, Allen MD, Lowe J, Bycroft M. Structure of the Jab1/MPN domain and its implications for proteasome function. Biochemistry. 2003;**42**:11460-11465. DOI: 10.1021/bi035033g

The Roles of Cullin RING Ligases and the Anaphase Promoting Complex/Cyclosome in the Regulation of DNA Double Strand Break Repair

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Additional information is available at the end of the chapter

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Abstract

Historically, genome maintenance has been viewed as the largely independent activities of (1) ubiquitin ligases driving unidirectional cell cycle progression and, (2) the activity of cellular checkpoints that monitor DNA integrity and DNA replication. It is well established that the DNA damage response (DDR) checkpoint machinery promotes the activation of repair mechanisms in addition to opening a window for repair. Emerging evidence demonstrates an integrated network of the central cell cycle driving E3 ubiquitin ligases and the checkpoint machinery, as well as deubiquitinating enzymes, which intermittently cooperate and antagonize one another to define windows of checkpoint and repair activities to optimize genome stability and cellular health. A growing number of components of the ubiquitin machinery are involved in the DDR. Herein, we focus on the regulation of cell cycle checkpoints and the DNA repair mechanisms for double strand breaks (DSBs) by the coordinated activities of Cullin RING ligases (CRLs) and the anaphase promoting complex/cyclosome (APC/C).

Keywords: DNA repair, deubiquitinating enzymes, E3 ubiquitin ligase, APC/C, Cullin-RING ligase, SCF, homologous repair, non-homologous end-joining

1. Introduction

Our cells face a multitude of DNA damaging insults, both internally and externally derived, on a daily basis. The majority of our cells is not cycling and must simply respond by rapidly repairing the damaged DNA to maintain homeostasis. For those cells that are cycling, however, the precise maintenance of the genome is of even more critical importance to ensure the faithful transmission of identical copies of an undamaged genome to the next generation of cells. Of critical concern for cycling cells is the precise duplication of one exact copy of the genome followed by the accurate segregation of the two copies. To ensure that these events happen once, only once, and in the proper order, cells utilize the periodic synthesis and ubiquitin-mediated degradation of a host of proteins to control the timely activity of multiple enzymatic activities, such as kinases and polymerases, to drive the unidirectional transit through the cell cycle.

Upon the occurrence of DNA damage, cycling cells must not only sense and respond to the insult, but must also coordinate cell cycle progression with repair. Moreover, as damage can occur during any of the many processes taking place during the cell cycle, proliferating cells have evolved a number of mechanisms for repairing and overcoming damage to maintain the genome. However, the proper selection of the repair mechanism to use is nearly as important as sensing the damage as some mechanism could be highly mutagenic if utilized at the wrong point in the cell cycle or if used without proper control. Such errors would clearly result in the generation of mutations that could lead to a number of human pathologies, most notably cancer. Moreover, the induction of DNA damage is a central therapeutic strategy for treatment of the majority of cancer types. And, while clinically useful, such treatments lack specificity and are often limited by toxicities. The dissection of genome maintenance pathways thus holds the potential to define new therapeutic targets that may ultimately lead to more effective therapeutic strategies. This review will focus on recent advances in our understanding of how the interplay of the major cell cycle-associated ubiquitin ligases, the DNA-monitoring checkpoint machinery, and deubiquitinating enzymes coordinate cell cycle progression with the response to the proper repair of DNA damage. In particular, the focus will be on the use of mechanisms of repairing DNA double strand breaks and stalled replication forks.

2. Modular E3 ubiquitin ligases in the cell cycle and genome repair

2.1. Cullin RING ligases (CRLs)

The cullin family proteins (Cul1, 2, 4A, 4B, 5, and 7) function as the central scaffolds for the assembly of multi-subunit ubiquitin ligases [1]. The cullin C-terminus adopts a globular conformation that provides a docking site for the RING finger proteins Rbx1 or Rbx2. The RING fingers recruit the ubiquitin E2 enzymes and catalyze the transfer of ubiquitin to substrates. At the cullin N-terminus is a helical domain that is the site of interaction with an adapter protein (s), which recruits substrate receptors. In general, each cullin associates with a distinct set of substrate receptors. For example, the CRL1 ligases utilize the adaptor protein Skp1 to interact the F-box family of proteins defined for a Skp1-interacting motif defined in the archetypical F-box, Cyclin F. The CRLs are denoted by the identity of the cullin family member and the associate receptor. For example, CRL1$^{\text{Cyclin F}}$ denotes a cullin1-based ligase complexed with the substrate adapter Cyclin F. The human genome contains nearly 200 cullin-associated substrate receptors thus allowing CRLs to regulate myriad cellular processes [1]. The extent of this functional diversity is exemplified by the fact that a single CRL can have either oncogenic or tumor suppressive activity depending on the substrate adaptor, for example CRL1$^{\text{Skp2}}$ and CRL1$^{\text{Fbw7}}$, respectively [2–5].

With nearly 200 E3 ligases regulating an estimated 20% of the human proteome and a growing number of cellular process, it is not surprising that the function of CRLs is highly regulated at multiple levels, including; regulation of substrate receptor availability (e.g., regulated expression and degradation of receptors), activation/inactivation by the reversible neddylation of the cullin subunit, CAND1-mediated exchange of substrate receptors, regulation of substrate-receptor interactions (e.g., post-translational modification of substrates such as phosphorylation and glycosylation) and the activity of deubiquitinating enzymes [1, 6].

2.1.1. CRL1 (a.k.a. SCF) complexes

The Cullin1-based CRL1 ligases are more commonly known as the Skp1-Cullin1-F-box (SCF) ligases. There are nearly 70 F-box proteins in the human genome, although only a subset has been studied in great detail. For the purposes of this review we will utilize the SCF rather than CRL1, nomenclature. Multiple SCF ligases are involved in cell cycle control and the response to and repair of DNA damage. In consideration of space constraints, we will give overviews of two key SCF, rather than CRL1, ligases as more specific examples of the function of this group of enzymes.

2.1.1.1. SCF^{Skp2}

SCF^{Skp2} functions as a driver of S-phase and exhibits oncogenic activity in multiple settings. Skp2 activity is regulated by its controlled expression and degradation. In addition, even when the Skp2 protein is present and complexed with Cullin1 and Skp1, its ability to recruit substrates for ubiquitination requires site-specific phosphorylation of its target proteins to create a phosphodegron that is recognized by Skp2. Many Skp2 substrates are phosphorylated in a cell cycle-specific fashion, adding an additional layer of control. Skp2 is predominantly known for its role in driving S-phase entry by degrading the Cdk inhibitors p21 and p27 to drive S-phase entry. It is frequently overexpressed in tumours of varying origins and exhibits oncogenic activity [7].

2.1.1.2. $SCF^{\beta TrCP}$

$SCF^{\beta TrCP}$ is a collective term for two SCF complexes defined by the F-box proteins βTrcp1 and βTrCp2, which are largely, but not exclusively interchangeable. In contrast to the fluctuating levels of Skp2, βTrcp levels are relatively constant throughout the cell cycle, and a major determinant $SCF^{\beta TrCP}$ activity is the creation of a consensus DpSGxxpS phosphodegron upon substrates. Multiple kinases are involved in the generation of phospho-DSGxxS in substrates, including GSK3β, CK2, Polo-like kinases (e.g., Plk1) and Chk1. Thus, some substrates, for example those directed to $SCF^{\beta TrCP}$ by Plk1, are degraded in a cell cycle specific manner owing to the regulated expression of Plk1 itself [7].

2.1.2. CRL4 complexes

The cullin 4-based ligases, encompassing cullin 4A or 4B, display almost complete functional redundancy and are generally referred to collectively as CRL4. These ligase complexes incorporate the adapter protein damage DNA-binding 1 (DDB1) and associate with ~25 substrate receptors known as the DDB1 and Cul4 associated factors (DCAFs) [8, 9]. As with the SCF ligases, the majority of CRL4 complexes have not been studied in detail, yet it is clear that the CRL4 ligases are involved in a multitude of processes, including embryogenesis and haematopoiesis and impact both tumorigenesis and tumour suppression depending on context. CRL4 ligases are best characterized for their roles in cell cycle progression (predominantly controlling replication) and DNA repair. In regard to the latter, CRL4^{CSA} and CRL4^{DDB2} are well characterized for their roles in nucleotide excision repair (NER) in response to UV irradiation [10].

2.1.2.1. CRL4^{Cdt2}

CRL4^{Cdt2} is a central component of the S-phase machinery, which acts to ensure that genome replication is limited to a single round per cell cycle. CRL4^{Cdt2} couples destruction of these targets to replication through a partnership with PCNA, which interacts with a host of proteins to maintain genomic integrity, including licensing factors, helicases, methyltransferase, repair enzymes, and the translesion (TLS) polymerases [11]. The regulated recruitment of these proteins is critical for preparing the genome for faithful transmission to the next generation as spurious engagement of several PCNA-binding proteins has been shown to have deleterious effects [8, 12–17]. Importantly, the majority of these factors engage the same interaction surface on PCNA via a PCNA-interacting protein (PIP)-box motif. Interestingly, the PIP-box of a subset of PCNA-interacting proteins, such as the Cdk inhibitor p21 and the replication licensing factor Cdt1, when bound to PCNA, acts to recruit the CRL4^{Cdt2} leading to the ubiquitination and destruction of these proteins [13, 18]. Notably, these CRL4^{Cdt2}-PCNA-substrate interactions only occur when PCNA is bound to DNA to allow recruitment of additional factors [11]. A number of mechanisms regulate these interactions with PCNA, but a critical determinant is the strength of the PCNA-PIP-box interface.20 The PIP-box of the tumour suppressor p21 has the highest known affinity for PCNA, allowing it to prevent PCNA interactions with other PIP-box proteins [19]. In this way, p21 acts to prevent spurious replication and prevent the inappropriate engagement of the error-prone polymerases, which are able to continue DNA replication despite damaged DNA. However, upon replication blocks such as UV-induced damage, p21 is degraded by CRL4^{Cdt2} to allow TLS. Subsequently, the bypassed sites of damage can be repaired by NER.

2.2. Anaphase promoting complex/cyclosome (APC/C)

The APC/C is a large, multi-subunit E3 ubiquitin ligase conserved from yeast to humans. By targeting a multitude of proteins for destruction by the 26S proteasome, the APC is a major driver of cell cycle progression, as well as regulating many diverse processes including meiosis, TFGβ signalling, synaptic maturation and differentiation [20–31]. Although not itself a cullin, the central APC2 subunit bares significant homology to the cullins and like these proteins provides a scaffold for the assembly of the multi-subunit APC/C E3 ligase. APC2 contains a binding site for APC11, the RING finger and catalytic component of the APC/C. The APC/C, like CRLs, is involved in numerous cellular processes. However, in contrast to the CRLs, substrate recognition by the APC/C is mediated by a bipartite receptor made up of the APC/C core component APC10 and one of only two substrate receptor/activator proteins, Cdc20 and Cdh1.

Recognition of substrates is mediated by several cis-acting sequence motifs (degrons). It is generally thought that D-boxes and KEN-boxes are responsible for the destruction of all APC substrates [32, 33]. Indeed, most substrates contain one (often multiple) of these two degrons;

however, there are a growing number of motifs identified as critical for APC/C-mediated ubiquitination in the ever-increasing number of APC/C substrates. Recent structural analyses have identified the molecular basis for the interaction of substrates with Cdc20 and Cdh1, which suggests that non-canonical APC/C degrons interact with the activators in manners analogous to the canonical degrons.

APC^{Cdc20}, essential for cell division and viability, is indirectly inhibited by clinically relevant agents (*e.g.* paclitaxel, an anti-cancer drug Taxol), and has received substantial evaluation for pharmacological manipulation. In contrast, APC^{Cdh1} activity is not required for viability, although increasing data demonstrate a role for APC^{Cdh1} in genomic stability and tumor suppression [34, 35]. Indeed, many APC^{Cdh1} substrates (*e.g*. Cyclin A, Skp2, Aurora A, Plk1, and Id2) are associated with oncogenesis, and the regulation of the stability of these substrates has been extensively linked to highly malignant cancers [36]. However, increased Cdh1 activity is also deleterious to cells.

APC/C activity must be tightly controlled and this is accomplished by several mechanisms. First, the activators are regulated at the level of expression with both Cdc20 and Cdh1 accumulating during S and G2 phases. At the end of mitosis, Cdc20 is then degraded by APC/C^{Cdh1}. APC/C^{Cdh1} activity remains high in G1 and its inactivation is critical for commitment to S-phase. Down regulation of APC/C^{Cdh1} activity involves APC/C-mediated degradation of its primary E2 enzyme, UbcH10, Cdk-mediated phosphorylation of Cdh1 which antagonizes its binding to the APC/C holoenzyme, degradation of Cdh1, and the interaction of APC/C with Emi1. Binding of Emi1 prevents substrate engagement and ubiquitination activities and is critical for inhibition of APC/C^{Cdh1}.

2.3. Crosstalk between CRL and APC/C ligases

There is increasing understanding that crosstalk between the CRL ligases and APC/C ligases is required for efficient cell cycle. For example, Skp2 is a substrate of APC/C^{Cdh1} and as cells near the G1/S transition, Cyclin E-Cdk2 complexes initiate the inactivation of APC/C^{Cdh1}, which promotes early accumulation of APC substrates such as Cyclin A and Skp2 (and the activation of SCF^{Skp2}) which promotes further Cdk activity as well as the expression of Emi1, leading to rapid abrogation of APC/C^{Cdh1} activity [37–47]. Then, as cells transit S and G2 the accumulation of the APC/C^{Cdh1} Plk1 leads to the SCF$^{\beta TrCP}$-mediated degradation of Emi1 at the G2/M transition to allow APC/C to become active in mitosis [48–51]. Recently, it was discovered that in addition to APC/C-mediated degradation of Cdh1 in late G1, Plk1 also directs the SCF$^{\beta TrCP}$-mediated degradation of Cdh1 as cells enter S-phase [52]. SCF^{Fbw7} via its ability to target and regulate the levels of Cyclin E and Plk1 adds another input to this regulatory circuit [53].

3. DNA damage responses

3.1. The double strand break response

The generation of double strand breaks (DSBs) is of potentially grave consequence to cells at any stage of the cell cycle and must be dealt with immediately. In response to DSBs the MRE11-RAD50-NBS1 (MRN) complex and the inactive dimers of the ATM kinase localize to the damaged site, resulting in the autophosphorylation and activation of ATM monomers (**Figure 1**). The MRN-dependent activation of ATM is facilitated by the non-degradative, K63 linked ubiquitination of NBS1 by SCF^{Skp2} [54]. Phosphorylation of histone H2AX by ATM leads to the recruitment of the checkpoint mediator MDC1, which recruits additional MRN-ATM complexes, to amplify the checkpoint signal, and promotes ubiquitination of histone H2AK15, by the concerted actions of the RNF8 and RNF168 ubiquitin ligases [55–61]. The PR-Set7 and MMSET methyltransferases are also recruited to sites of DNA damage where they catalyse methylation of histone H4K20 [62–66]. Together the ubiquitination of H2A and the methylation of H4 provide a high-affinity binding sight for the checkpoint mediator 53BP1 at sites of damage [67, 68]. 53BP1 further stimulates ATM activity by interacting with MRN complexes and sets the stage for repair. While ATM provides local regulation of the DDR, global regulation is carried out by the effector kinase Chk2, which is activated by ATM. Chk2 phosphorylates numerous proteins, including Cdc25 family phosphatases (to promote/maintain inhibitory phosphorylation of Cdks and cell cycle arrest), p53, and the repair protein BRCA1.

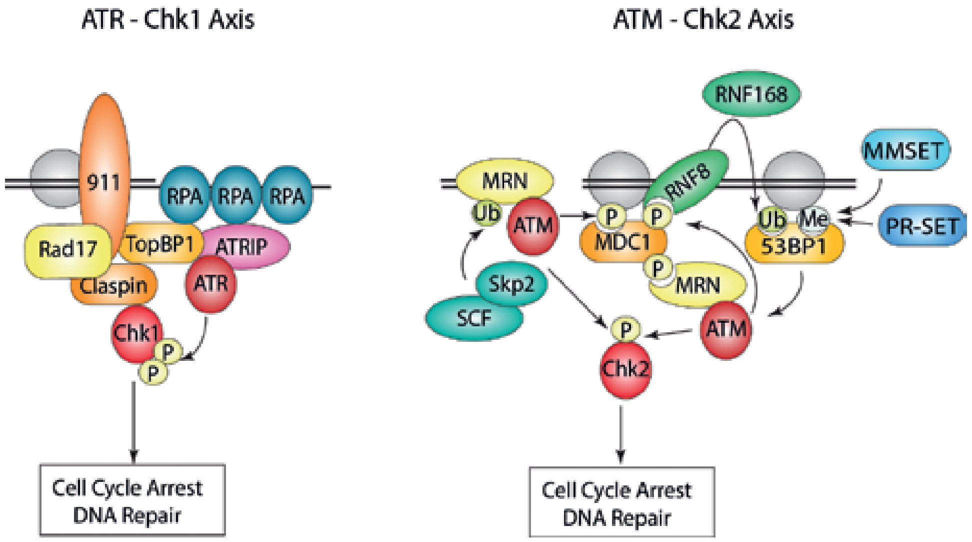

Figure 1. Activation of the ATR and ATM kinase cascades upon DNA damage. Left—Single stranded DNA, generated by blocks to DNA replication or the resection of double strand breaks (DSBs) is coated by RPA, which acts to recruit the ATR-ATRIP heterodimer. The RAD9-RAD1-HUS1 [1] complex is loaded by RAD17. 9-1-1 recruits the ATR activator TopBP1. The mediator protein, Claspin then recruits Chk1 to the site of damage where it is activated by ATR to effect the checkpoint. Right—The induction of a DSB leads to the direct binding of the MRE11-RAD50-NBS1 (MRN)—ATM complex, which phosphorylates histone H2AX (grey spheres represent the histone octamer). The checkpoint mediator MDC1 binds to the phosphorylated histone and is then bound by another MRN-ATM complex. Phosphorylation of MDC1 by ATM recruits the E3 ubiquitin ligase RNF8, which in conjunction with RNF168, ubiquitinates histone H2K15. The ubiquitin ligase SCF^{Skp2} also promotes the MRN-ATM complex formation. The methyltransferases MMSET and PR-SET catalyse methylation of histone H4K20. The H2K15-Ub and H4K20me marks are recognized by 53BP1 leading to further stimulation of ATM activity and the ultimate induction of cell cycle arrest and DNA repair by the effector kinase Chk2.

3.2. Mechanisms of DSB repair

Cells with DNA damage in the form of double strand breaks (DSBs) predominantly use two mechanisms to repair these lesions (**Figure 2**) [69, 70]. The least error-prone of these mechanisms, homologous recombination (HR), utilizes the non-damaged sister chromatid as a template to inform repair of the damaged DNA and is thus limited to S and G2 phases of the cell cycle, where the sister template is available [69]. Indeed, damage incurred during S-phase, whether DSB, interstrand cross-links, or collapsed replication forks rely heavily on HR for repair. The alternative repair pathway, non-homologous end-joining (NHEJ), as the name suggests, involves the sequence-independent ligation of broken DNA ends. Although, some NHEJ (alt-NHEJ or microhomology-mediated, mmNHEJ) do utilize very small regions of homology to identify DNA ends for ligation, canonical NHEJ has no requirement for any

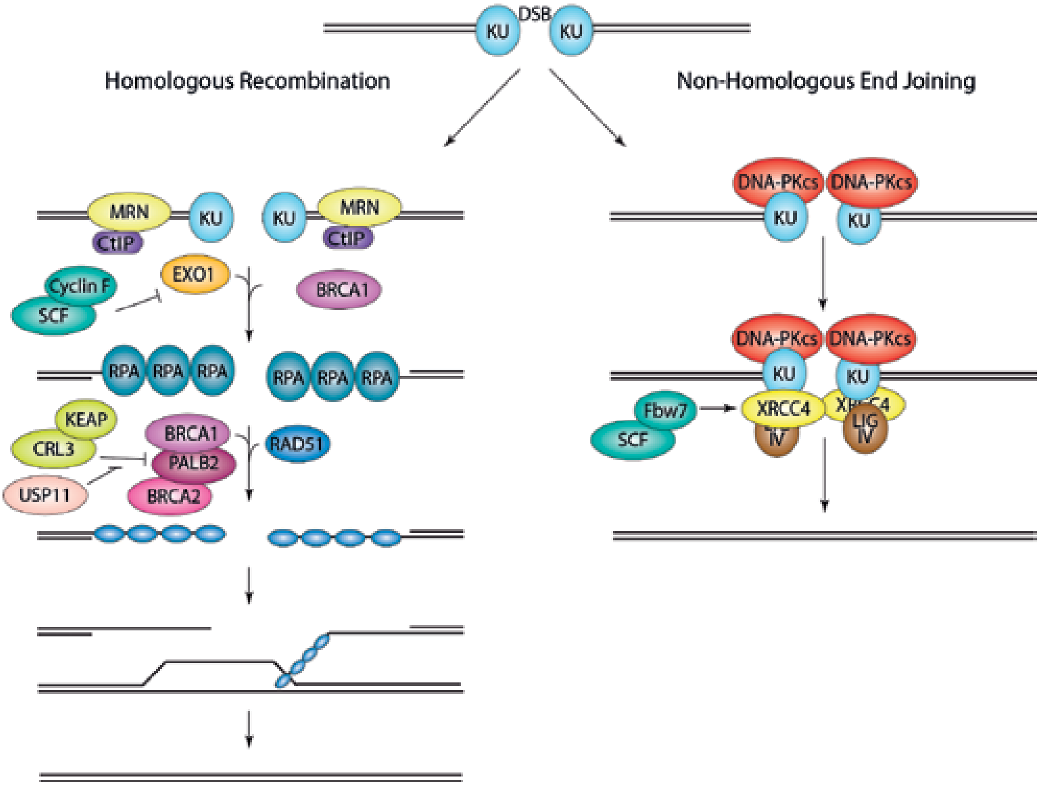

Figure 2. Mechanisms of DSB repair. Upon induction of DSBs, KU proteins are recruited to the broken DNA, protecting the ends. MRN complexes are then recruited. In the presence of BRCA1 and CtIP, the DNA ends are resected, recruiting additional nucleases, including EXO1. Resection leads to the removal of KU proteins. $SCF^{Cyclin\ F}$ prevents excessive resection by targeting EXO1 for degradation. ssDNA generated by resection is coated by RPA. The BRCA1, BRCA2, PALB2 complex then stimulates the replacement of RPA with RAD51, which promotes strand invasion of the sister chromatid template, leading to homology directed repair of the break. PALB2 function is negatively regulated by the $CRL3^{KEAP}$ ligase and promoted by USP11. In the absence of resection, DNA-PKcs is recruited by the KU proteins, which leads to the recruitment of additional factors, including XRCC4, which is stimulated by SCF^{Fbw7} ligase activity, and DNA ligase IV, which ultimately joins the DNA fragments together.

sequence homology in the selection of ends to be ligated and NHEJ is thus potentially error prone and mutagenic [71]. A key step in NHEJ is the rapid recruitment of Ku70/80 proteins to the severed DNA ends, which function to hold the broken fragments together, limiting the mutagenic potential of this mechanism (**Figure 2**) [72, 73]. Ku70/80 recruits the DNA-PKcs to form the functional DNA-dependent protein kinase, which directs NHEJ. Small gaps in the broken DNA are filled by polymerase μ in to generate blunt ends, which are then ligated by DNA ligase IV in conjunction with XRCC4 [70, 74]. NHEJ is further stimulated by the K63-linked ubiquitination of XRCC4 by SCF^{Fbw7} [74]. The end-joining process is rapid and likely of relatively little genetic consequence [75]. Small deletions could readily occur [71]. If, however, the damage is extensive, processing of DNA ends in an ATM and MRN dependent manner is required, which may lead to larger deletions and in the case of multiple damage sites can produce mutagenic evens on the scale of chromosomal rearrangements [71].

In contrast to blunt-ended ligation of NHEJ, the use of the sister chromatid as a template for HR requires the formation of a synapse between the damaged DNA and the undamaged sister (**Figure 2**). Synapse formation requires resection of the DNA from at the site of the break to generate ssDNA. Resection is driven by stimulation of the nuclease activity of the MRN complex by CtIP [76–79]. The ability of CtIP to drive resection is controlled by the balance of BRCA1 and 53BP1 on the chromatin [79–86]. The presence of 53BP1 forms a barrier that limits the accessibility of chromatin to HR-driving nucleases (**Figure 3**). A major role of BRCA1 in HR is to antagonize the binding of 53BP1 to chromatin to al-low resection and repair. Indeed, loss of 53BP1 function in BRCA1 mutant cells improves resection and overall genomic stability (**Figure 2**) [81, 85]. BRCA1 recruitment to damaged chromatin is multifactorial and it is thought that BRCA1-Ctip-MRN complex accesses chromatin directly while BRCA1-PALB2-BRCA2 complexes promote the loading of Rad51 on the resected DNA [78, 79, 87]. Rad51 functions to coat the ssDNA and facilitates synapse formation with the template DNA. In contrast the BRCA1-A (BRCA1-MERIT40-BRCC36-BRCC45-ABRAXAS) complex is recruited

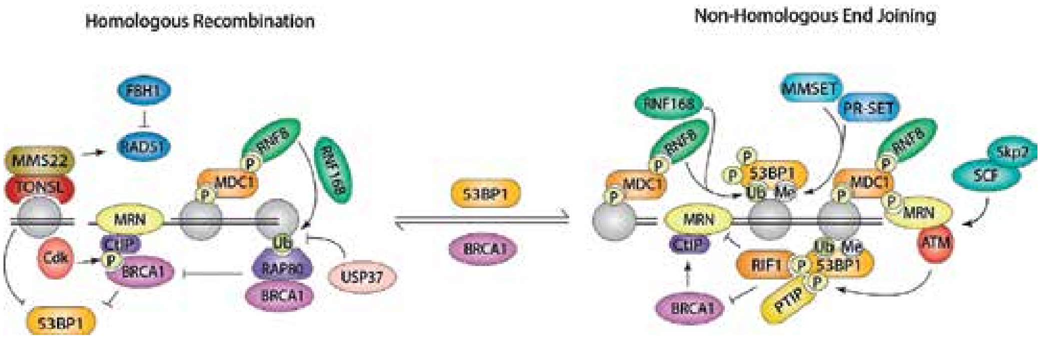

Figure 3. 53BP1 and BRCA1 determine the choice between DNA repair mechanisms. Homologous recombination, takes place in S- and G2 phases after DNA replication. Histones (gray circles) of newly replicated DNA lack methylation at H4K20, which weakens the interaction with 53BP1. Moreover, MMS22-TONSL binds the non-methylated H4, which may further antagonize 53BP1, and promotes RAD51 function, which is antagonized by FBH1. BRCA1 is then able to complex with CDK phosphorylated CtIP to drive end resection, preventing NHEJ and setting the stage for HR. BRCA1 further blocks 53BP1 binding, in part by competing for the histone H2K15-Ub marks when complexed with RAP80. This complex also limits resection and is antagonized by the deubiquitinase USP37. In G1 and G2 upon *de novo* H4K20 methylation, 53BP1 is recruited to chromatin to form a barrier to resection. ATM phosphorylation of 53BP1 recruits RIF1 and PTIP, which antagonize BRCA1 and promote recruitment of additional factors to promote repair by NHEJ.

to chromatin by the RNF168-mediated ubiquitination of histones, via the ubiquitin-binding RAP80 protein (**Figure 3**) [88–90]. Interestingly, formation of this complex limits the access of BRCA1 to the damaged DNA, suppressing resection [88].

The critical distinction between these two pathways is the dependency of HR on the resection of the broken DNA end to generate ssDNA to form the synapse with the template DNA. In addition, because NHEJ relies on the ligation of blunt DNA ends and once resection is initiated NHEJ cannot be used. Thus, regulation of resection is central to the choice between mechanisms of repair. Moreover, the inappropriate induction of resection can also give rise to the use of alt-NHEJ. Given that cells that are HR-deficient, (e.g., BRCA1 mutant cells) exhibit sensitivity to DSBs suggests that NHEJ is either too mutagenic or simply does not function efficiently during S-phase, when HR normally predominates. These two possibilities are not mutually exclusive. In addition to DSB repair, HR is also critical for the stabilization and restart of replication forks after prolonged replication stress. As discussed below, multiple layers do, in fact, limit the use of NHEJ during S-phase.

3.3. The replication stress checkpoint

Cells encounter a multitude of intrinsic and extrinsic barriers in attempting to achieve accurate DNA replication. To ensure that replication is error free, eukaryotes possess a conserved checkpoint that monitors replication progress. Upon replication stress (e.g., stalled replication fork, nucleotide deficiency, DNA damage), extensive regions of ssDNA are formed, which are coated by Replication Protein A (RPA) which mediates the recruitment of the apical kinase ATR to the DNA [91]. The Rad17 protein then promotes the loading of a protein complex including the ATR activator, TopBP1, and the checkpoint mediator, Claspin, that then recruits the effector kinase Chk1, which is ultimately phosphorylated by ATR at S317 and S345 that allow Chk1 to adopt an open, active conformation. In turn, Chk1 phosphorylates many proteins, including the Cdk-activating Cdc25 phosphatases, the CDK inhibitory kinase WEE1, and the key HR protein Rad51 [92]. Notably, phosphorylation of Cdc25A by Chk1 leads to $SCF^{\beta TrCP}$-mediated degradation. Chk1 ultimately controls origin firing and entry into mitosis as well as promoting replication fork restart and repair, which is predominantly dependent upon the HR machinery.

In the absence of Chk1 recruitment and activation, cells undergoing replication stress maintain high levels of Cdk activity and, continue to fire origins. Under these conditions, replication forks may be prone to stalling and will likely collapse to form DSBs leading to chromosomal abnormalities. These cells are thus highly sensitive to additional replication stress. Importantly, high levels of replication stress are associated with high rates of proliferation during early development and expression of multiple oncogenes (e.g., Cyclin E, c-Myc) [93–98]. Chk1 activity is essential for embryonic development and it follows that surviving the process of transformation requires Chk1 function to survive with abnormal levels of replication stress [99]. As a result, transformed cells are highly dependent on the ATR-Claspin-Chk1 pathway for survival and are sensitive to agents that either induce additional stress or inhibit this critical checkpoint [94, 97, 98]. Indeed, mice possessing an extra copy of Chk1 are more susceptible to oncogenic stimuli. Intriguingly, premature Chk1 activation may drive S-phase entry and failure to down-regulate Chk1 activation is also detrimental.

3.4. Crosstalk between the ATM-Chk2 and ATR-Chk1 axes

As described above, it would seem that the ATM-Chk2 and ATR-Chk1 pathways function in isolation, depending on cell cycle stage and type of insult. However, there is clear cross-talk between the two and, at least in some cell types, the G2 DDR is dramatically weakened, if not abrogated, in the absence of Chk1 function. Resection of damaged DNA ends upon initiation of the HR pro-cess yields ssDNA similar to replication stress, which is also coated by RPA and serves as a scaffold upon which to activate the ATR-Chk1 cascade.

4. CRLs and APC/C in DNA damage checkpoint responses

4.1. The G2 DNA damage checkpoint

Initial evidence that Cdh1 possesses a function in the DDR was obtained from chicken DT40 cells in which Cdh1 gene had been deleted [100]. Surprisingly, these Cdh1 knock-out cells were unable to maintain a G2 arrest in the presence of DNA damage. This result was unexpected as APC/C^{Cdh1} is largely thought to be inactive in S and G2 cells due to Cyclin A- and Cyclin E-Cdk-mediated phosphorylation of Cdh1, which both prevents its binding to the APC/C holoenzyme and, at least at the G1/S transition promotes the creation of a phosphodegron that is recognized by SCF$^{\beta TrCP}$ leading to Cdh1 degradation [40, 41, 52, 53, 101]. In addition, Emi1, which binds to the APC/C with high affinity and prevents ubiquitination of substrates, is maximally expressed from late G1 through early mitosis [44, 46, 47, 49–51, 102]. Moreover, key APC/C substrates, including Cyclin A, Cyclin B, and Skp2 remain stable during a G2 arrest [103]. Indeed, nearly all APC/C targets tested do remain stable upon DNA damage in G2, with the exception of Plk1 [103]. This is an important distinction as APC/C substrates can have dramatically different impacts on the checkpoint. Plk1 dampens the checkpoint by phosphorylating 53BP1 and Chk2 to inhibit ATM signalling (**Figure 4**) [104]. In addition, Plk1 catalyzes the SCF$^{\beta TrCP}$-mediated inhibition of ATR-Chk1 signalling (**Figure 4**) [105–109]. Down-regulation of Plk1 protein levels upon DNA damage was demonstrated to be the result of APC/C^{Cdh1} activation (**Figure 4**) [103]. A critical question stemming from these studies is how APC/C^{Cdh1} targets only Plk1 under these conditions.

The studies in both chicken and human cells indicate that active APC/C^{Cdh1} complexes form upon DNA damage in G2 [100, 103]. Previous analyses had suggested the existence of an Emi1-free pool of the APC/C during interphase [44]. Consistent with this idea, an increased APC/C-Cdh1 association was detected upon damage whereas changes in the abundance of either Emi1 protein or in amount of Emi1-bound APC/C were not observed upon DNA damage [103]. Given that Cdk activity is diminished upon DNA damage (**Figure** 4), these data suggest that a pool of APC/C exists that is independent of Emi1 and regulated largely by inhibitory phosphorylation of Cdh1. The failure to phosphorylate Cdh1 may result in the dephosphorylation and activation of this pool of Cdh1 due to a shift in the balance of kinase phosphatase activities brought about by the inactivation of Cdks by the DDR. In addition, it has been shown that specific release of the Cdc14B phosphatase from the nucleolus upon DNA damage contributes to the dephosphorylation of Cdh1, promoting APC/C^{Cdh1} formation [103].

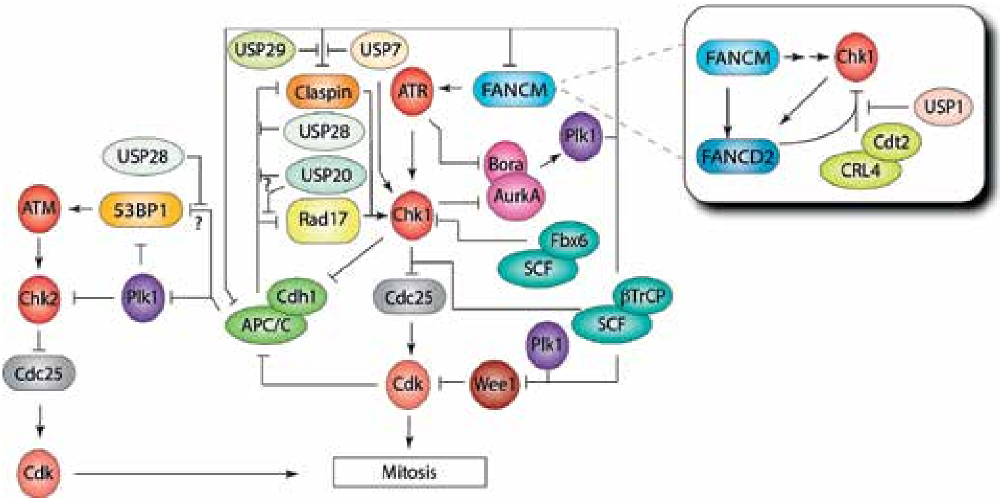

Figure 4. Interplay between Plk1 kinase, ubiquitin machinery and DNA damage checkpoint activity. The circuitry in the main figure depicts the crosstalk between APC/C, SCF ligases and the checkpoint. Plk1 is a critical factor in checkpoint recovery, silencing both the ATM and ATR cascades. Plk1 phosphorylates and inhibit both 53BP1 and Plk1 to allow Cdk1 activity. Similarly, Plk1 triggers the SCF$^{\beta TrCP}$-dependent destruction of Claspin and FANCM to silence the ATR-Chk1 axis. Plk1 activity may also contribute to the silencing of APC/C^{Cdh1} activity upon replication stress and during recovery from APC/C^{Cdh1} activation during the G2 DDR. In G2 APC/C^{Cdh1} targets Plk1 for degradation, while USP28 prevents it from targeting Claspin. USP28 also stabilizes 53BP1 after DNA damage as well, possibly from APC/C^{Cdh1} (represented by, "?"). Chk1 activation requires Claspin function, which is protected from SCF$^{\beta TrCP}$-mediated degradation by USP29 and USP7. USP20 stabilizes both Claspin and Rad17 to promote Chk1 activity, possibly from APC/C^{Cdh1} ("?") as they are both substrates of the ligases. ATR and Chk1 prevent checkpoint recovery by inhibiting the Plk1 activators Aurora and Bora. Irreversible checkpoint activation is prevented by the degradation of active Chk1 by SCF^{Fbx6}. USP7 prevents the complete destabilization of Chk1. The inset shows a potential feedback loop between ATR-Chk1 and the Fanconi pathway. FANCM promotes Chk1 activation (indirectly via ATR). Chk1 promotes FANCM-promoted FANCD2 monoubiquitination. In turn, FANCD2-Ub promotes the CRL4^{Cdt2}-mediated degradation of Chk1. USP1 deubiquitinates FANCD2, stabilizing Chk1. The negative feedback loop favors silencing of Chk1 due to the inactivation of USP1 upon DNA damage.

However, whether this Cdc14B contributes to the G2 checkpoint activity or DNA repair functions of APC/C^{Cdh1} is unclear and could be influenced by cell type [110–112].

Activation of this pool of APC/C^{Cdh1} may not be sufficient to target the majority of APC/C substrates. As dephosphorylated Cdh1 localizes to the nucleus, substrates such as Cyclin B, which are localized in the cytoplasm in G2 would be likely to remain safe from this pool of APC/C^{Cdh1} [113]. How other nuclear APC/C substrates (e.g., Cyclin A) remain stable is an open question. One potential mechanism by which APC/C substrates may evade degradation is via the antagonistic activity of DUBs. Indeed, there is evidence that USP28 activity prevents APC/C^{Cdh1}-mediated degradation of Claspin after DNA damage (**Figure** 4) [103, 114]. Given the apparently small size of the APC/C pool activated by DNA damage, it is likely that selective targeting of APC/C substrates may also be achieved by specific localization of DNA damage-activated APC/C^{Cdh1}, for example to sites of DNA damage where proteins such as Claspin and Plk1 are expected be found. This idea remains to be tested, but it is worth noting that APC/C^{Cdh1} is found on chromatin in S-phase and APC/C^{Cdh1}- mediated regulation of Cdc7-Dbf4 activity appears to be via specific targeting of the chromatin-bound fraction of the kinase [115]. Thus it is possible that the apparent substrate-specificity may be due to limited access to substrates in conjunction with antagonism by DUBs. In keeping with this idea, it is worth noting that Plk1 levels are diminished, but not abolished by damage-activated APC/C^{Cdh1}, perhaps reflecting degradation of a pool of Plk1 in the vicinity of the sites of damage, where phosphorylation of key substrates such as Claspin and 53BP1 will eventually be phosphorylated to promote checkpoint recovery [103, 104]. In addition, 53BP1 has recently been identified as an APC/C substrate and, intriguingly, was also identified as an USP28 substrate raising the possibility that it too may be protected from APC/C at sites of DNA damage, but idea has not been tested (**Figure** 4) [114, 116].

4.2. The replication stress checkpoint

Whereas it is clearly established that APC/C^{Cdh1}controls entry into S-phase, multiple recent lines of evidence suggest that the E3 is also a key regulator of the replication stress response as well. However, in contrast to its role as a positive regulator of the G2 DNA damage checkpoint, APC/C^{Cdh1} appears to be a negative regulator of the replication stress checkpoint, as it targets two critical regulators of the checkpoint, Rad17 and Claspin, which are central to the activation of Chk1 (**Figure 4**) [103, 117, 118]. Indeed, in the absence of Cdh1, failure to degrade Claspin leads to unscheduled Chk1 activation, which is associated with premature S-phase entry [117]. Given the importance of these proteins for the stress response, both UV-irradiation and induction of replication stress by treatment with hydroxyurea lead to the degradation of Cdh1 and, at least in the case of UV, to the stabilization of Rad17 [115, 118]. Activation of Chk1 then feeds back to further enhance its own activation by triggering Cdh1 destruction [115]. Claspin stability is also dependent on Chk1 activity, suggesting that down-regulation of Cdh1 contributes to this arm of a Chk1 auto-amplification loop as well [119]. Notably, Claspin stability is also dependent on context dependent DUB activity as well. ATR activation leads to the stabilization of USP20, which promotes Claspin stability during S-phase [120, 121]. USP20 has also been demonstrated to stabilize Rad17, suggesting perhaps that this DUB may antagonize APC/C^{Cdh1}-mediated destruction of these proteins to promote ATR-Chk1 function (**Figure 4**) [122, 123]. USP9x has also been identified as a DUB for Claspin during replication stress and USP7 has been found to counteract the degradation of Claspin by $SCF^{\beta TrCP}$, but not APC/C^{Cdh1}, during replication stress as well [123, 124]. A similar role has been demonstrated for USP29 (**Figure 4**).

The mechanism for Chk1-mediated degradation of Cdh1 is not well-defined, but in the case of HU-induced stress, APC/C-mediated destruction has been implicated [115]. However, degradation induced by UV exposure, which would presumably be augmented by Chk1 as well, involves a region of Cdh1, which is not known to mediate interactions with the APC/C, but does lie between two regions of the Cdh1 N-terminal domain (NTD) that make critical contacts with the APC/C and are negatively regulated by phosphorylation [125, 126]. Thus, Chk1 may directly or indirectly alter the association of Cdh1 with the APC/C to promote its degradation. In addition, the region containing the UV-responsive degron in Cdh1 also encompasses the $SCF^{\beta TrCP}$ phosphodegron [53, 115, 125]. Notably, while phosphorylation by Plk1 has been identified as critical for creating this phosphodegron there are additional phosphorylation events, mediated by unknown kinases, which contribute to recognition by

SCF$^{\beta TrCP}$ [53]. It is tempting to speculate that Chk1 directly or indirectly promotes the SCF$^{\beta TrCP}$-mediated destruction of Cdh1 as well.

Chk1 itself is also targeted for destruction. Upon activation, Chk1 adopts an open conformation, which exposes degrons that are recognized by SCF^{Fbw6} and CRL4^{Cdt2} ubiquitin ligases (**Figure 4**) [127–129]. DUB activity also plays a role in the maintenance of Chk1 levels. Surprisingly, there are few examples of Chk1 stabilization by DUBs in comparison to their involvement stabilizing Claspin to promote Chk1 activation. To date, only USP7 and USP1 have been implicated in the maintenance of active Chk1 levels. USP7 directly deubiquitinates Chk1 and this activity is enhanced by ATM activation [124, 130, 131]. Whether ATR may also promote USP7-mediated Chk1 activity is not clear. Active Chk1 levels are indirectly protected by USP1 via its ability to antagonize the ubiquitination FANCD2, which induces CRL4-mediated degradation of Chk1 (**Figure 4**) [132]. USP1 is also an APC/C^{Cdh1} substrate adding another level of complexity to the Chk1-Cdh1 feedback loop [133].

The relationship between Chk1 and USP1 also begins to lend some insight into how the feedback loop is faulted to allow checkpoint recovery (**Figure 4**). First, the ATR-Chk1 axis promotes FANCD2 ubiquitination, which would begin to induce down-regulation of active Chk1 [132, 134–136]. Second, USP1 activity is inhibited by multiple mechanisms after UV-damage or the induction of replication stress [137–140]. Thus as the damage or stress-inducing events are resolved and ATR signalling is diminished, active Chk1 becomes susceptible to degradation, which would allow the accumulation of Cdh1 protein. Stabilization of Cdh1 leads to degradation of Rad17 to further inhibit activation of additional Chk1 [118]. Diminished activity of ATR and Chk1 promotes the stabilization of Bora and allows Aurora A activity, respectively, which are critical for Plk1 activation **(Figure 4**) [108, 134, 141]. Plk1, in turn, phosphorylates FANCM and Claspin to promote their SCF$^{\beta TrCP}$-mediated degradation to further silence ATR and Chk1 activity, respectively, and further promote loss of Chk1 activity (**Figure 4**) [109, 142]. A key remaining question is how APC/C activity is then restrained to allow normal cell cycle progression. The increase in Plk1 activity also triggers SCF$^{\beta TrCP}$-mediated degradation of Wee1, preventing the inhibitory phosphorylation of Cdks [143, 144]. A straightforward mechanistic model is that increased Cdk activity following stabilization of Cdc25A levels and loss of Wee1 promote increased Cdk-mediated inhibitory phosphorylation of Cdh1 to return to normal levels of APC/C^{Cdh1} activity. It is currently unclear, however, why Cdh1 is able reaccumulate during checkpoint recovery despite rising activity of the SCF$^{\beta TrCP}$-targeting kinase, Plk1.

5. CRLs and APC/C influence the selection of DSB repair mechanism

Given their many roles in the regulation and execution of checkpoints that monitor the integrity of DNA, it is not surprising that the CRL and APC/C ligases also have roles in regulating DNA repair pathways. SCF^{Fbw7} has been demonstrated to promote NHEJ by catalyzing K63-linked ubiquitination of XRCC4 to enhance its interaction with Ku70/80 complex [74]. However, for the most part the concerted efforts of these ligases do not appear to exert a predominant effect on the decision between NHEJ and HR repair pathways at the moment of damage. Rather their activities appear to promote proper and efficient use of NHEJ and HR.

A critical distinction between these two pathways is the dependency of HR on the resection of the broken DNA end to generate ssDNA that forms a synapse with the template DNA.

APC/C^{Cdh1} activity is required for faithful repair, possibly independent of its checkpoint role. Indeed, APC/C^{Cdh1} regulates multiple components of these pathways. Recently, it was shown that CtIP levels are regulated by APC/C^{Cdh1} upon mitotic exit and after DNA damage, thus limiting the potential for attempting HR in G1, which would likely be mutagenic, and limiting the potential frequency of HR upon damage in G2 (**Figure 5**) [145]. Interestingly, failure to

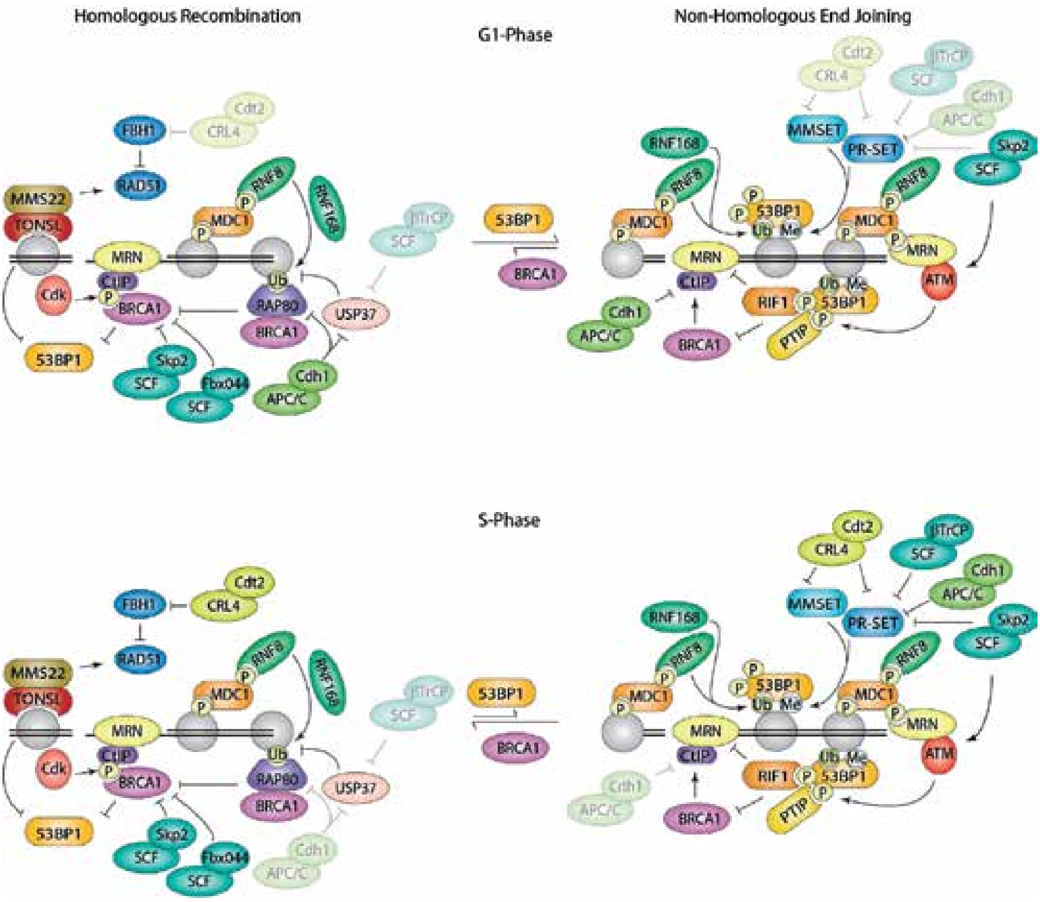

Figure 5. Cell cycle ligases set the stage for the selection of NHEJ and HR in G1 and S phases, respectively. During G1 (upper panel) APC/C^{Cdh1} mediates the degradation of pro-HR factors, CtIP, USP37 and RAP80, whereas pro-NHEJ factors MMSET and PR-SET remain stable. Together these factors promote the recruitment of 53BP1 to chromatin favoring NHEJ. The anti-HR factor FBH1 also remains stable. During S-phase (lower panel) replication coupled, CRL4^{Cdt2}-mediated degradation of FBH1 favors HR. Similarly, CRL4^{Cdt2} targets the methyltransferase MMSET2 and PR-SET7 for destruction. PR-SET7 is also targeted for destruction by SCF^{Skp2} and SCF$^{\beta TrCP}$. This destruction hinders the recruitment of 53BP1 to chromatin favoring HR. Finally, Cdk activity, positively regulated by SCF^{Skp2} and the downregulation of APC/C^{Cdh1}activity in S-phase further promotes the use of HR. The activities of SCF^{Skp2} and SCF^{Fbxo44} limit BRCA1-CtIP–mediated resection are depicted in both phases, although the cell cycle-dependence of these events is not clear. Similarly, the potential for APC/C^{Cdh1}–mediated regulation of PR-SET7 is depicted, but remains unclear.

down-regulate CtIP levels by APC/C^{Cdh1} leads to increased resection and inefficient repair, potentially due to interference with the use of NHEJ as well [145]. Although it remains to be tested, it would stand to reason that degradation of Cdh1 upon replication stress may also lead to enhanced stabilization of CtIP to promote HR. In contrast to limiting resection by targeting CtIP, APC/C^{Cdh1} also targets the HR-limiting factor RAP80, which localizes BRCA1 to regions flanking DSB in an ubiquitin-dependent manner, but represses BRCA1-mediated HR [88, 146]. APC/C-mediated destruction limits RAP80 expression during G1, presumably to diminish competition for H2AK15-Ub binding with 53BP1 at DSBs to promote the use of NHEJ in the absence of a homologous template (**Figure 5**). During S and G2, BRCA1-dependent HR is thought to involve the degradation of RAP80 and, although the activation of APC/C^{Cdh1} by DSBs in G2 suggests that it may be, it remains to be determined whether the APC/C^{Cdh1} is involved in this destruction event. In addition, APC/C^{Cdh1} and SCF$^{\beta TrCP}$ cooperate to limit the expression of USP37 to S-phase and early G2 (**Figure 5**). USP37, along with the related USP26, has been shown to antagonize RAP80 to promote BRCA1-dependent HR [147–149]. SCF^{Skp2} is also required for efficient HR, in part via promotion of checkpoint signaling [54]. In addition, SCF^{Skp2} and SCF^{Fbxo44} ubiquitinate BRCA1 to control the extent of resection (**Figure 5**) [150]. The balance of CRL3^{Keap} and USP11 activities also regulates HR by targeting PALB2 (**Figure 2**) [151]. CRL4^{Cdt2} catalyzes the degradation of FBH1, which negatively regulates Rad51 function to limit HR prior to replication-dependent generation of the template. Interestingly, the interaction of FBH1 with PCNA may promote the use of TLS [152–156]. The APC/C may also contribute to the use of HR by antagonizing the expression of the NHEJ-promoting protein 53BP1 [116]. However, it is not clear whether APC/C impacts NHEJ activation via regulation 53BP1. Interestingly, a proteomic screen identified several additional pro-NHEJ factors in association with APC/C^{Cdh1} [145]. Yet, it remains to be determined whether these are substrates of the ligase.

In addition to restricting the use of HR to S-phase and G2 by regulating the levels of key HR factors to these phases, the coordinated efforts of APC/C and CRL ligases also limit the use of NHEJ during S-phase. The methyltransferases PR-SET7 and MMSET promote NHEJ by directing recruitment methylating H4K20 to recruit 53BP1 (**Figure 5**) [62–65]. Whereas global H4K20 methylation is not significantly altered by the induction of DSBs, de novo methylation of H4K20 at sites of damage has been demonstrated to mediate recruit 53BP1 and promote NHEJ. Importantly, histones deposited during replication lack H4K20 methylation. Multiple ligases, APC/C^{Cdh1}, SCF$^{\beta TrCP}$, SCF^{Skp2}, and CRL4^{Cdt2} restrict expression and activity of the methyltransferase PR-Set7 to G2, mitosis, and early G1 (**Figure 5**) [66, 157–161]. In addition, CRL4^{Cdt2} targets MMSET for replication-coupled degradation (**Figure 5**) [162]. Thus, with little capacity to generate NHEJ promoting methylation marks, DSBs occurring in S-phase, and likely early G2 as well, are not permissive for the recruitment of NHEJ factors allowing relatively uncontested access to the damaged sites by the HR machinery. In addition, the deposition of histones lacking H4K20 methylation marks in newly replicated DNA recruits MMS22L-TONSL complex, which directly promotes HR (**Figure 5**) [163–166]. Finally, the window of kinase activities, (cyclin-Cdk activity in particular) opened to promote the transition into and through S-phase also catalyze the phosphorylation of multiple components of the HR machinery, which promote the activity of this pathway [77, 78, 167, 168].

6. Conclusion

For many years, the importance of the CRL and APC/C ligases in cancer and genome stability has been appreciated. It was long thought that these roles were attributed to their ability to control cell cycle transitions, particularly their abilities to regulate one another. As discussed herein, we have more recently begun to elucidate that these ligases possess more direct, highly regulated and interconnected roles in the response to and repair of DNA damage as well.

While alterations in the mechanisms controlling genome stability lead to disease such as cancer, the induction of DNA damage is a tested and potent anti-cancer strategy. Moreover, manipulating these pathways has obvious therapeutic potential. Indeed, recent advances in inhibitors of DNA checkpoint and repair proteins (e.g., Chk1) suggest that manipulating the DDR response offers a therapeutic advantage over DNA damage based therapies alone. However, these strategies have faced challenges in translation. As we move ever closer to the realization of personalized medicine, it is of increasing importance that we understand not only the full cadre of players in a given pathway, but also those regulating it as well. Only with this knowledge can we fully appreciate the impact of altering that pathway, whether in dissecting pathophysiological changes of disease or in the development of potential therapeutic manipulations. We are increasingly successful in targeting components of the ubiquitin proteasome system and there are now small molecules capable of inhibiting specific SCF complexes with potential for substrate specificity. Similar accomplishments have been made in the targeting of the APC/C as well as DUBs, including USP1 and USP7. Finally, while we have focused on the role of these ligases in the major responses to DNA damage and the impact they have on DSB repair, there is mounting evidence that the activities of these enzymes impact multiple damage response and repair pathways. Thus, as we increase our understanding of the how these components of the ubiquitin machinery impact the choice and efficient use of DNA repair mechanisms we also increase our opportunities for improved therapeutic options.

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References

[1] Lydeard JR, Schulman BA, Harper JW. Building and remodelling Cullin-RING E3 ubiquitin ligases. EMBO Reports. 2013;**14**:1050-1061

[2] Zheng N, Zhou Q, Wang Z, Wei W. Recent advances in SCF ubiquitin ligase complex: Clinical implications. Biochimica et Biophysica Acta. 2016;**1866**:12-22

[3] Kitagawa K, Kitagawa M. The SCF-type E3 ubiquitin ligases as cancer targets. Current Cancer Drug Targets. 2016;**16**:119-129

[4] Kitagawa K, Kotake Y, Kitagawa M. Ubiquitin-mediated control of oncogene and tumor suppressor gene products. Cancer Science. 2009;**100**:1374-1381

[5] Nakayama KI, Nakayama K. Regulation of the cell cycle by SCF-type ubiquitin ligases. Seminars in Cell & Developmental Biology. 2005;**16**:323-333

[6] Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009;**458**:732-736

[7] Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: Tipping the scales of cancer. Nature Reviews. Cancer. 2008;**8**:438-449

[8] Jin J, Arias EE, Chen J, Harper JW, Walter JC. A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Molecular Cell. 2006;**23**:709-721

[9] Angers S, et al. Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature. 2006;**443**:590-593

[10] Hannah J, Zhou P. Regulation of DNA damage response pathways by the cullin-RING ubiquitin ligases. DNA Repair (Amst). 2009;**8**:536-543

[11] Mailand N, Gibbs-Seymour I, Bekker-Jensen S. Regulation of PCNA-protein interactions for genome stability. Nature Reviews. Molecular Cell Biology. 2013;**14**:269-282

[12] Mansilla SF, et al. UV-triggered p21 degradation facilitates damaged-DNA replication and preserves genomic stability. Nucleic Acids Research. 2013;**41**:6942-6951

[13] Havens CG, Walter JC. Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. Molecular Cell. 2009;**35**:93-104

[14] Soria G, Speroni J, Podhajcer OL, Prives C, Gottifredi V. P21 differentially regulates DNA replication and DNA-repair-associated processes after UV irradiation. Journal of Cell Science. 2008;**121**:3271-3282

[15] Soria G, Podhajcer O, Prives C, Gottifredi V. P21Cip1/WAF1 downregulation is required for efficient PCNA ubiquitination after UV irradiation. Oncogene. 2006;**25**:2829-2838

[16] Gottifredi V, McKinney K, Poyurovsky MV, Prives C. Decreased p21 levels are required for efficient restart of DNA synthesis after S phase block. The Journal of Biological Chemistry. 2004;**279**:5802-5810

[17] Jones MJ, Colnaghi L, Huang TT. Dysregulation of DNA polymerase kappa recruitment to replication forks results in genomic instability. The EMBO Journal. 2012;**31**:908-918

[18] Havens CG, Walter JC. Mechanism of CRL4(Cdt2), a PCNA-dependent E3 ubiquitin ligase. Genes & Development. 2011;**25**:1568-1582

[19] Bruning JB, Shamoo Y. Structural and thermodynamic analysis of human PCNA with peptides derived from DNA polymerase-delta p66 subunit and flap endonuclease-1. Structure 2004;**12**:2209-2219

[20] Pesin JA, Orr-Weaver TL. Regulation of APC/C activators in mitosis and meiosis. Annual Review of Cell and Developmental Biology. 2008;**24**:475-499

[21] Manchado E, Eguren M, Malumbres M. The anaphase-promoting complex/cyclosome (APC/C): Cell-cycle-dependent and -independent functions. Biochemical Society Transactions. 2010;**38**:65-71

[22] Peters JM. The anaphase promoting complex/cyclosome: A machine designed to destroy. Nature Reviews. Molecular Cell Biology. 2006;**7**:644-656

[23] Wasch R, Robbins JA, Cross FR. The emerging role of APC/CCdh1 in controlling differentiation, genomic stability and tumor suppression. Oncogene. 2010;**29**:1-10

[24] Simpson-Lavy KJ, et al. Fifteen years of APC/cyclosome: A short and impressive biography. Biochemical Society Transactions. 2010;**38**:78-82

[25] Bassermann F, Pagano M. Dissecting the role of ubiquitylation in the DNA damage response checkpoint in G2. Cell Death and Differentiation. 2010;**17**:78-85

[26] Skaar JR, Pagano M. Control of cell growth by the SCF and APC/C ubiquitin ligases. Current Opinion in Cell Biology. 2009;**21**:816-824

[27] Li M, Zhang P. The function of APC/CCdh1 in cell cycle and beyond. Cell Division. 2009;**4**:2

[28] van Leuken R, Clijsters L, Wolthuis R. To cell cycle, swing the APC/C. Biochimica et Biophysica Acta. 2008;**1786**:49-59

[29] Lindon C. Control of mitotic exit and cytokinesis by the APC/C. Biochemical Society Transactions. 2008;**36**:405-410

[30] Sullivan M, Morgan DO. Finishing mitosis, one step at a time. Nature Reviews. Molecular Cell Biology. 2007;**8**:894-903

[31] Kim AH, Bonni A. Thinking within the D box: Initial identification of Cdh1-APC substrates in the nervous system. Molecular and Cellular Neurosciences. 2007;**34**:281-287

[32] Pfleger CM, Kirschner MW. The KEN box: An APC recognition signal distinct from the D box targeted by Cdh1. Genes & Development. 2000;**14**:655-665

[33] Glotzer M, Murray AW, Kirschner MW. Cyclin is degraded by the ubiquitin pathway. Nature. 1991;**349**:132-138

[34] Garcia-Higuera T, et al. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. Nature Cell Biology. 2008;**10**:802-811

[35] Engelbert D, Schnerch D, Baumgarten A, Wasch R. The ubiquitin ligase APC(Cdh1) is required to maintain genome integrity in primary human cells. Oncogene. 2008;**27**:907-917

[36] Lehman NL, et al. Oncogenic regulators and substrates of the anaphase promoting complex/cyclosome are frequently overexpressed in malignant tumors. The American Journal of Pathology. 2007;**170**:1793-1805

[37] Wei W, et al. Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature. 2004;**428**:194-198

[38] Bashir T, Dorrello NV, Amador V, Guardavaccaro D, Pagano M. Control of the SCF(Skp2-Cks1) ubiquitin ligase by the APC/C(Cdh1) ubiquitin ligase. Nature. 2004;**428**:190-193

[39] Cappell SD, Chung M, Jaimovich A, Spencer SL, Meyer T. Irreversible APC(Cdh1) inactivation underlies the point of no return for cell-cycle entry. Cell. 2016;**166**:167-180

[40] Sorensen CS, et al. A conserved cyclin-binding domain determines functional interplay between anaphase-promoting complex-Cdh1 and cyclin A-Cdk2 during cell cycle progression. Molecular and Cellular Biology. 2001;**21**:3692-3703

[41] Sorensen CS, et al. Nonperiodic activity of the human anaphase-promoting complex-Cdh1 ubiquitin ligase results in continuous DNA synthesis uncoupled from mitosis. Molecular and Cellular Biology. 2000;**20**:7613-7623

[42] Lukas C, et al. Accumulation of cyclin B1 requires E2F and cyclin-A-dependent rearrangement of the anaphase-promoting complex. Nature. 1999;**401**:815-818

[43] Verschuren EW, Ban KH, Masek MA, Lehman NL, Jackson PK. Loss of emi1-dependent anaphase-promoting complex/cyclosome inhibition deregulates E2F target expression and elicits DNA damage-induced senescence. Molecular and Cellular Biology. 2007;**27**: 7955-7965

[44] Miller JJ, et al. Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. Genes & Development. 2006;**20**:2410-2420

[45] Eldridge AG, et al. The evi5 oncogene regulates cyclin accumulation by stabilizing the anaphase-promoting complex inhibitor emi1. Cell. 2006;**124**:367-380

[46] Hsu JY, Reimann JD, Sorensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). Nature Cell Biology. 2002;**4**: 358-366

[47] Reimann JD, et al. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. Cell. 2001;**105**:645-655

[48] Moshe Y, Boulaire J, Pagano M, Hershko A. Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome.

Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:7937-7942

[49] Hansen DV, Loktev AV, Ban KH, Jackson PK. Plk1 regulates activation of the anaphase promoting complex by phosphorylating and triggering SCFbetaTrCP-dependent destruction of the APC inhibitor Emi1. Molecular Biology of the Cell. 2004;**15**:5623-5634

[50] Margottin-Goguet F, et al. Prophase destruction of Emi1 by the SCF(betaTrCP/Slimb) ubiquitin ligase activates the anaphase promoting complex to allow progression beyond prometaphase. Developmental Cell. 2003;**4**:813-826

[51] Guardavaccaro D, et al. Control of meiotic and mitotic progression by the F box protein beta-Trcp1 in vivo. Developmental Cell. 2003;**4**:799-812

[52] Lau AW, et al. Regulation of APC(Cdh1) E3 ligase activity by the Fbw7/cyclin E signaling axis contributes to the tumor suppressor function of Fbw7. Cell Research. 2013;**23**:947-961

[53] Fukushima H, et al. SCF-mediated Cdh1 degradation defines a negative feedback system that coordinates cell-cycle progression. Cell Reports. 2013;**4**:803-816

[54] Wu J, et al. Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. Molecular Cell. 2012;**46**:351-361

[55] Mailand N, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**:887-900

[56] Kolas NK, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science. 2007;**318**:1637-1640

[57] Huen MS, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell. 2007;**131**:901-914

[58] Mochan TA, Venere M, DiTullio RA Jr, Halazonetis TD, 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. Cancer Research. 2003;**63**:8586-8591

[59] Mattiroli F, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell. 2012;**150**:1182-1195

[60] Stewart GS, et al. The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. Cell. 2009;**136**:420-434

[61] Doil C, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446

[62] Pei H, et al. The histone methyltransferase MMSET regulates class switch recombination. Journal of Immunology. 2013;**190**:756-763

[63] Pei H, et al. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature. 2011;**470**:124-128

[64] Tuzon CT, et al. Concerted activities of distinct H4K20 methyltransferases at DNA double-strand breaks regulate 53BP1 nucleation and NHEJ-directed repair. Cell Reports. 2014;**8**:430-438

[65] Dulev S, Tkach J, Lin S, Batada NN. SET8 methyltransferase activity during the DNA double-strand break response is required for recruitment of 53BP1. EMBO Reports. 2014;**15**:1163-1174

[66] Oda H. et al. Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. Molecular Cell. 2010;**40**:364-376

[67] Wilson MD, et al. The structural basis of modified nucleosome recognition by 53BP1. Nature. 2016;**536**:100-103

[68] Fradet-Turcotte A, et al. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature. 2013;**499**:50-54

[69] Karanam K, Kafri R, Loewer A, Lahav G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Molecular Cell. 2012;**47**:320-329

[70] Deriano L, Roth DB. Modernizing the nonhomologous end-joining repertoire: Alternative and classical NHEJ share the stage. Annual Review of Genetics. 2013;**47**:433-455

[71] Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends in Cell Biology. 2016;**26**:52-64

[72] Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. Genes & Development. 2001;**15**:3237-3242

[73] Mari PO, et al. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:18597-18602

[74] Zhang Q, et al. FBXW7 facilitates nonhomologous end-joining via K63-linked polyubiquitylation of XRCC4. Molecular Cell. 2016;**61**:419-433

[75] Betermier M, Bertrand P, Lopez BS. Is non-homologous end-joining really an inherently error-prone process? PLoS Genetics. 2014;**10**:e1004086

[76] Sartori AA, et al. Human CtIP promotes DNA end resection. Nature. 2007;**450**:509-514

[77] Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. The Journal of Biological Chemistry. 2009;**284**:9558-9565

[78] Wang H, et al. The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. PLoS Genetics. 2013;**9**:e1003277

[79] Escribano-Diaz C, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Molecular Cell. 2013;**49**:872-883

[80] Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. Science. 2013;**339**:700-704

[81] Li M, et al. 53BP1 ablation rescues genomic instability in mice expressing 'RING-less' BRCA1. EMBO Reports. 2016;**17**:1532-1541

[82] Feng L, et al. Cell cycle-dependent inhibition of 53BP1 signaling by BRCA1. Cell Discovery. 2015;**1**:15019

[83] Feng L, Fong KW, Wang J, Wang W, Chen J. RIF1 counteracts BRCA1-mediated end resection during DNA repair. The Journal of Biological Chemistry. 2013;**288**:11135-11143

[84] Chapman JR, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Molecular Cell. 2013;**49**:858-871

[85] Bunting SF, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;**141**:243-254

[86] Bouwman P, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nature Structural & Molecular Biology. 2010;**17**:688-695

[87] Park JY, Zhang F, Andreassen PR. PALB2: The hub of a network of tumor suppressors involved in DNA damage responses. Biochimica et Biophysica Acta. 2014;**1846**:263-275

[88] Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. The Journal of Biological Chemistry. 2011;**286**:13669-13680

[89] Shao G, et al. The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**: 3166-3171

[90] Sobhian B, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science. 2007;**316**:1198-1202

[91] Nam EA, Cortez D. ATR signalling: More than meeting at the fork. The Biochemical Journal. 2011;**436**:527-536

[92] Zhang Y, Hunter T. Roles of Chk1 in cell biology and cancer therapy. International Journal of Cancer. 2014;**134**:1013-1023

[93] Bester AC, et al., Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell. 2011;**145**:435-446

[94] Hoglund A, et al., Therapeutic implications for the induced levels of Chk1 in Myc-expressing cancer cells. Clin Cancer Res. 2011;**17**:7067-7079

[95] Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science. 2008;**319**:1352-1355

[96] Bartkova J. et al., Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature. 2006;**444**:633-637

[97] Murga M. et al., Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. Nat Struct Mol Biol. 2011;**18**:1331-1335

[98] Schoppy DW. et al., Oncogenic stress sensitizes murine cancers to hypomorphic suppression of ATR. J Clin Invest. 2012;**122**:241-252

[99] Zachos G, Rainey MD, Gillespie DA, Chk1-dependent S-M checkpoint delay in vertebrate cells is linked to maintenance of viable replication structures. Mol Cell Biol. 2005;**25**:563-574

[100] Sudo T, et al. Activation of Cdh1-dependent APC is required for G1 cell cycle arrest and DNA damage-induced G2 checkpoint in vertebrate cells. The EMBO Journal. 2001;**20**: 6499-6508

[101] Keck JM, et al. Cyclin E overexpression impairs progression through mitosis by inhibiting APC(Cdh1). The Journal of Cell Biology. 2007;**178**:371-385

[102] Machida YJ, Dutta A. The APC/C inhibitor, Emi1, is essential for prevention of rereplication. Genes & Development. 2007;**21**;184-194

[103] Bassermann F, et al. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell. 2008;**134**:256-267

[104] van Vugt MA, et al. A mitotic phosphorylation feedback network connects Cdk1, Plk1, 53BP1, and Chk2 to inactivate the G(2)/M DNA damage checkpoint. PLoS Biology. 2010;**8**:e1000287

[105] Peschiaroli A, et al. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. Molecular Cell. 2006;**23**:319-329

[106] Mamely I, et al. Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. Current Biology. 2006;**16**:1950-1955

[107] Mailand N, Bekker-Jensen S, Bartek J, Lukas J. Destruction of Claspin by SCFbetaTrCP restrains Chk1 activation and facilitates recovery from genotoxic stress. Molecular Cell. 2006;**23**:307-318

[108] Qin B, Gao B, Yu J, Yuan J, Lou Z. Ataxia telangiectasia-mutated- and Rad3-related protein regulates the DNA damage-induced G2/M checkpoint through the Aurora A cofactor Bora protein. The Journal of Biological Chemistry. 2013;**288**:16139-16144

[109] Schwab RA, Blackford AN, Niedzwiedz W. ATR activation and replication fork restart are defective in FANCM-deficient cells. The EMBO Journal. 2010;**29**:806-818

[110] Lin H, et al. Cdc14A and Cdc14B redundantly regulate DNA double-strand break repair. Molecular and Cellular Biology. 2015;**35**:3657-3668

[111] Wei Z, et al. Early-onset aging and defective DNA damage response in Cdc14b-deficient mice. Molecular and Cellular Biology. 2011;**31**:1470-1477

[112] Mocciaro A, et al. Vertebrate cells genetically deficient for Cdc14A or Cdc14B retain DNA damage checkpoint proficiency but are impaired in DNA repair. The Journal of Cell Biology. 2010;**189**:631-639

[113] Zhou Y, Ching YP, Chun AC, Jin DY. Nuclear localization of the cell cycle regulator CDH1 and its regulation by phosphorylation. The Journal of Biological Chemistry. 2003; **278**:12530-12536

[114] Zhang D, Zaugg K, Mak TW, Elledge SJ. A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. Cell. 2006;**126**:529-542

[115] Yamada M, et al. ATR-Chk1-APC/CCdh1-dependent stabilization of Cdc7-ASK (Dbf4) kinase is required for DNA lesion bypass under replication stress. Genes & Development. 2013;**27**:2459-2472

[116] Kucharski TJ, Minshall PE, Moustafa-Kamal M, Turnell AS, Teodoro JG. Reciprocal regulation between 53BP1 and the anaphase-promoting complex/cyclosome is required for genomic stability during mitotic stress. Cell Reports. 2017;**18**:1982-1995

[117] Gao D, et al. Cdh1 regulates cell cycle through modulating the claspin/Chk1 and the Rb/ E2F1 pathways. Molecular Biology of the Cell. 2009;**20**:3305-3316

[118] Zhang L. et al. Proteolysis of Rad17 by Cdh1/APC regulates checkpoint termination and recovery from genotoxic stress. The EMBO Journal. 2010;**29**:1726-1737

[119] Chini CC, Wood J, Chen J. Chk1 is required to maintain claspin stability. Oncogene. 2006;**25**:4165-4171

[120] Zhu M, Zhao H, Liao J, Xu X. HERC2/USP20 coordinates CHK1 activation by modulating CLASPIN stability. Nucleic Acids Research. 2014;**42**:13074-13081

[121] Yuan J. et al. HERC2-USP20 axis regulates DNA damage checkpoint through Claspin. Nucleic Acids Research. 2014;**42**:13110-13121

[122] Shanmugam I, et al. Ubiquitin-specific peptidase 20 regulates Rad17 stability, checkpoint kinase 1 phosphorylation and DNA repair by homologous recombination. The Journal of Biological Chemistry. 2014;**289**:22739-22748

[123] McGarry E, et al. The deubiquitinase USP9X maintains DNA replication fork stability and DNA damage checkpoint responses by regulating CLASPIN during S-Phase. Cancer Research. 2016;**76**:2384-2393

[124] Faustrup H, Bekker-Jensen S, Bartek J, Lukas J, Mailand N. USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. The Journal of Cell Biology. 2009;**184**:13-19

[125] Liu W, Li W, Fujita T, Yang Q, Wan Y. Proteolysis of CDH1 enhances susceptibility to UV radiation-induced apoptosis. Carcinogenesis. 2008;**29**:263-272

[126] Chang L, Zhang Z, Yang Y, McLaughlin SH, Barford D. Atomic structure of the APC/C and its mechanism of protein ubiquitination. Nature. 2015;**522**:450-454

[127] Zhang YW, et al. The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. Molecular Cell. 2009;**35**:442-453

[128] Leung-Pineda V, Huh J, Piwnica-Worms H. DDB1 targets Chk1 to the Cul4 E3 ligase complex in normal cycling cells and in cells experiencing replication stress. Cancer Research. 2009;**69**:2630-2637

[129] Huh J, Piwnica-Worms H. CRL4(CDT2) targets CHK1 for PCNA-independent destruction. Molecular and Cellular Biology. 2013;**33**:213-226

[130] Zhang P, et al. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. Nature Cell Biology. 2014;**16**:864-875

[131] Alonso-de Vega I, Martin Y, Smits VA. USP7 controls Chk1 protein stability by direct deubiquitination. Cell Cycle. 2014;**13**:3921-3926

[132] Guervilly JH, Renaud E, Takata M, Rosselli F. USP1 deubiquitinase maintains phosphorylated CHK1 by limiting its DDB1-dependent degradation. Human Molecular Genetics. 2011;**20**:2171-2181

[133] Cotto-Rios XM, Jones MJ, Busino L, Pagano M, Huang TT. APC/CCdh1-dependent proteolysis of USP1 regulates the response to UV-mediated DNA damage. The Journal of Cell Biology. 2011;**194**:177-186

[134] Zhi G, et al. Fanconi anemia complementation group FANCD2 protein serine 331 phosphorylation is important for fanconi anemia pathway function and BRCA2 interaction. Cancer Research. 2009;**69**:8775-8783

[135] Guervilly JH, Mace-Aime G, Rosselli F. Loss of CHK1 function impedes DNA damage-induced FANCD2 monoubiquitination but normalizes the abnormal G2 arrest in Fanconi anemia. Human Molecular Genetics. 2008;**17**:679-689

[136] Wang X, et al. Chk1-mediated phosphorylation of FANCE is required for the Fanconi anemia/BRCA pathway. Molecular and Cellular Biology. 2007;**27**:3098-3108

[137] Kim JM, et al. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. Developmental Cell. 2009;**16**:314-320

[138] Cohn MA, et al. A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. Molecular Cell. 2007;**28**:786-797

[139] Huang TT, et al. Regulation of monoubiquitinated PCNA by DUB autocleavage. Nature Cell Biology. 2006;**8**:339-347

[140] Nijman SM, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**:331-339

[141] Macurek L, et al. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. Nature. 2008;**455**:119-123

[142] Kee Y, Kim JM, D'Andrea AD. Regulated degradation of FANCM in the Fanconi anemia pathway during mitosis. Genes & Development. 2009;**23**:555-560

[143] Watanabe N, et al. Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:11663-11668

[144] Watanabe N, et al. M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFbeta-TrCP. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:4419-4424

[145] Lafranchi L, et al. APC/C(Cdh1) controls CtIP stability during the cell cycle and in response to DNA damage. The EMBO Journal. 2014;**33**:2860-2879

[146] Cho HJ, et al. Degradation of human RAP80 is cell cycle regulated by Cdc20 and Cdh1 ubiquitin ligases. Molecular Cancer Research. 2012;**10**:615-625

[147] Burrows AC, Prokop J, Summers MK. Skp1-Cul1-F-box ubiquitin ligase (SCF (betaTrCP))-mediated destruction of the ubiquitin-specific protease USP37 during G2-phase promotes mitotic entry. The Journal of Biological Chemistry. 2012;**287**:39021-39029

[148] Huang X, et al. Deubiquitinase USP37 is activated by CDK2 to antagonize APC(CDH1) and promote S phase entry. Molecular Cell. 2011;**42**:511-523

[149] Typas D, et al. The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucleic Acids Research. 2015;**43**:6919-6933

[150] Parameswaran B, et al. Damage-induced BRCA1 phosphorylation by Chk2 contributes to the timing of end resection. Cell Cycle. 2015;**14**:437-448

[151] Orthwein A, et al. A mechanism for the suppression of homologous recombination in G1 cells. Nature. 2015;**528**:422-426

[152] Chu WK, et al. FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51. Nature Communications. 2015;**6**:5931

[153] Simandlova J, et al. FBH1 helicase disrupts RAD51 filaments in vitro and modulates homologous recombination in mammalian cells. The Journal of Biological Chemistry. 2013;**288**:34168-34180

[154] Bacquin A, et al. The helicase FBH1 is tightly regulated by PCNA via CRL4(Cdt2)-mediated proteolysis in human cells. Nucleic Acids Research. 2013;**41**:6501-6513

[155] Laulier C, Cheng A, Huang N, Stark JM. Mammalian Fbh1 is important to restore normal mitotic progression following decatenation stress. DNA Repair (Amst). 2010;**9**: 708-717

[156] Lorenz A, Osman F, Folkyte V, Sofueva S, Whitby MC. Fbh1 limits Rad51-dependent recombination at blocked replication forks. Molecular and Cellular Biology. 2009;**29**: 4742-4756

[157] Wang Z, et al. SCF(beta-TRCP) promotes cell growth by targeting PR-Set7/Set8 for degradation. Nature Communications. 2015;**6**:10185

[158] Jorgensen S, et al. SET8 is degraded via PCNA-coupled CRL4(CDT2) ubiquitylation in S phase and after UV irradiation. The Journal of Cell Biology. 2011;**192**:43-54

[159] Wu S, et al. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes & Development. 2010;**24**:2531-2542

[160] Centore RC, et al. CRL4(Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. Molecular Cell. 2010;**40**:22-33

[161] Abbas T, et al. CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Molecular Cell. 2010;**40**:9-21

[162] Evans DL, et al. MMSET is dynamically regulated during cell-cycle progression and promotes normal DNA replication. Cell Cycle. 2016;**15**:95-105

[163] Saredi G, et al. H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. Nature. 2016;**534**:714-718

[164] Piwko W, et al. The MMS22L-TONSL heterodimer directly promotes RAD51-dependent recombination upon replication stress. The EMBO Journal. 2016;**35**:2584-2601

[165] O'Donnell L, et al. The MMS22L-TONSL complex mediates recovery from replication stress and homologous recombination. Molecular Cell. 2010;**40**:619-631

[166] Duro E, et al. Identification of the MMS22L-TONSL complex that promotes homologous recombination. Molecular Cell. 2010;**40**:632-644

[167] Tomimatsu N, et al. Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. Nature Communications. 2014;**5**:3561

[168] Hustedt N, Durocher D. The control of DNA repair by the cell cycle. Nature Cell Biology. 2016;**19**:1-9

Chapter 6

The Cross Talk among Autophagy, Ubiquitination, and DNA Repair: An Overview

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Additional information is available at the end of the chapter

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Abstract

Cellular plasticity is modulated by protein posttranslational modifications, which act on most intracellular pathways. Ubiquitination is a versatile posttranslational modification (PTM) that influences protein fate, controlling their degradation or modulating their activity and subcellular localization. The ubiquitin proteasome system, UPS, and the autophagic pathway are the main degradative intracellular machineries, which rely on ubiquitination for their activation and/or the selective recycling of proteins and organelles. Recent findings indicate that the cross talk between UPS and autophagy plays a key role in controlling DNA repair pathways. Even being a cytoplasmic process, it is now clear that autophagy can directly impact on the correct activation of DNA repair. Of note, defects on autophagy are related to the impairment of homologous recombination repair and to an increase of the nonhomologous end joining repair activity. These evidences give new insights into the molecular processes underlying the DNA damage response and provide further explanation for the tumorigenesis associated with autophagy impairment. Moreover, these findings introduce new examples of synthetic lethality between autophagy and DNA repair genes and lead to the possible development of target therapies for tumors with defective autophagy.

Keywords: autophagy, DNA repair, ubiquitination, ubiquitin proteasome system, p62

1. Introduction

In eukaryotic cells, protein homeostasis is essential to maintain cell survival and it occurs through two major pathways: the ubiquitin-proteasome system (UPS) and autophagy. The UPS is responsible for degradation of both cytosolic and nuclear short-lived or damaged proteins, and it is involved in the removal of 80–90% of cellular proteins. It regulates several

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processes, including maintenance of cellular quality control, transcription, cell cycle progression, DNA repair, receptor-mediated endocytosis, cell stress response, and apoptosis [1]. By contrast, autophagy mediates the degradation of long-lived proteins, entire organelles (e.g., mitochondria and peroxisomes), or pathogens and aggregates *via* the lysosome. On the one hand, autophagy is related to cell growth, survival, and development; on the other hand, it is involved in cell death and it has been implicated in human pathologies such as cancer, neurodegeneration, myopathies, and heart and liver diseases [2].

The ubiquitin-proteasome system and autophagy were long viewed as independent and parallel processes. However, it becomes increasingly clear that the UPS and autophagy crosstalk to each other [3]. The need for energetic homeostasis and protein balance requires that both these degradation systems are tightly controlled and coordinated during a cell life. In particular, the balance of cellular homeostasis needs to be carefully regulated and this is made possible by protein posttranslational modifications (PTMs) such as phosphorylation, acetylation, methylation, and ubiquitination [4–6]. PTMs, indeed, due to their reversible or irreversible nature, provide the necessary flexibility in order to adapt the cells rapidly to different environmental stress.

Accumulating evidence indicates that ubiquitination regulates autophagy through at least two mechanisms [7]. One is controlling the stability of upstream autophagy-related (ATG) genes. In this context, many E3 ligase and deubiquitinase (DUBS) enzymes have been identified as crucial for autophagy induction, maturation, or termination [8–14]. The other one facilitates the recruitment of ubiquitinated substrates to the autophagy machinery [15]. In this case, ubiquitination plays an essential role in determining the selectivity of autophagy cargos.

There are different interfaces between autophagy and UPS. First, ubiquitin or ubiquitin-like proteins are common degradative tags; ubiquitin, indeed, is a very small molecule that can be attached to the substrate by several ways, generating a broad repertoire of signals. These degradative tags are then recognized by specific adaptor proteins, such as p62/sequestosome 1 (SQSTM1) or neighbor of BRCA1 gene 1 (NBR1), that are molecules capable of directing ubiquitinated target proteins to both systems [15]. They act through specific domains, such as the ubiquitin-associated domain (UBA) or the ubiquitin-binding domain (UBD), able to specifically recognize the substrate for mediating its degradation. The other point in common is the participation of these mechanisms to general cellular programs, such as the ER stress response [16] or the atrophy program [17]. Moreover, recent studies have revealed that autophagy and UPS participate together also in DNA damage response (DDR) [18, 19]. DDR is an essential mechanism to maintain genome integrity; similar to protein homeostasis, maintenance of genomic integrity is essential for an organism's survival. Although these mechanisms occur in spatially distinct cellular compartments, evidence has been accumulated about a strict cross talk among autophagy, ubiquitination, and DNA repair. When a DNA lesion occurs, chromatin undergoes a relaxed conformation through a series of histone PTMs, recruitment of DDR sensors, and additional proteins to further regulate DNA replication, cell cycle, repair, and cell survival *versus* cell death. In this context, a key role is played by p62 that has been recently found to be able to shuttle between cytoplasm and nucleus, where it is able to inhibit homologous recombination (HR) or the recruitment of DNA-binding factors [20–22]. In this chapter, we provide an overview of the current knowledge about the coordination among autophagy, ubiquitination, and DNA repair pathways, and its importance to maintain cell homeostasis and survival.

2. Cross talk between autophagy and UPS

UPS and autophagy are two crucial mechanisms that are involved in cellular catabolism in normal physiology and development, but also in human pathologies such as cancer, neurodegeneration, and aging. By these processes, cells are able to recycle proteins, aggregates, or entire organelles to obtain energy. Although these pathways differ for specificity, kinetics, and substrates, it is increasingly clear that they are cooperative and complementary to ensure cellular homeostasis and survival.

2.1. Autophagy: an overview of its main actors and functions

Autophagy is a catabolic process occurring in all eukaryotic cells to maintain cellular viability and homeostasis in basal conditions, by controlling long-lived proteins and damaged organelles. However, autophagy can also be stimulated in response to sublethal stresses, such as nutrient or growth factor deprivation, hypoxia, reactive oxygen species (ROS), or viral and pathogen invasion to maintain cell survival [23]. During autophagy, cells undergo rapid changes to adapt their metabolism and protect themselves against potential damages. Depending on the delivery route of cytoplasmic material to the lysosomal lumen, three different forms of autophagy are known: microautophagy, chaperone-mediated autophagy, and macroautophagy. In microautophagy, portions of cytosol are instantly engulfed by the lysosomal membrane. In chaperone-mediated autophagy, proteins characterized by a specific sequence signal are recognized by lysosomal receptors and then degraded by lysosomal proteases. During macroautophagy (hereafter, more simply, autophagy), cytoplasmic material (e.g., proteins, lipids, and organelles) is sequestered by a cup-shaped membrane (called isolation membrane or phagophore), which expands while becoming spherical to urn into a double-membraned vesicle, termed autophagosome; this slides along cytoskeletal structures and fuses with lysosomes, thus forming a single vesicle called the autophagolysosome, in which both autophagosome membrane and contents are degraded by lytic enzymes [24].

Taking advantages from yeast genetics, more than 35 ATG genes have been identified and characterized, with most of them being well-conserved from yeast to mammals [25]. The autophagy process is divided into mechanistically distinct steps, including induction, autophagosome formation, and autophagosome-lysosome fusion, followed by the release of the degradation products back into the cytosol. Different sets of ATG proteins are involved in these steps and constitute the core autophagic machinery.

Indeed, the core pathway of mammalian autophagy involves at least five molecular complexes including (1) the ULK1 complex, (2) the BECLIN 1/class III PI3K complex, (3) two transmembrane proteins: ATG9 and VMP1, (4) two ubiquitin-like protein (ATG12 and LC3) conjugation systems, and (5) proteins that mediate the formation of autophagolysosomes [24].

The activation of this molecular machinery is extremely complicated and it involves multiple signaling inputs. According to current knowledge, the most important sensor of cellular stress is mammalian target of rapamycin complex 1 (mTORC1). This serine-threonine kinase shuts off autophagy in cells growing in the presence of nutrients and growth factors; in basal conditions, mTORC1 negatively regulates the ULK1 complex, the early most important structural complex of the core autophagic machinery.

As a consequence of the autophagy role on cellular homeostasis, increasing evidence reveal that alteration in autophagy occurs in many human diseases, such as neurodegenerations, myopathies, infectious disease, aging, and cancer, contributing to their pathogenesis. Autophagy results to be deregulated in many neurodegenerative diseases, causing the accumulation of aggregates of mutated toxic proteins [26]. Autophagy has also been identified as a crucial process in oncogenesis and cancer progression [27, 28]. Many autophagy-related proteins are considered tumor suppressor genes and are mutated in cancer (Beclin 1, ATG5, Bif-1, ATG4C, and UVRAG), leading to an accumulation of DNA damage and genome instability [28]. Finally, the activity and recruitment of ATG proteins are important also for antigen presentation, innate immune signaling, and pathogen degradation.

2.2. UPS

Ubiquitin proteasome system (UPS) is the major pathway responsible for the degradation of cytosolic short-lived proteins and of proteins residing in the nucleus and the endoplasmic reticulum (ER) [29]. The tagging molecule is ubiquitin, a small protein of 76 amino acids that is covalently linked to thousands of different proteins by a bond between the glycine at the C-terminal end of ubiquitin and the side chains of lysine on proteins. The earmarked proteins are then degraded by the 26S proteasome, a highly conserved multicatalytic ATP-dependent protease complex. Conjugation of ubiquitin to a substrate is mediated by the action of three ubiquitin-activating enzymes called E1, E2, and E3. E1 binds ubiquitin and transfers it to the active site of E2; finally E3 enzyme transfers the ubiquitin molecule directly to the substrate. Regarding the selection of the substrates, many strategies could exist; in some cases, the E3 enzyme recognizes and binds a signal in the protein sequence [30].

In the human genome, 2 E1s, 50 E2s, and 600 E3s have been identified [31]. The classification of ubiquitin ligases is based on their biochemical and structural features. The best known domain subclasses include HECT (homologous to E6-associated protein carboxy-terminus), RING-fingers (RING, really interesting new gene), and U-box domains (a modified RING motif without the full complement of Zn2 + −binding ligands).

Ubiquitination is a reversible, specific, and adaptable PTM, similar to phosphorylation; by means of seven lysine residues in ubiquitin (at positions 6, 11, 27, 29, 33, 48, and 63) that act as acceptors of other ubiquitin molecules, this PTM is considered very versatile.

2.3. Connections between autophagy and UPS

The different molecular machinery characterizing UPS and autophagy is just one of the differences between these two processes; they are also responsible for the disposal of different substrates. The proteasome is responsible for degradation of short-life proteins, while those with long-life, organelles and aggregates, are autophagic substrates. At variance with UPS, autophagy is restricted to the cytoplasm; moreover, the two processes differ in the time window in which they act, since autophagy is considered slower than UPS (**Figure 1**). However, several recent lines of evidence have suggested that UPS and autophagy are functionally connected [32]. Indeed, the need for energetic homeostasis and protein balance requires that both degradation systems are tightly controlled and coordinated during a cell life.

The first unifying factor linking UPS and autophagy is ubiquitin. Although autophagy was considered originally a nonspecific process, it has recently emerged as a selective mechanism that specifically removes damaged organelles, such as mitochondria, or defective proteins. This specificity may be accounted for by special proteins called autophagy receptors and adaptors that are able to recognize and bind the ubiquitinated proteins listed for degradation by the autophagy machinery. They include p62/SQSTM1, neighbor of BRCA1 gene 1(NBR1), histone deacetylase 6 (HDAC6), the BH3-only family protein BNIP3L/Nix, the ubiquitin receptor nuclear dot protein 52kd (Ndp52), and optineurin [15]. These receptors recognize ubiquitin chains (including Lys-63-poly Ub and others) through their UBA domain on one side and directly bind LC3 or other ATG8 proteins via their LC3-interacting region (LIR). This allows the incorporation of autophagy substrates into the autophagosome. Among them, p62 has been extensively studied. P62 molecules are distributed not only in the cytosol but also in the nucleus, as well as they localize with autophagosomes and lysosomes. Besides its role in macroautophagy and selective autophagy (such as mitophagy) that has been fully investigated, there are several evidence that p62 is the main actor in mediating the cross talk between autophagy and UPS. First, the proteasome is inhibited in autophagy-deficient cells due to accumulation of p62; second, pharmacological inhibition of the proteasome also increases p62 expression [33]; third, p62 silencing attenuates the accumulation of proteasome substrates [34]. One explanation is that accumulation of p62 sequesters ubiquitinated proteins that aggregate and become inaccessible to the proteasome.

Intriguingly, p62/SQSTM1 is also known as an inhibitor of proteasomal degradation of LC3 [35]. In linking proteasomal degradation and autophagy, an important role is also played by HDAC6, the enzyme that regulates the acetylation of γ-tubulin and facilitates the transport of polyubiquitinated protein aggregates to the nascent phagophore [36]. HDAC6 has been shown to be involved in both aggresome formation and the fusion of autophagosomes with lysosomes, thus making it an attractive target to regulate protein aggregation.

A second important link is that ubiquitination can affect stability and function of ATG proteins and their upstream regulators. Many ubiquitin E3 ligases have been demonstrated to regulate autophagy: for instance, RNF5, which directly modulates the stability of ATG4B, or TRAF6, Nedd4 or NEDD4L, which mediate ubiquitination of Beclin 1 and ULK1, respectively [8]. Intriguingly, a catalytic activity–independent role for ubiquitin ligases such as TRIM13 and c-Cbl in autophagy is emerging by regulating the recruitment of autophagy adaptors like LC3 and p62 [37].

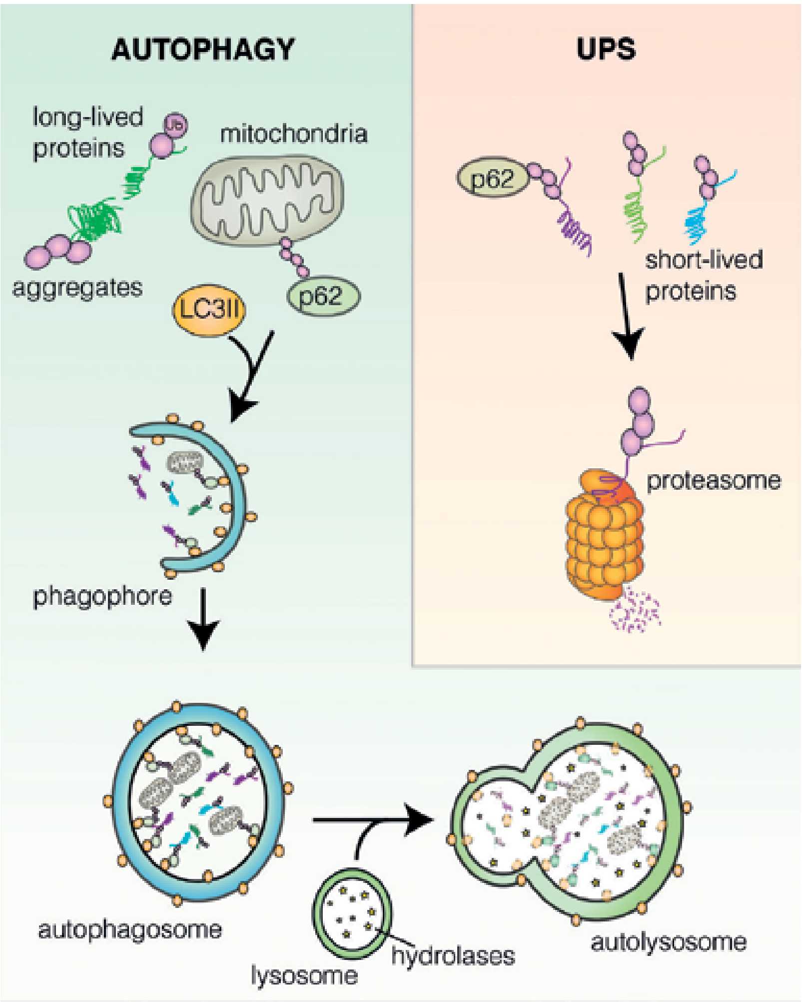

Figure 1. Overview of autophagy and the ubiquitin proteasome system (UPS). Autophagy and UPS are the main intracellular recycling processes. While autophagy degrades long-lived proteins, protein aggregates, and whole organelles (e.g. mitochondria), UPS is involved in degrading short-lived proteins. Proteins and organelles that need to be degraded are labeled by ubiquitin. Ubiquitin chains can be recognized by adapters, such as p62, that mediate the binding of the target with the proteasome (UPS) or with the protein LC3II (autophagy). Autophagy begins with the formation of the phagophore that embeds the material to be recycled and maturates into the autophagosome. The autolysosome is then formed through fusion with the lysosome, and hydrolases are responsible for the content degradation.

3. Autophagy and DNA repair

Genome integrity is preserved by an evolutionary conserved machinery named DNA damage response (DDR). Upon DNA damage, molecular key players of DNA repair pathways induce arrest of cell cycle progression and enhance activation of DNA repair pathways [38]. Programmed cell death mechanisms are then activated if the DNA lesions are not repaired and, so far, defects in DNA repair and death processes are considered the major source of genomic instability and malignant transformation [39]. Although autophagy is a cytoplasmic process, autophagy-deficient cells display genomic instability and accumulation of DNA damage [28]. To date, a range of mechanisms have been found to be involved in linking autophagy and DNA repair, this opening important questions that need to be addressed.

3.1. DNA damage response (DDR): an overview

The DDR is comprehensively a set of intracellular pathways and specialized molecules that are activated in response to different types of damage to facilitate repair and prevent cell transformation and death. This process has been widely investigated and well-reviewed elsewhere [40–43]. We here provide only a brief overview of its function and components that is relevant to understand the cross talk between DNA repair pathways and autophagy.

DNA damage can be caused by several exogenous (e.g. ultraviolet light or ionizing radiation) or endogenous agents (e.g. reactive oxygen species (ROS)). The most common types of lesion can be single-strand breaks (SSBs), double-strand breaks (DSBs), and interstrand cross-links (ICLs). Sensing DNA damage results in the initiation of some programs, including cell cycle arrest, checkpoint activation, and DNA damage repair [38–43]. When a DNA lesion occurs, histones undergo PTMs, such as phosphorylation and acetylation, that lead to chromatin relaxation. This provides access to DDR sensors that bind DNA lesions. Initially, DSBs are bound by the Mre11 complex (MRN), including Mre11/Rad50-Nbs1, that recruits the ataxia telangiectasia mutated protein kinase ATM [44]. ATM activation is then induced by a series of PTMs that trigger the recruitment of additional proteins, including checkpoint kinase 2 (Chk2), involved in cell cycle control, the tumor suppressor protein p53 that controls cell survival, and HDAC1 and HDAC2 that regulate chromatin remodeling to further orchestrate and amplify the DSB response. In the case of DSBs, the ATM-DNAPK pathway induces phosphorylation of the histone variant γ-H2AX that flanks DBS sites.

SSBs, instead, favor the activation of ataxia telangiectasia and Rad3-related (ATR) kinase that is recruited by the replication protein A (RPA) complex [45]. ATR activity is, in turn, amplified by the recruitment of several factors, leading to the spread of SSB signal.

There are five main DNA repair mechanisms: mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), nonhomologous end joining (NHEJ), and homologous recombination (HR). MMR, BER, and NER are used for different types of base-associated lesions that require a single strand incision. NHEJ and HR repair mechanisms are involved in DSB repair [38, 39].

DNA repair is carried out by a large number of enzymes that chemically modify DNA to repair the damage, including nucleases, helicases, polymerases, topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and phosphatases. PTMs of these proteins by ubiquitin and ubiquitin-like modifiers (UBLs) are essential in regulating the enzymes that reestablish genome integrity after damage.

Mutations and dysfunctions in genes involved in DNA repair pathways have been implicated in many human diseases, such as neurological and immunological defects, aging, and cancer [38]. In the nervous system, indeed, neurons exhibit high oxygen consumption by mitochondrial respiration, which can result in oxidative stress and subsequent DNA damage; given that neurons display limited capacity of replacement, DNA repair pathways play an essential role to maintain their homeostasis [39]. Deficiency in multiple DNA repair pathways, including NER, BER, and DSB repair, has been linked to premature aging. Finally, the maintenance of genomic integrity by DNA repair pathways is critical to prevent tumorigenesis, as indicated by the cancer predisposition of several DDR syndromes [46].

3.2. Connections between autophagy and DNA repair

The first evidence that linked autophagy to DNA damage came out to understand why defects in autophagy rendered cells susceptible to metabolic stress promoting tumorigenesis. In 2007, Mathew and colleagues reported for the first time that autophagy could function to protect the genome [28]. Knockdown of autophagy genes such as ATG5 and Beclin 1 results in gene amplification, chromosomal instability, and aneuploidy, facilitating tumor progression. In detail, in autophagy-deficient cells, they found an increase in the levels of γ-H2AX and other DNA damage responses, suggesting that constitutive and stress-induced autophagy is important to prevent DNA damage and maintain the integrity of the genome [28, 47–51].

Interestingly, it has been reported that in murine embryonic fibroblasts (MEFs), knockout for the 200 kDa FAK-family interacting protein (FIP200), there is a significant decrease in DNA damage repair in response to ionizing radiation as well as to chemotherapeutic agents [52]. FIP200 is a component of the ULK1 complex and is essential for activation of autophagy. In this study, at variance with its potential tumor suppression function, inactivation of FIP200 and subsequent deficiency in autophagy sensitize cells to apoptosis-inducing agents probably due to the defective DNA damage repair.

From then onwards, several studies have demonstrated that autophagy participates, directly or indirectly, in DNA repair pathways. Indeed, it is now accepted that autophagy, in particular mitophagy—the selective removal of damaged mitochondria, can prevent genomic instability by removing ROS-producing mitochondria, since ROS are one of the major sources of DNA damage as they could directly modify the DNA or indirectly generate different lesions, both affecting cell viability [53]. Moreover, autophagy is also necessary for providing energy and metabolites required for an efficient DNA repair. In fact, many evidence show that, by sustaining the energy demand required to support DNA repair processes, autophagy can help the development of chemoresistance mechanisms in cancer cells, delaying apoptotic cell death upon DNA damage [54, 55]. Besides that, it is now clear that autophagy can be activated by DNA damage at multiple levels. The use of the DNA-damaging agents such as camptothecin, etoposide and temozolomide, p-anilioaniline, and ionizing radiation (IR), in addition to initiate cell cycle arrest, also initiates autophagy [54, 56, 57].

As described above, ATM is a central regulator of the DDR response. In response to DNA damage, the transcription factor FOXO3a binds ATM, thus leading to its activation and promoting repair. Both ATM and FOXO3a have been linked to autophagy. ATM induces the activation of the energy sensor AMP-activated protein kinase (AMPK), leading to autophagy progression [58, 59]. On the one hand, AMPK interacts with the main negative regulator of autophagy, the mTORC1 complex *via* a pathway involving tuberous sclerosis complex 1 and

2 (TSC1/2); on the other hand, AMPK directly phosphorylates one of the key protein kinases that initiate autophagy, ULK1 [60, 61]. ATM also mediates the activation of Che-1, a RNA polymerase II-binding protein that regulates the transcription of two mTOR inhibitors: Redd1 and Deptor [62]. Otherwise, FOXO3a controls the transcription of autophagy-related genes, such as LC3 and Bnip3 [63–65]. Another DDR protein involved in autophagy is poly[ADP-ribose] polymerase 1 (PARP1). After a DNA lesion, PARP1 synthesizes poly(ADP-ribose) chains that recruit the DNA damage repair proteins. Recently, it has been demonstrated that hyperactivated PARP1 causes a depletion of ATP that leads to AMPK activation and, consequently, to autophagy induction [66].

A crucial regulator of DNA repair pathways is the tumor suppressor protein p53. P53 has a dual role in autophagy [67, 68]: on one hand, p53 together with other members of its family (p63 and p73) regulates transcriptionally autophagy-related proteins; on the other hand, p53 acts directly on AMPK signaling.

Moreover, HDAC proteins represent a significant link between autophagy and DNA repair pathways. HDACs are histone deacetylases that influence DNA damage response through acetylation of key DNA repair and checkpoint proteins. Robert and colleagues found that HDACs control chromosome stability by coordinating the ATR checkpoint and DSB processing with autophagy [36]. In particular, HDAC inhibition triggers degradation of the recombination protein SAE1 (in human CtIP) by promoting autophagy that affects the DNA damage sensitivity of *HDAC* mutants.

Recent studies have suggested that another family of proteins called sirtuins could play an important role in autophagy and DNA repair pathways. Sirtuins are protein deacetylases dependent on NAD+ that are involved in autophagosome formation by deacetylating ATG5, ATG7, and ATG8. In DNA repair pathways, sirtuins regulate transcriptional activity of p53, thus affecting cell cycle and cell death under DNA damage conditions [69].

Recently, an interesting connection between DNA repair signaling and mitophagy has also been provided. As mentioned above, damaged mitochondria may produce elevated levels of ROS, thus inducing DNA damage. In addition, blockage of mitophagy can result in the accumulation of dysfunctional mitochondria, damaged mtDNA, and an increased rate of apoptotic cell death. Feng and colleagues found that in ataxia telangiectasia patients, characterized by ATM dysfunctions, the defect in the nuclear DNA damage repair leads to defective mitophagy [70]. This occurs through the impairment of Sirtuin1 activity that, in turn, affects the expression of the mitochondrial uncoupling protein 2 (UCP2), responsible for the import, cleavage, and removal of PINK1, a key molecule in mitophagy induction.

Intriguingly, new evidence suggests a direct role for autophagy in the function of "error proof" HR, NER, or MMR. About the involvement of autophagy in NER regulation, (an adaptable DNA repair pathway that corrects helix-distorting base lesions induced by environmental carcinogens), it has been found implicated in downregulating the transcription of XPC and impairing the recruitment of DDB2 to UV-induced lesion sites through TWIST1-mediated inhibition of EP300 [71]. MMR defects also impair autophagy induced by chemotherapeutic drugs [72]. Mispairs induced by nucleoside analogs, such as 6-thioguanine (6-TG) and 5-fluorouracil (5-FU), have been reported to induce autophagy in a p53-, mTOR-dependent manner by upregulation of BNIP3. These studies suggest that targeted inhibition of the autophagic pathway may enhance the cytotoxicity of those anticancer agents that are recognized and processed by the MMR system.

Of note, the protein UVRAG (UV-irradiation-resistance-associated gene) plays a dual role acting both in autophagosome formation and maturation and chromosomal stability [73], independently from autophagy. In autophagy, UVRAG is responsible for the activation of PI(3) class III (PI(3)KC3) kinase through Beclin 1 interaction. During NHEJ, UVRAG interacts and helps the assembly of the upstream protein kinase of the NHEJ pathway, DNA-PK. Moreover, UVRAG is found to be associated with centrosomes by its interaction with CEP63. Affecting the UVRAG-centrosome interaction destabilizes centrosomes, resulting in extensive aneuploidy. In the same way, Beclin 1 exerts a specific role on the NHEJ pathway [74]. Conversely, the genomic instability characterizing autophagy-defective mice models underlines how autophagy-deficient cells rely on the error-prone NHEJ repair process.

Of note, one of the most important connections between autophagy and DNA repair pathways is highlighted by the mediator of autophagy on UPS, the adaptor protein p62. These very recent and fascinating discoveries will be better explained in the next paragraph.

4. Autophagy, ubiquitination, and DNA repair

As previously described, p62 is an autophagic receptor and substrate that selectively targets polyubiquitinated proteins for degradation via both proteasome and autophagy. P62 levels are impaired in many diseases, such as cancer, proteinopathies, neurodegeneration, obesity, and liver diseases [75, 76]. P62 protein levels are strongly induced by different proteotoxic stresses such as oxidants, proteasomal inhibitors, or ionophores; many p62 functions are carried out by its N-terminal PB1 and C-terminal UBA domains that are necessary for protein-protein interactions and for its polymerization. P62 is commonly found in the cytosol, together with ubiquitinated proteins or aggregates; however, by now, it is evident that p62 is able to shuttle between cytoplasm and nucleus by a specific nuclear export signal (NES) and two nuclear localization signals (NLS) [22]. In the nucleus, p62 is found associated to promyelocytic leukemia bodies (PML) that usually contain proteasomes, chaperones, and ubiquitinated proteins [77]. Its nucleocytosolic distribution is finely regulated by several mechanisms, including self-association, phosphorylation, and binding to ubiquitinated proteins. In particular, accumulation of ubiquitinated proteins is able to retain p62, resulting in its accumulation in aggregates both in the cytosol and in the nucleus. It has been recently found that nuclear p62 is able to associate with markers of DNA repair, providing the first important link among autophagy, UPS, and DNA repair [20]. More in detail, Hewitt and colleagues found that after DNA damage (X-ray irradiation), p62 was associated with DNA damage *foci* (DDF); this association decreased after autophagy induction and it was impaired in autophagy-deficient cells, thus suggesting a role for p62 in mediating the effect of autophagy on DNA repair. The molecular mechanism by which p62 carries out its functions in this context involves the proteasomal degradation of two essential DNA repair-related proteins: filamin A (FLNA) and the RAD51 recombinase. FLNA is known to mediate the recruitment of RAD51 to DSB and facilitate

HR. The p62-mediated proteasomal degradation of FLNA results in reducing RAD51 protein levels and slower DNA repair. Therefore, autophagy is able to control HR by reducing the levels of its substrate p62. Overall, these findings explain how autophagy impairment leads to an increase in DNA damage and consequently to genomic instability. This is particularly relevant during aging, when nuclear levels and co-localization of p62 with DNA damage *foci* have been reported to increase and when autophagy is gradually impaired, underlining the important role of this pathway to age-related diseases.

Novel important mechanistic insights into the connection among autophagy, ubiquitination, and DNA damage response have been presented in the same year, and also in those cases, a main role was played by p62. Wang and colleagues identified the E3 ligase RNF168 as a novel p62-binding protein [21]. RNF168 is an ubiquitin E3 ligase that binds ubiquitinated histones H1 and H2A, thus propagating the H2A ubiquitination at sites of DNA damage [78]. RNF168 catalyzes Ub-K63 chains on Lys13-15 of H2A and H2AX [79–81]. The RNF168 pathway has an important function in regulating the DSB repair pathway choice, by promoting the recruitment of the key repair factors for both NHEJ and HR at chromatin areas near DSBs. Wang and colleagues discovered that p62 inhibits RNF168 E3 ligase activity, leading to a decrease in RNF168-dependent polyubiquitination of histone H2A [21]. It has been reported that the LB domain of p62 is responsible for the binding and repression of RNF168. After DNA damage, p62 dissociates from RNF168, presumably because p62 is degraded by autophagy. In autophagy-deficient cells, indeed, p62 accumulates at DNA damage sites and impairs chromatin ubiquitination. When histone ubiquitination decreases, the recruitment of DNA repair proteins such as BRCA1, RAD51, and RAP80 to sites of DSBs is compromised and consequently also the repair of radiation-induced DNA damage.

Besides the direct role of p62 in DNA repair, some key factors of DNA damage response have also been found to be degraded by autophagy. The heterochromatin component HP1a is necessary to maintain chromatin in a condensed state, and this hides the RAD51 binding site at DSBs. A recent study showed that, after X-ray irradiation, the E2 ligase RAD6 interacts with HP1a, leading to its ubiquitination and degradation *via* autophagy. HP1a autophagy-mediated degradation makes chromatin more permissive for the catalysis of HR [82]. In addition, these findings are supported by another work showing that RAD6 is important for Parkin-dependent mitophagy [83]. Interestingly, checkpoint kinase 1 (Chk1), a regulator of DNA damage repair by HR, is another target of autophagy. A recent paper shows that loss of autophagy results in decreased levels of total and phospho-Chk1; the authors propose that decreased levels of Chk1, in the absence of autophagy, are due to increased proteasomal activity and this, in turn, impairs both DNA damage repair by HR (but not NHEJ) and genomic integrity [84]. Another study identified Chk1 as a target of chaperone-mediated autophagy (CMA) [85]. Park and colleagues found that CMA is upregulated by DNA damage after both irradiation and chemotherapy, thus inducing degradation of p-Chk1 [86]. Interestingly, CMA is able to degrade Chk1 only after its phosphorylation on Ser345; by contrast, Ser317-phosphorylated Chk1 is the preferred substrate of the proteasome. When CMA is defective, Chk1 accumulates in the nucleus and leads to destabilization of the MRN complex involved in the initial processing of DSBs prior to DNA repair by HR, thus facilitating genomic instability. A schematic representation of the cross talk among autophagy, ubiquitination, and DNA repair machinery is reported in **Figure 2**.

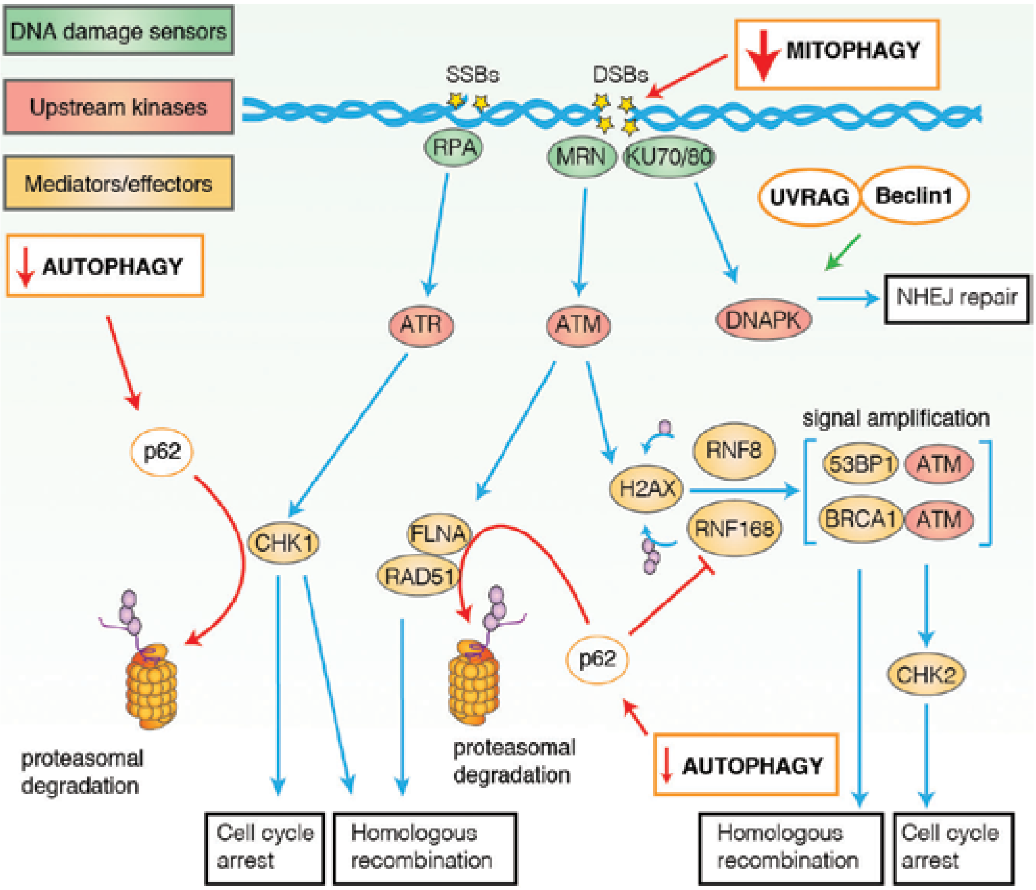

Figure 2. Model of autophagy in the DNA damage response. Autophagy impairment is directly associated with the modulation of different DNA repair pathways and with the formation of DNA double strand breaks. Mitophagy defects lead to the accumulation of malfunctioning mitochondria and to the increase of reactive oxygen species (ROS) that cause the formation of DNA double strand breaks. Upon DNA damage, different DNA repair pathways are induced, depending on the type of DNA lesion and on the phase of cell cycle. It has been demonstrated that impairment on autophagy decreases the functionality of homologous recombination (HR). P62 levels increase upon autophagy downregulation, thus inducing the proteasome-dependent degradation of CHK1 or Rad51/FLNA proteins. Moreover, p62 affects HR repair by directly binding and inhibiting the histone ubiquitin ligase RNF168. On the other hand, autophagy-related proteins, such as Beclin 1 and UVRAG, can shuttle into the nucleus and promote the nonhomologous end joining pathway of DNA repair.

Recent publications reported that autophagy can also positively regulate NER, acting on the levels of NER-specific damage recognition proteins such as XPC, UVRAG, and DDB1/2. As previously mentioned, UVRAG is involved in both autophagy and DNA repair. In this work, they found that, after irradiation, UVRAG localizes to DNA lesions and associates with DDB1 to promote assembly and activity of the DDB2-DDB1-Cullin4A-Roc1 ubiquitin ligase complex, thus leading to XPC recruitment and NER [87]. Moreover, impairment of autophagy leads to both transcriptional suppression and ubiquitination of XPC, a key process for DNA damage recognition [71]. Intriguingly, the DDB1-Cul4 ubiquitin complex is also known to be directly involved in autophagy [11]. In fact, the pro-autophagy protein AMBRA1 is degraded by Cullin-4 in a time-dependent manner during autophagy. In nutrient-rich conditions, Cullin-4 association limits AMBRA1 abundance. ULK1 activation by nutrient deprivation causes a rapid release of AMBRA1 from Cullin-4 and consequent AMBRA1 protein stabilization. Several hours later, Cullin-4 reassociates with AMBRA1 and triggers its degradation, initiating autophagy termination.

How the mechanism of autophagy termination upon starvation can be applied also to other types of stress remains unknown. Recent evidence shows that Cullin-1 is responsible for termination of autophagy after DNA damage [88]. Cullin-1, *via* binding its receptor FBXL20, mediates the proteasomal degradation of VPS34, a key component of Beclin 1 complex in autophagy. Degradation of VPS34 occurs during the mitotic arrest induced by DNA damage agents by CDK1-mediated phosphorylation and after transcriptional induction of FBXL20 and p53.

5. Conclusion and perspectives

Autophagy is a central player in the regulation of DNA repair pathways and it may have evolved as a quality control system that responds to many stressful conditions, including DNA damage.

In recent years, there have been impressive advances in our understanding of the principles and mechanisms by which autophagy cross talks with the DNA damage machinery and how integration with PTMs, in particular ubiquitination, allows for optimal context-dependent DSB repair. Impairments in autophagy have been linked to increased susceptibility of the cells to genotoxic agents, and this could be important in anticancer therapy. However, it should be taken into account that this process plays a context-dependent role in cancer development.

Interestingly, defects in DNA damage repair impair autophagy. Contrarily, an impairment of autophagy causes the production of protein and free radicals increasing mutation rate, which might promote human diseases such as cancer and neurodegeneration. However, the question about the exact role of autophagy in DNA repair pathways and its implication for cancer therapy is still waiting for a complete answer.

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References

[1] Varchavsky A. The ubiquitin system. Trends in Biochemical Sciences. 1997;**22**(10):383-387

[2] Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. Molecular Cell. 2010;**40**(2):280-293. DOI: 10.1016/j.molcel.2010.09.023

[3] Clague MJ, Urbe S. Ubiquitin: Same molecule, different degradation pathways. Cell. 2010;**143**:682-685. DOI: 10.1016/j.cell.2010.11.012

[4] Jensen ON. Modification-specific proteomics: Characterization of post-translational modifications by mass spectrometry. Current Opinion in Chemical Biology. 2004;**8**(1): 33-41. DOI: 10.1016/j.cbpa.2003.12.009

[5] Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nature Biotechnology. 2003;**21**(3):255-261. DOI: 10.1038/nbt0303-255

[6] Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. Nature Structural & Molecular Biology. 2010;**17**(6):666-672. DOI: 10.1038/nsmb.1842

[7] McEwan DG, Dikic I. The three musketeers of autophagy: Phosphorylation, ubiquitination and acetylation. Trends in Cell Biology. 2011;**21**:195-201. DOI: 10.1016/j.tcb.2010.12.006

[8] Kuang E, Qi J, Ronai Z. Emerging roles of E3 ubiquitin ligases in autophagy. Trends in Biochemical Sciences. 2013;**38**(9):453-460. DOI: 10.1016/j.tibs.2013.06.008

[9] Abrahamsen H, Stenmark H, Platta HW. Ubiquitination and phosphorylation of Beclin 1 and its binding partners: Tuning class III phosphatidylinositol 3-kinase activity and tumor suppression. FEBS Letters. 2012;**586**(11):1584-1591. DOI: 10.1016/j.febslet.2012.04.046

[10] Nazio F, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nature Cell Biology. 2013;**15**(4): 406-416. DOI: 10.1038/ncb2708

[11] Antonioli M, et al. AMBRA1 interplay with cullin E3 ubiquitin ligases regulates autophagy dynamics. Developmental Cell. 2014;**31**(6):734-746. DOI: 10.1016/j.devcel.2014.11.013

[12] Liu CC, et al. Cul3-KLHL20 ubiquitin ligase governs the turnover of ULK1 and VPS34 complexes to control autophagy termination. Molecular Cell. 2016;**61**(1):84-97. DOI: 10.1016/j.molcel.2015.11.001

[13] Nazio F, et al. Fine-tuning of ULK1 mRNA and protein levels is required for autophagy oscillation. The Journal of Cell Biology. 2016;**215**(6):841-856. DOI: 10.1083/jcb.201605089

[14] Liu J, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011;**147**(1):223-234. DOI: 10.1016/j.cell.2011.08.037

[15] Kim BW, Kwon do H, Song HK. Structure biology of selective autophagy receptors. BMB Reports. 2016;**49**(2):73-80. DOI: 10.5483/BMBRep.2016.49.2.265

[16] Chen X, Yin XM. Coordination of autophagy and the proteasome in resolving endoplasmic reticulum stress. Veterinary Pathology. 2011;**48**:245-253. DOI: 10.1177/0300985810385154

[17] Zheng Q, Wang X. Autophagy and the ubiquitin-proteasome system in cardiac dysfunction. Panminerva Medica. 2010;**52**:9-25

[18] Hewitt G, Korolchuk VI. Repair, reuse, recycle: The expanding role of autophagy in genome maintenance. Trends in Cell Biology. 2016;S0962-8924(16)30209-4. DOI: 10.1016/j.tcb.2016.11.011

[19] Eliopoulos AG, Havaki S, Gorgoulis VG. DNA damage response and autophagy: A meaningful partnership. Frontiers in Genetics. 2016;**7**:204. eCollection 2016. DOI: 10.3389/fgene.2016.00204

[20] Hewitt G et al. SQSTM1/p62 mediates crosstalk between autophagy and the UPS in DNA repair. Autophagy. 2016;**12**(10):1917-1930. DOI: 10.1080/15548627.2016.1210368

[21] Wang Y et al. Autophagy regulates chromatin ubiquitination in DNA damage response through elimination of SQSTM1/p62. Molecular Cell. 2016;**63**:34-48. DOI: 10.1016/j.molcel.2016.05.027

[22] Pankiv S et al. Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to promyelocytic leukemia bodies. The Journal of Biological Chemistry. 2010;**285**(8):5941-5953. DOI: 10.1074/jbc.M109.039925

[23] Klionsky DJ, Emr SD. Autophagy as regulated pathway of cellular degradation. Science. 2000;**290**:1717-1721. DOI: 10.1126/science.290.5497.1717

[24] Kawabata T, Yoshimori T. Beyond starvation: An update on the autophagic machinery and its functions. Journal of Molecular and Cellular Cardiology. 2016;**95**:2-10. DOI: 10.1016/j.yjmcc.2015.12.005

[25] Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. Current Opinion in Cell Biology. 2010;**22**:124-131. DOI: 10.1016/j.ceb.2009.11.014

[26] Richter-Landsberg C, Leyk J. Inclusion body formation, macroautophagy, and the role of HDAC6 in neurodegeneration. Acta Neuropathologica. 2013;**126**:793-807. DOI: 10.1007/s00401-013-1158-x

[27] Lin L, Baehrecke EH. Autophagy, cell death, and cancer. Molecular and Cellular Biology. 2015;**2**(3):e985913. DOI: 10.4161/23723556.2014.985913

[28] Mathew R, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes & Development. 2007;**21**(11):1367-1381. DOI: 10.1101/gad.1545107

[29] Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annual Review of Biochemistry. 1996;**65**:801-847. DOI: 10.1146/annurev.bi.65.070196.004101

[30] Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. Nature Reviews. Molecular Cell Biology. 2008;**9**(9):679-690. DOI: 10.1038/nrm2468

[31] Buetow L, Huang DT. Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nature Reviews Molecular Cell Biology. 2016;**17**:626-642. DOI: 10.1038/nrm.2016.91

[32] Cohen-Kaplan V, et al. The ubiquitin-proteasome system and autophagy: Coordinated and independent activities. The International Journal of Biochemistry & Cell Biology. 2016;**79**:403-418. DOI: 10.1016/j.biocel.2016.07.019

[33] Nakaso K, Yoshimoto Y, Nakano T, et al. Transcriptional activation of p62/A170/ZIP during the formation of the aggregates: Possible mechanisms and the role in Lewy body formation in Parkinson's disease. Brain Research. 2004;**1012**:42-51. DOI: 10.1016/j.brainres.2004.03.029

[34] Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Molecular Cell. 2009;**33**:517-527. DOI: 10.1016/j.molcel.2009.01.021

[35] Gao Z, et al. Processing of autophagic protein LC3 by the 20S proteasome. Autophagy. 2010;**6**:126-137. DOI: 10.4161/auto.6.1.10928

[36] Robert T, Vanoli F, Chiolo I, Shubassi G, Bernstein KA, Rothstein R, et al. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature. 2011;**471**:74-79. DOI: 10.1038/nature09803

[37] Tomar D, et al. TRIM13 regulates ER stress induced autophagy and clonogenic ability of the cells. Biochimica et Biophysica Acta. 2012;**1823**:316-326. DOI: 10.1016/j.bbamcr.2011.11.015

[38] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;**461**(7267):1071-1078. DOI: 10.1038/nature08467

[39] Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science. 2008;**319**(5868):352-355. DOI: 10.1126/science.1140735

[40] Ciccia A, Elledge SJ. The DNA damage response: Making it safe to play with knives. Molecular Cell. 2010;**40**(2):179-204. DOI: 10.1016/j.molcel.2010.09.019

[41] Marechal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harbor Perspectives in Biology. 2013;**5**(9). DOI: 10.1101/cshperspect.a012716

[42] Ribezzo F, Shiloh Y, Schumacher B. Systemic DNA damage responses in aging and diseases. Seminars in Cancer Biology. 2016;**37-38**:26-23. DOI: 10.1016/j.semcancer.2015.12.005

[43] Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death and Differentiation. 2015;**22**(3):377-388. DOI: 10.1038/cdd.2014.150

[44] Shiloh Y. The ATM-mediated DNA-damage response: Taking shape. Trends in Biochemical Sciences. 2006;**31**:402-410. DOI: 10.1016/j.tibs.2006.05.004

[45] Cortez D, et al. ATR and ATRIP: Partners in checkpoint signaling. Science. 2001;**294**: 1713-1716. DOI: 10.1126/science.1065521

[46] Spry M, Scott T, Pierce H, D'Orazio JA. DNA repair pathways and hereditary cancer susceptibility syndromes. Frontiers in Bioscience. 2007;**12**:4191-4207

[47] Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(25): 15077-15082. DOI: 10.1073/pnas.2436255100

[48] Liang C, Feng P, Ku B, Dotan I, Canaani D, BH O, Jung JU. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. Nature Cell Biology. 2006;**8**(7):688-699. DOI: 10.1038/ncb1426

[49] Karantza-Wadsworth V et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes & Development. 2007;**21**(13):1621-1635. DOI: 10.1101/gad.1565707

[50] White E. The role of autophagy in cancer. The Journal of Clinical Investigation. 2015; **125**(1):42-46. DOI: 10.1172/JCI73941

[51] Takamura A et al. Autophagy-deficient mice develop multiple liver tumors. Genes & Development. 2011;**25**(8):795-800. DOI: 10.1101/gad.2016211

[52] Bae H, Guan JL. Suppression of autophagy by FIP200 deletion impairs DNA damage repair and increases cell death upon treatments with anticancer agents. Molecular Cancer Research. 2011;**9**:1232-1241. DOI: 10.1158/1541-7786.MCR-11-0098

[53] Scheibye-Knudsen M et al. Cockayne syndrome group B protein prevents the accumulation of damaged mitochondria by promoting mitochondrial autophagy. The Journal of Experimental Medicine. 2012;**209**:855-869. DOI: 10.1084/jem.20111721

[54] Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death and Differentiation. 2007;**14**:500-510. DOI: 10.1038/sj.cdd.4402039

[55] Bordin DL, Lima M, Lenz G, Saffi J, Meira LB, Mesange P, et al. DNA alkylation damage and autophagy induction. Mutation Research. 2013;**753**:91-99. DOI: 10.1016/j.mrrev.2013.07.001

[56] Rieber M, Rieber MS. Sensitization to radiation-induced DNA damage accelerates loss of Bcl-2 and increases apoptosis and autophagy. Cancer Biology & Therapy. 2008;**7**:1561-1566

[57] Katayama M, Kawaguchi T, Berger MS, Pieper RO. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death and Differentiation. 2007;**14**(3):548-558. DOI: 10.1038/sj.cdd.4402030

[58] Alexander A, Kim J, Walker CL. ATM engages the TSC2/mTORC1 signaling node to regulate autophagy. Autophagy. 2010;**6**:672-673. DOI: 10.4161/auto.6.5.12509

[59] Alexander A, et al. ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:4153-4158. DOI: 10.1073/pnas.0913860107

[60] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;**115**:577-590

[61] Zhao M, Klionsky DJ. AMPK-dependent phosphorylation of ULK1 induces autophagy. Cell Metabolism. 2011;**13**:119-120. DOI: 10.1016/j.cmet.2011.01.009

[62] Desantis A. Che-1-induced inhibition of mTOR pathway enables stress-induced autophagy. The EMBO Journal. 2015;**34**(9):1214-1230. DOI: 10.15252/embj.201489920

[63] Mammucari C, et al. FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metabolism. 2007;**6**:458-471. DOI: 10.1016/j.cmet.2007.11.001

[64] Salminen A, Kaarniranta K. Regulation of the aging process by autophagy. Trends in Molecular Medicine. 2009;**15**:217-224. DOI: 10.1016/j.molmed.2009.03.004

[65] Chiacchiera F, Simone C. The AMPK-FoxO3A axis as a target for cancer treatment. Cell Cycle. 2010;**15, 9**(6). DOI: 10.4161/cc.9.6.11035

[66] Munoz-Gamez JA et al. PARP-1 is involved in autophagy induced by DNA damage. Autophagy. 2009;**5**(1):61-74

[67] Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:8204-8209. DOI: 10.1073/pnas.0502857102

[68] Valbuena A, Castro-Obregón S, Lazo PA. Down-regulation of VRK1 by p53 in response to DNA damage is mediated by the autophagic pathway. PLoS One. 2011;**6**:e17320. DOI: 10.1371/journal.pone.0017320

[69] Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. Biochimica et Biophysica Acta. 2010;**1804**:1684-1689. DOI: 10.1016/j.bbapap.2010.05.002

[70] Fang EF, et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/ SIRT1 reduction. Cell. 2014;**157**:882-896. DOI: 10.1016/j.cell.2014.03.026

[71] Qiang L. Autophagy positively regulates DNA damage recognition by nucleotide excision repair. Autophagy. 2016;**12**(2):357-368. DOI: 10.1080/15548627.2015.1110667

[72] Zeng X, Kinsella TJ. A novel role for DNA mismatch repair and the autophagic processing of chemotherapy drugs in human tumor cells. Autophagy. 2007;**3**:368-370. DOI: 10.4161/auto.4205

[73] Zhao Z, et al. A dual role for UVRAG in maintaining chromosomal stability independent of autophagy. Developmental Cell. 2012;**22**(5):1001-1016. DOI: 10.1016/j.devcel.2011.12.027

[74] Park JM. Beclin 1 and UVRAG confer protection from radiation-induced DNA damage and maintain centrosome stability in colorectal cancer cells. PLoS One. 2014;**9**(6):e100819. DOI: 10.1371/journal.pone.0100819

[75] Bitto A. P62/SQSTM1 at the interface of aging, autophagy, and disease. Age. 2014;**36**(3): 9626. DOI: 10.1007/s11357-014-9626-3

[76] Nagaoka U. Increased expression of p62 in expanded polyglutamine-expressing cells and its association with polyglutamine inclusions. Journal of Neurochemistry. 2004;**91**(1): 57-68. DOI: 10.1111/j.1471-4159.2004.02692.x

[77] Rockel TD, Stuhlmann D, von Mikecz A. Proteasomes degrade proteins in focal subdomains of the human cell nucleus. Journal of Cell Science. 2005;**118**(Pt 22):5231-5242. DOI: 10.1242/jcs.02642

[78] Doil C, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446. DOI: 10.1016/j.cell.2008.12.041

[79] Mattiroli F, et al. RNF168 ubiquitinates K13-15 on H2A/ H2AX to drive DNA damage signaling. Cell. 2012;**150**:1182-1195. DOI: 10.1016/j.cell.2012.08.005

[80] Stewart GS, et al. RIDDLE immunodeficiency syndrome is linked to defects in 53BP1-mediated DNA damage signaling. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:16910-16915. DOI: 10.1073/pnas.0708408104

[81] Fradet-Turcotte A, et al. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature. 2013;**499**:50-54. DOI: 10.1038/nature12318

[82] Chen S, et al. RAD6 promotes homologous recombination repair by activating the autophagy- mediated degradation of heterochromatin protein HP1. Molecular and Cellular Biology. 2015;**35**:406-416. DOI: 10.1128/MCB.01044-14

[83] Haddad DM, et al. Mutations in the intellectual disability gene Ube2a cause neuronal dysfunction and impair parkin-dependent mitophagy. Molecular Cell. 2013;**50**(6): 831-843. DOI: 10.1016/j.molcel.2013.04.012

[84] Liu EY, et al. Loss of autophagy causes a synthetic lethal deficiency in DNA repair. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(3):773-778. DOI: 10.1073/pnas.1409563112

[85] Cuervo M. Chaperone-mediated autophagy: Selectivity pays off. Trends in Endocrinology and Metabolism. 2010;**21**:142-150. DOI: 10.1016/j.tem.2009.10.003

[86] Park C, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. Nature Communications. 2015;**6**:6823. DOI: 10.1038/ncomms7823

[87] Yang Y, et al. Autophagic UVRAG promotes UV-induced photolesion repair by activation of the CRL4(DDB2) E3 ligase. Molecular Cell. 2016;**62**:507-519. DOI: 10.1016/j.molcel.2016.04.014

[88] Xiao J, et al. FBXL20-mediated Vps34 ubiquitination as a p53 controlled checkpoint in regulating autophagy and receptor degradation. Genes & Development. 2015;**29**:184-196. DOI: 10.1101/gad.252528.114

Chapter 7

Interlace between Chromatin Structure, DNA Repair and Ubiquitination

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Peter John

Additional information is available at the end of the chapter

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Abstract

Chromatin remodeling, ubiquitylation, and DNA damage repair may be regarded as three discrete processes, but in fact, they are three extremely important interlinked processes that are imperative for the sustenance for life. Discrepancies in one will have outcomes that will affect the other processes direly. Exogenous and endogenous factors persistently affect the DNA by inducing damage and modifications. To sustain the integrity of life, these challenges need to be combated efficiently. For the preservation of the structural and functional components of the genome, nature has allowed them to evolve numerous pathways that constantly work to repair the induced damage. This sort of response is termed as DDR (DNA damage response) that include BER and NER (base excision and nucleotide excision repair, respectively) and non-homologous end joining and homologous recombination (NHEJ & HR). Since the DNA in cells is exceedingly organized and compressed, hence any process that utilizes DNA as its substrate requires essential remodeling of the chromatin structure. The chapter emphasizes on the phenomenon of chromatin remodeling and ubiquitylation which subsequently affects the integral process of DNA damage repair.

Keywords: chromatin remodeling, ubiquitylation, DDR, NHEJ, HR, BER, NER, exogenous and endogenous factors

1. Introduction

The dynamic structure of chromatin not only aids in wrapping the entire colossal genome into the boundaries of the nucleus but also plays an imperative role in regulating the accessibility of the DNA for various processes and mechanisms like recombination, transcription, replication, and repair. At the cytological level, the structure of nucleosome may appear inflexible; however, the repeating subunits of chromatin are highly dynamic and flexible in nature [1].

Chromatin is an intricate macromolecular structure that is basically found in cells, which consist of DNA, protein, and RNA. The protein component of chromatin is the histones, which are primarily responsible for the compaction of DNA [2]. The main functions of chromatin include packaging of DNA into a compact shape, reinforcement of the DNA molecules in such a way to allow the process of mitosis and controlling gene expression and DNA replication. The compaction of chromatin can vary depending on the type of cell and the phase of cell cycle that the cell is in. Chromatin in the nucleus can exist as euchromatin or heterochromatin. At the time of interphase cell is not dividing actively, this is euchromatin which is in less packed and compact form. DNA is usually exposed in euchromatin form and processes like replication, and transcription can take place readily. However, a small amount of chromatin exists as heterochromatin. It consists of repeating units known as nucleosomes which consist of around 150 bp of DNA wrapped around a core of octamer of histones. Core consists of two of each of the following subunits H2A, H2B, H3, and H4. The DNA is tightly packed and is not in an unwind state to facilitate the processes like replication, gene transcription, etc. During staining procedures, heterochromatin stains more darkly than euchromatin [3].

Histones play an important role in maintaining the dynamicity of the chromatin structure. Histone exchange is a process that is utilized by the cell to maintain the dynamicity and subtlety of the chromatin structure. The process involves the removal of entire nucleosome or some designated part of it which is trailed by replacement with newly synthesized histones or different components of it. This crucial mechanism of swapping is commonly known as histone turnover. Histone turnover has dominant applications in sustaining the structure composition and functions of different expanses of the genome. For instance, hyperactive exchange of histones will lead to an eventual increase in the accessibility of a specific genomic area to the different components of the cell such as the enzyme DNA polymerase II, thus facilitating and enabling the process of transcription. Nevertheless, if the components of the nucleosome are replaced with other alternatives which are not compatible with cellular processes and hinder subsequent exchange, then this will hinder the availability of DNA for important processes like transcription. A category of histones known as canonical histones can be potentially replaced by histone variants but alter both the physical and chemical structure of nucleosome ultimately direly affecting various cellular process discreetly. Factors that regulate the exchange of histones during transcription include PTMs, chromatin remodelers, and histone chaperones which work individually or in accordance with each other [1].

Histones are one of the most copiously ubiquitinated proteins. The ubiquitination of the histones plays a crucial role in many processes undergoing in the nucleus. The processes include transcription, maintenance, and regulation of the chromatin structure along with DNA repair [4]. The protein ubiquitin is involved in the process of ubiquitination. Ubiquitin is a regulatory protein weighing 8.5 kDa found in many tissues of eukaryotic organism which was discovered back in 1975 [5] by Gideon Goldstein and was further characterized and categorized throughout the span of 1970s and 1980s [6]. It is encoded by a total of four genes in the human genome, namely UBB, UBC, UBA52, and RPS27A [7]. The addition of the regulatory protein ubiquitin to a substrate protein is known as ubiquitination. The process of ubiquitination is known to affect proteins in many ways: it usually marks them for destruction or degradation via the proteasome pathway and it is also known to change the cellular location of the proteins and sometimes promoting or inhibiting various protein interactions along with playing an important role in signal transduction and protein trafficking [8–10]. Ubiquitination involves three main steps: activation, conjugation, and ligation. These three major steps are performed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s), respectively. The result of this consecutive cascade of reactions is finally the binding of ubiquitin to lysine on the substrate protein by an isopeptide bond, cysteine residues through a thioester bond, serine and threonine residues via an ester bond, or the amino group of the protein's N-terminus via a peptide bond resulting in one of the fates mentioned above [11–13].

The process of DNA repair is closely linked to histone ubiquitination, deubiquitination, and chromatin remodeling [4, 14]. DNA repair is a systematic process by which the cells of body recognizes and consequently corrects the damage to which DNA molecules are exposed that subsequently encodes the genome of an organism. In case of the human cells, DNA damage can be caused by abnormal cell cycle, metabolic activities as well as environmental factors such as radiations especially the UV radiation. All these can cause as many as 1 million lesions and abrasions to the DNA in a single day [15]. These lesions are responsible for causing basic impairment and damage to the DNA molecule which resultantly removes or changes the ability of the cells to transcribe a gene that the affected part of the DNA encodes. Furthermore, other lesions may be able to induce destructive mutations in the genome of a cell consequently disturbing the overall survival of the progeny cells as it undergoes mitosis. Resultantly, the repair processes for DNA are continuously active because they retort to the damages in the DNA structure. When these normal repair processes of the DNA molecules fail to repair the damage and when programmed cellular death (apoptosis) does not take place, irreversible DNA occurs which include cross linkages also known as inter-stand cross links, double and single breaks in the DNA molecule which will eventually lead to the formation of malignant tumors [16, 17], or other sort of cancers according to the two-hit hypothesis. Like all other processes, DNA repair is also dependent on factors, including type and age of cell and extracellular environment of the cell. A cell that has hoarded a great amount of damaged DNA or a cell that no longer effectively repairs the damaged DNA will face one of the three possible fates:

1. An irreversible state of latency, known as senescence.
2. Suicide of the cell, also known as apoptosis.
3. Unchecked cell division leading tumor formation that can eventually lead to cancer.

Repairing of damaged DNA is vital for the maintenance of the integrity of the genome and to preserve the normal functioning of the genome [18].

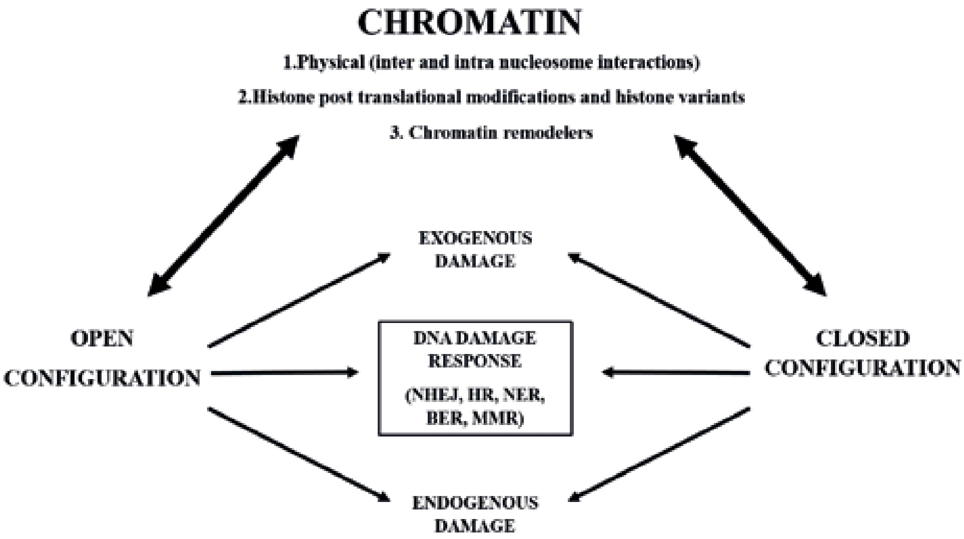

Figure 1. Interplay between chromatin rearrangement and DNA damage response, adapted from [19].

Figure 1 explains the close association between DNA damage response and repair and remodeling of the chromatin. As the subtleties of the chromatin structure play a part in maintaining the stability of the genome, intra and internucleosomal interactions along with post translational modifications of histones, histone variants, and the function of ATP-dependent chromatin remodelers all collectively play their due share in controlling and maintaining the structural assembly of the chromatin. Collectively, all these factors safeguard appropriate and suitable chromatin conformation during different stages of the diverse cell cycle and during numerous DNA templated processes. The OPEN state is vulnerable to both external and internal damage consequently leading to increased DDR. The CLOSED state on the other hand successfully overpowers the dual genomic invectives and acts as a determent to DDR. The closed state also hinders various processes such as transcription, recombination repair, etc. Hence the subtleties of the chromatin arrangement aid not only in repairing DNA lesions and damages but also in permitting access to cellular machineries to accomplish processes that depend on DNA, thus ensuring the maintaining of the steadiness of the genome [19].

In this chapter, we aim to explain the link between these three extremely important processes as current research advances have defined central roles of all the three processes, histone ubiquitination, remodeling of chromatin, and DNA repair.

2. Mechanism of gene activation and regulation

2.1. DNA repair and chromatin remodeling

Chromatin decondensation and reorganization has a crucial role in all cellular processes that use DNA as template or substrate like DNA repair mechanism, replication, and transcription. For example, base excision repair (BER) that requires the removal of altered or damaged base relies in chromatin remodeling, similarly for the nucleotide excision repair (NER) that counteracts with helix distorting lesions caused by UV radiations [20]. NER has two modes of action which are dependent on the nature of lesion caused, the transcription coupled-NER (TC-NER) only operates in genes that are transcriptionally active where polymerase-II triggers the DNA damage response while the global genomic NER branch (GG-NER) operates when lesion is in chromatin environment but both of the pathways fill the gap by same core machinery [21, 22]. The open and compact structure of chromatin affects the activation and efficiency of DNA damage response (DDR), as it is difficult for repair proteins to reach a damaged structure in compact or highly condensed chromatin. In case of a double stranded break (DSB), chromatin relaxation along with the recruitment of break-sensing proteins at the damaged site is induced via an ATP-dependent mechanism that works independently of DDR kinases [23].

The ATP-dependent chromatin remodeling enzymes are a source of chromatin reorganization and transformations. The Snf2- or SWI/SNF enzymes, that were first discovered as chromatin remodeling enzymes during the characterization of yeast, consists of a conserved sequence of seven amino acids which is present in all eukaryotes [24, 25]. Depending on the sequence homology in the ATPase core, the Snf2 proteins have been assigned 24 subfamilies [26]. These chromatin remodeling enzymes interact with each other and induce a range of chromatin transformations such as histone octamer sliding across DNA, change in nucleosomal DNA conformation, and composition of histone octamer. DNA is tightly bound to histone octamer, which is disrupted by chromatin remodeling which is disrupted by chromatin remodeling enzymes during chromatin de-condensation and reorganization [27].

However, DDR kinase-dependent chromatin changes promote the local environment favorable for DNA repair mechanism of which the most important is regulation of nuclear organization. Studies in yeast suggest that there are repair centers for DSB repair; however, the unrepairable DSBs move towards the nuclear periphery, and these are merged into a single repair focus [28]. Moreover, increased mobility of chromatin has been observed in yeast nuclei as a consequence of DSB which increases the DNA repair efficiency, and this movement is attributed to Mec1ATR kinase, RAD51recombinase, and resection of DNA end [29, 30]. RAD51-coated DNA is efficient in finding its homologous sequence [31], thereby promoting the repair machinery to act. Double stranded breaks (DSBs) are either repaired by error-free homologous recombination (HR) that involves sister chromatids or by error-prone nonhomologous end joining (NHEJ) that involves the damage recognition by Ku70/80 that bind to damaged DNA and recruit DNA-PK, a serine/threonine protein kinase that induce conformational changes on damage site after which protein kinase ataxia-telangiectasia, mutated (ATM), and ATM and Rad3-related protein (ATR) are recruited that interact with XRCC4 and DNA ligase IV that proceed to DNA relegation as shown in **Figure 2** [22, 23, 24, 33].

The damaged region of heterochromatin moves toward the outside boundary as a consequence of heterochromatin expansion caused by the break and DDR kinases that lead to RAD51 dependent homology search [35]. The exact mechanism is still not known, but DDR kinases have been observed to modify nucleoporins that in turn breaks the interaction of chromosome and pores [36]. Another possibility is the phosphorylation of KAP1 that binds heterochromatin protein HP1 and chromatin remodeling factors like INO80 and H2A are recruited which

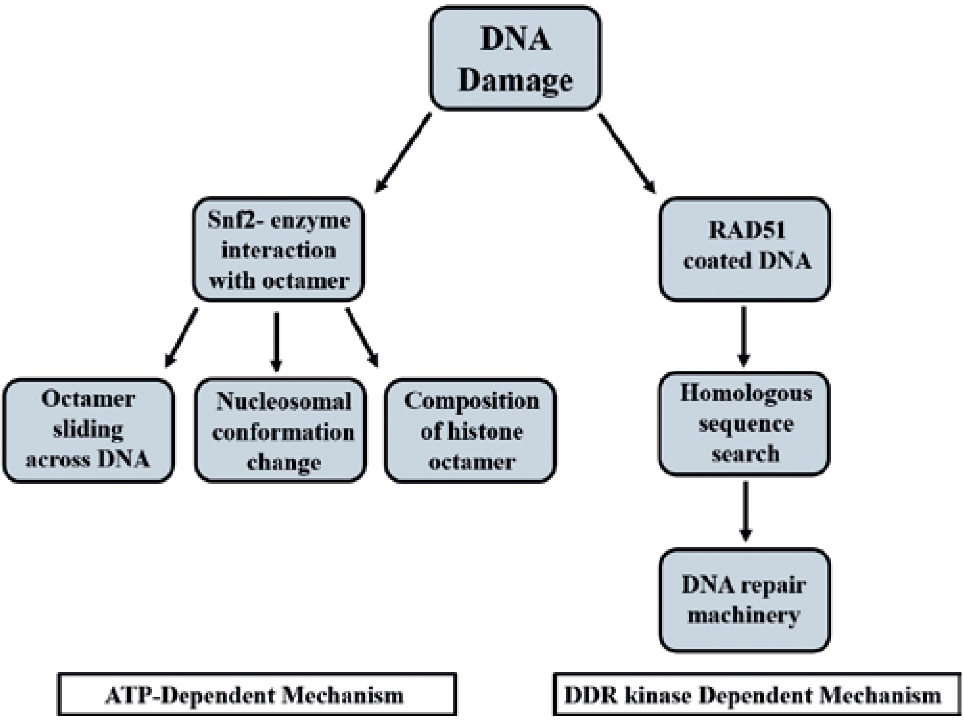

Figure 2. DSBs induces histone modification and DDR.

facilitate the mobility and repair machinery [37–39]. Damage recognition is highly dependent on chromatin conformation changes and signaling cascades based on phosphorylation, ubiquitylation, and PARylation, these pathways also facilitate DDR by halting cell cycle.

2.2. Chromatin ubiquitylation

Amongst chromatin ubiquitination, modification of H2A, core histones, H2B, H3, H4, and linker H1 are modified by ubiquitin. This modification of histone plays its vital role in transcriptional control and DDR [40]. The exact role and mechanism is still to be explored, but H2A ubiquitination has been proposed in chromatin folding [40, 41]. The ubiquitination of linker H1 occurs through TAFII250 that is a part of transcription factor TFIID [42].

2.3. DNA repair and histone ubiquitylation

Most common histone modification is histone ubiquitination that has been observed to play a vital role in DDR. Impairment of DNA repair has been identified as a major culprit as defense mechanism is evoked against cells that have cell cycle arrest, apoptosis, and DNA damage [43]. A DSB evokes the phosphorylation of H2AX at γ position and tracks the damage by ATM, ATR, and DNA-PK [44]. This phosphorylation facilitates the accumulation of Mdc1/NFBD1, RNF8, RNF168, and response regulators [45]. The K63-linked polyubiquitination on histone H2A and H2AX is catalyzed by RNF168 and RNF8 and acts as an recognition element that in turn recruits RAP80 which consequently recruits BRCA1 [46–52]. Apart from polyubiquitination, the monoubiquitination of histones H2A, H2B, and H2AX also occurs at DNA damage site. This monoubiquitination of histones is catalyzed by RING1B/BMI1 and RNF20/RNF40, moreover, the depletion of RNF20 disrupts monoubiquitination which ultimately halts the DNA repair machinery in both HR and NHEJ pathways [53].

Histone modification at DNA damage loci is ubiquitination of H2A histone, variant H2AX, and H1 linker histone [32, 49, 54]. MDC1-dependent recruitment of E3 ligase RNF8 along with Ubc13 catalyzes K63-linked polyubiquitination of histone H1 at DSBs [55]. This ubiquitinated histone H1 mediates the recruitment of E3 ligase RNF168 and RNF8 which triggers the catalysis of histones H2A and H2AX at lysine 13–15 [49, 54, 56, 57]. These histones provoke the effector proteins BRCA1 and 53BP1 to damage site promoting homologous recombination (HR). BRAC1 through its binding partner within BRCA1-A complex RAP80 tether to histone H2A and is considered essential for HR, whereas 53BP1 is a mediator of NHEJ **Figure 3** [34, 47, 52, 58].

Ubiquitination is a prominent feature of chromatin signaling in NER, as during GG-NER E3 ligases catalyze the ubiquitination of histone H2A by UV-RING1B complex, which has DDB1, DDB2, CUL4B, and the E3 ligase RING1B as subunits that operate the early damage recognition [59]. Ubiquitylation of lysine 119 of histone H2A is catalyzed and it provides an attachment platform for H2A-ubiquitin binding protein ZRF1. CUL4B-RBX1 subunits are removed, ZFR1 thus mediating UV-DDB CUL4A complex generation at damage site, and then factor XPC is polyubiquitylated which stabilizes it at the damaged site [59, 60]. This ubiquitylation of XPC acts as a timing device for damage recognition and verification [61]. Henceforth, the ubiquitination and deubiquitination of histones mediate the DDR and compaction of chromatin [53, 62].

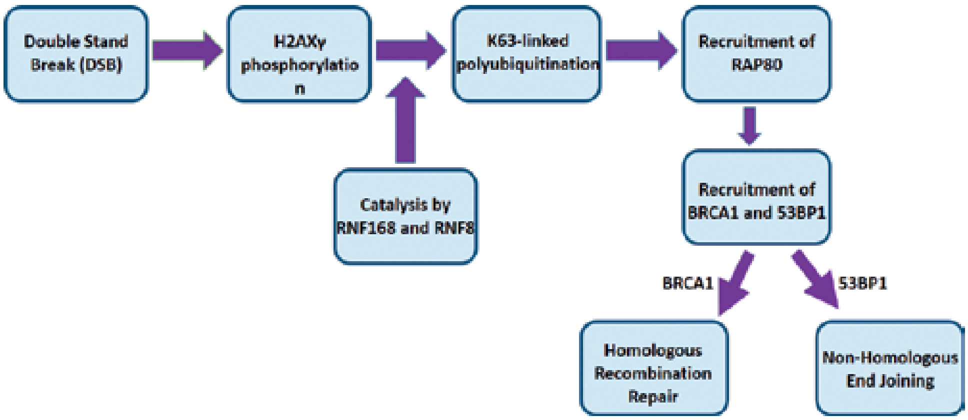

Figure 3. Chromatin remodeling and DNA repair.

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References

[1] Venkatesh S, Workman JL. Histone exchange, chromatin structure and the regulation of transcription. Nature Reviews Molecular Cell Biology. 2015;**16**(3):178

[2] Mondal T, Rasmussen M, Pandey GK, Isaksson A, Kanduri C. Characterization of the RNA content of chromatin. Genome Research. 2010;**20**(7):899-907

[3] Cooper GM. The cella molecular approach. Sunderland (ma) sinauer associates. Tools of Cell Biology. 6th ed. 2013. p. 108

[4] Cao J, Yan Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Frontiers in Oncology. 2012;**2**:26

[5] Goldstein G, Scheid M, Hammerling U, Schlesinger D, Niall H, Boyse E. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proceedings of the National Academy of Sciences. 1975;**72**(1): 11-15

[6] Wilkinson KD. The discovery of ubiquitin-dependent proteolysis. Proceedings of the National Academy of Sciences. 2005;**102**(43):15280-15282

[7] Kimura Y, Tanaka K. Regulatory mechanisms involved in the control of ubiquitin homeostasis. Journal of Biochemistry. 2010;**147**(6):793-798

[8] Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. Physiological Reviews. 2002;**82**(2):373-428

[9] Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science. 2007;**315**(5809):201-205

[10] Schnell JD, Hicke L. Non-traditional functions of ubiquitin and ubiquitin-binding proteins. Journal of Biological Chemistry. 2003;**278**(38):35857-35860

[11] Komander D, Rape M. The ubiquitin code. Annual Review of Biochemistry. 2012;**81**: 203-229

[12] McDowell GS, Philpott A. Non-canonical ubiquitylation: Mechanisms and consequences. The International Journal of Biochemistry & Cell Biology. 2013;**45**(8):1833-1842

[13] Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. BBA Molecular Cell Research. 2004;**1695**(1-3):55-72

[14] Price BD, D'Andrea AD. Chromatin remodeling at DNA double-strand breaks. Cell. 2013;**152**(6):1344-1354

[15] Lodish HF. Molecular Cell Biology. New York: W.H. Freeman and Company; 2004

[16] Acharya P. The isolation and partial characterization of age-correlated oligo-deoxyribo-ribonucleotides with covalently linked aspartyl-glutamyl polypeptides. Johns Hopkins Medical Journal. 1972;(Suppl. 1):254-260

[17] Bjorksten J, Acharya P, Ashman S, Wetlaufer DB. Gerogenic fractions in the tritiated rat. Journal of the American Geriatrics Society. 1971;**19**(7):561-574

[18] Browner WS, Kahn AJ, Ziv E, Reiner AP, Oshima J, Cawthon RM, et al. The genetics of human longevity. The American Journal of Medicine. 2004;**117**(11):851-860

[19] Nair N, Shoaib M, Sørensen CS. Chromatin dynamics in genome stability: Roles in suppressing endogenous DNA damage and facilitating DNA repair. International Journal of Molecular Sciences. 2017;**18**(7):1486

[20] de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. Genes & Development. 1999;**13**(7):768-785

[21] Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammalian cells: Molecular mechanisms and biological effects. Cell Research. 2008;**18**(1):73

[22] Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH. Understanding nucleotide excision repair and its roles in cancer and ageing. Nature Reviews. Molecular Cell Biology. 2014;**15**(7):465

[23] Kruhlak MJ, Celeste A, Dellaire G, Fernandez-Capetillo O, Müller WG, McNally JG, et al. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. The Journal of Cell Biology. 2006;**172**(6):823-834

[24] Côté J, Quinn J, Workman JL, Peterson CL. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science. 1994;**265**(5168):53-60

[25] Gorbalenya AE, Koonin EV. Helicases: Amino acid sequence comparisons and structure-function relationships. Current Opinion in Structural Biology. 1993;**3**(3):419-429

[26] Flaus A, Martin DM, Barton GJ, Owen-Hughes T. Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Research. 2006;**34**(10): 2887-2905

[27] Narlikar GJ, Sundaramoorthy R, Owen-Hughes T. Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. Cell. 2013;**154**(3):490-503

[28] Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, et al. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science. 2008;**322**(5901):597-602

[29] Dion V, Kalck V, Horigome C, Towbin BD, Gasser SM. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. Nature Cell Biology. 2012;**14**(5):502

[30] Miné-Hattab J, Rothstein R. Increased chromosome mobility facilitates homology search during recombination. Nature Cell Biology. 2012;**14**(5):510

[31] Forget AL, Kowalczykowski SC. Single-molecule imaging of DNA pairing by RecA reveals a three-dimensional homology search. Nature. 2012;**482**(7385):423

[32] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;**461**(7267):1071

[33] Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annual Review of Biochemistry. 2010;**79**:181-211

[34] Marnef A, Legube G. Organizing DNA repair in the nucleus: DSBs hit the road. Current Opinion in Cell Biology. 2017;**46**:1-8

[35] Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes SV, Karpen GH. Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell. 2011;**144**(5):732-744

[36] Bermejo R, Capra T, Jossen R, Colosio A, Frattini C, Carotenuto W, et al. The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell. 2011;**146**(2):233-246

[37] Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Löbrich M, Jeggo PA. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Molecular Cell. 2008;**31**(2):167-177

[38] Kalocsay M, Hiller NJ, Jentsch S. Chromosome-wide Rad51 spreading and SUMO-H2A. Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Molecular Cell. 2009;**33**(3):335-343

[39] Neumann FR, Dion V, Gehlen LR, Tsai-Pflugfelder M, Schmid R, Taddei A, Gasser SM. Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. Genes & Development. 2012;**26**(4):369-383

[40] Zhang Y. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes & Development. 2003;**17**(22):2733-2740

[41] Jason LJ, Moore SC, Lewis JD, Lindsey G, Ausió J. Histone ubiquitination: A tagging tail unfolds? BioEssays. 2002;**24**(2):166-174

[42] Pham A-D, Sauer F. Ubiquitin-activating/conjugating activity of TAFII250, a mediator of activation of gene expression in drosophila. Science. 2000;**289**(5488):2357-2360

[43] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001;**411**(6835):366

[44] Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature. 2005;**434**(7033):605

[45] Stewart GS, Wang B, Bignell CR, Taylor AMR, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature. 2003;**421**(6926):961

[46] Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell. 2007;**131**(5):901-914

[47] Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science. 2007;**316**(5828):1202-1205

[48] Klose RJ, Yan Q, Tothova Z, Yamane K, Erdjument-Bromage H, Tempst P, et al. The retinoblastoma binding protein RBP2 is an H3K4 demethylase. Cell. 2007;**128**(5):889-900

[49] Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**(5):887-900

[50] Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science. 2007;**316**(5828):1198-1202

[51] Wang B, Elledge SJ. Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. Proceedings of the National Academy of Sciences. 2007;**104**(52):20759-20763

[52] Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science. 2007;**316**(5828):1194-1198

[53] Moyal L, Lerenthal Y, Gana-Weisz M, Mass G, So S, Wang S-Y, et al. Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. Molecular Cell. 2011;**41**(5):529-542

[54] Pan M-R, Peng G, Hung W-C, Lin S-Y. Monoubiquitination of H2AX protein regulates DNA damage response signaling. Journal of Biological Chemistry. 2011;**286**(32):28599-28607

[55] Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature. 2015;**527**(7578):389-393

[56] Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**(3):435-446

[57] Mattiroli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell. 2012;**150**(6):1182-1195

[58] Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. Nature Reviews. Molecular Cell Biology. 2014;**15**(1):7

[59] Gracheva E, Chitale S, Wilhelm T, Rapp A, Byrne J, Stadler J, et al. ZRF1 mediates remodeling of E3 ligases at DNA lesion sites during nucleotide excision repair. The Journal of Cell Biology. 2016;**213**(2):185-200

[60] Sugasawa K, Okuda Y, Saijo M, Nishi R, Matsuda N, Chu G, et al. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Cell. 2005; **121**(3):387-400

[61] Chitale S, Richly H. Timing of DNA lesion recognition: Ubiquitin signaling in the NER pathway. Cell Cycle. 2017;**16**(2):163-171

[62] Shao G, Lilli DR, Patterson-Fortin J, Coleman KA, Morrissey DE, Greenberg RA. The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. Proceedings of the National Academy of Sciences. 2009;**106**(9):3166-3171

Maintenance of Genome Stability by Ubiquitination of DNA Repair Proteins in Mammalian Development and Disease

Mikio Shimada

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/

Abstract

To maintain genome DNA, DNA repair machinery has been developed in cellular life cycle. Multiple DNA repair pathways such as base excision repair, nucleotide excision repair, DNA cross link damage repair, DNA single strand break repair and DNA double strand break repair including nonhomologous end joining and homologous recombination are regulated by protein signal cascade. Because of limited gene number, protein posttranslational modification signal has advantage to control cell dynamics during development and senescence. This chapter focuses on how DNA repair proteins molecular modification including phosphorylation and ubiquitination contribute to genome stability pathway during mammalian development and disease.

Keywords: DNA repair, BRCA1, FA pathway, NBS1, mammalian development, inherited disease

1. Introduction

Genome DNA is damaged by several environmental factors such as ionizing radiation (IR), ultraviolet (UV), environmental mutagen and metabolic products including reaction oxygen species (ROS). It is well known that IR induces base damage, DNA cross link and DNA strand breaks defined as, single strand breaks (SSB) and double strand breaks (DSB) [1] For instance, 1Gy gamma-ray irradiation induces 1000 SSBs and 40 DSBs per cells [2] Although base damage causes genome DNA mutation leading to cancer, DSB is a catastrophic damage that leads to severe chromosome breakage and cell death. To prevent this, in mammalian cells, there are two major DSB repair pathway: nonhomologous end joining (NHEJ) repair [3] and homologous recombination (HR) repair [4] (**Figure 1**). NHEJ repair joint DNA damage ends directly and act as dominant repair pathway through cell cycle. HR repair is a precise pathway to repair completely with sister chromatid during S-G2 phase.

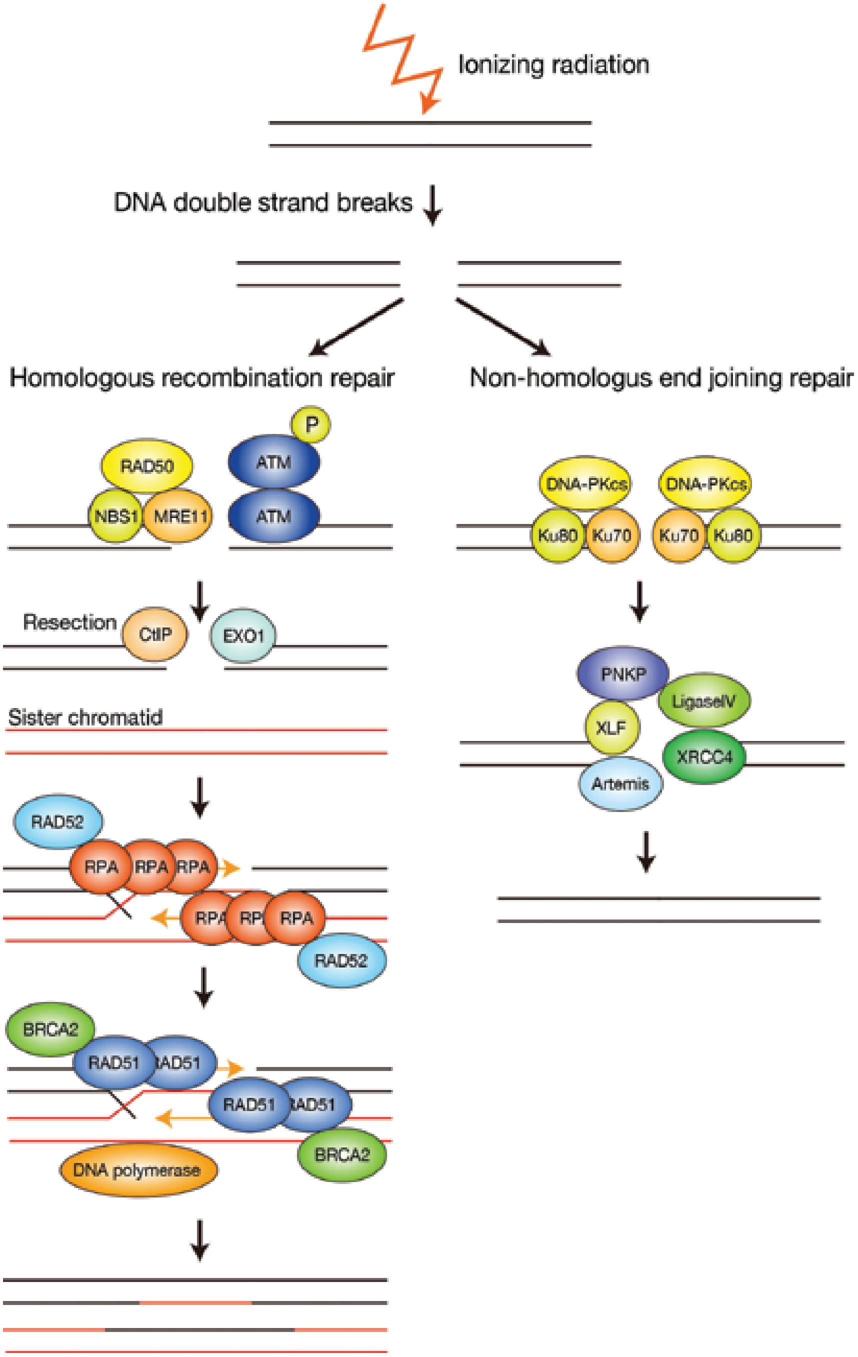

Figure 1. Schematic model of HR repair and NHEJ repair.

Both pathways cooperate to maintain genome DNA stability. Defect or depletion of related proteins of DNA repair result in hypersensitivity to the IR, severe developmental failure especially central nervous system and predisposition to the cancer. Recently, it is reported that abnormal expression of DSB repair proteins causes neurodegeneration, and small head phenotype called microcephaly. Neural stem cells and progenitors actively proliferate and produce ROS from mitochondria respiration [5, 6], which attack genome DNA. Decreasing of DNA repair activity is critical for cell survival. Furthermore, atomic bomb survivors show microcephaly when they are exposed in the womb [7]. DNA damage and centrosome amplification are critical for mammalian embryonic brain development [8, 9]. To regulate DNA repair machinery, several protein posttranslational modification systems such as phosphorylation, ubiquitination, SUMOylation, and NEDDylation are involved [10]. In this chapter, to identify how DNA repair signaling pathways are involved in mammalian development and disease, ubiquitination system in DNA repair machinery are focused on and discussed.

1.1. DSB NHEJ repair pathway

DNA-PKcs, Ku70, Ku80 are main components of NHEJ pathway and recruited to DNA damaged ends immediately when DSB occurred. Binding of Ku70/Ku80 to the DNA ends is important for protection from resection and recognition as telomere ends [11, 12]. DNA-PKcs act as a signal inducer by phosphorylation of several substrates [13]. DNA-PKcs also phosphorylates itself to activate this pathway. Subsequently, XRCC4 is recruited to damage sites as scaffold and then related proteins such as XLF, PAXX and Artemis are accumulated [14]. Artemis has endonuclease activity to resect DNA ends to facilitate DNA ligation. Polynucleotide kinase phosphatase (PNKP) is also recruited to DNA ends to remove 3′-P groups or add 5′-P residues for ligation [15, 16]. Finally, DNA ligase IV joint DNA ends. Because of broken ends by physical damage and resection by Artemis, NHEJ pathway sometimes generates DNA mutation and deletion. NHEJ pathway is not only important for DNA repair pathway, but also immune-systems, V(D)J recombination [17, 18]. Human patients with deficiency of Artemis, XLF, Ligase IV show severe combined immunodeficiency (SCID) and microcephaly phenotype. To investigate the role of DNA repair machinery in central nervous system, brain-specific conditional knockout mice of Ligase IV and PNKP were generated [19]. These mice show severe DNA damage and apoptosis in the cerebral cortex during embryonic development. This suggests that NHEJ repair machinery is important for genome maintenance of neural stem cells and progenitors during brain development.

1.2. DSB HR repair pathway

HR repair is a precise repair pathway that requires sister chromatid as DNA template. Ataxia telangiectasia mutated (ATM) and MRE11/RAD50/NBS1 (MRN complex) are accumulated to DNA damage sites as DNA damage sensor and ATM phosphorylates ATM itself (auto-phosphorylation) to activate HR signal [20, 21]. ATM is master regulator of DNA damage response signaling and has many substrates such as p53, SMC1, NBS1, MDC1, 53BP1 and CHK2. ATM regulates G1/S, S and G2/S cell cycle checkpoint. Ataxia telangiectasia (AT) patients show hypersensitivity to the IR, immunodeficiency, predisposition to malignancy and progressive cerebellar neurodegeneration. *MRE11* is responsible gene for AT like disorder (ATLD). ATLD patients show similar phenotype with AT. MRE11 has nuclease activity to generate 3′ ssDNA tails. Detail of NBS1 discussed below. After ATM activation, signal mediator such as BRCA1 and 53BP1 are accumulated depending on ubiquitination of histone protein by RNF8. RNF8 and RNF168 are key E3 ubiquitin ligase in HR pathway [22, 23]. Poly-ubiquitination of histone H1 by RNF8 is important for RNF168 recruitment to the DNA damage sites. Chromatin remodeling associates with DNA damage response to facilitate DNA repair. Histone protein modification is trigger of this process. NBS1 and ATM interact with E3 ubiquitin ligase RNF20 which mono-ubiquitinates H2B after DNA damage [24, 25]. RNF20 dependent H2B mono-ubiquitination is important process of DNA repair, because depletion of RNF20 by siRNA reduces accumulation of RAD51 and BRCA1 to the DNA damage sites. DNA exonucleases CtIP and Exo1 are recruited to damage sites to resect DNA ends [26]. Then, Replication protein A (RPA) and RAD52 binds to single strand DNA ends to protect DNA and replace with RAD51 and BRCA2 to promote DNA recombination [27, 28]. Simultaneously, cell cycle checkpoint proteins, p53, CHK1 and CHK2 activate to give repair time. HR factors are not only involved in DNA repair, but also meiosis.

Both DNA repair pathway are strictly regulated, and several E3 ubiquitination ligases are involved in these [29]. Defect of DNA repair machinery leads to chromosome aneuploidy and several diseases such as neurodegeneration, inflammation and cancer [30].

1.3. NHEJ and HR proteins and centrosomes

As mentioned earlier, NHEJ and HR proteins are important for DNA repair to maintain genome stability. In fact, defect of NHEJ and HR proteins leads to severe inherited disease such as immunodeficiency, neurodegeneration, developmental defect, predisposition to the malignancy. Recent reports uncover that NHEJ proteins and HR proteins localize centrosomes. Centrosome is an organelle consists of two centrioles surrounded by pericentriolar material (PCM) [31, 32]. γ-tubulin ring complex (γ-TuRC) attaches PCM to form microtubule extension. Centrosome plays pivotal role for the proper cell division [33]. Mammalian cells usually have one or two centrosomes depending on cell cycle. Ionizing radiation (IR) or some genotoxic reagents trigger centrosome amplification, which cause multipolar cell division and chromosome aneuploidy [8, 34–37]. Centrosome duplication is basically regulated cell cycle machinery. NHEJ factors such as DNA-PKcs localize centrosomes [38]. We found that DNA-Pkcs or Ku70 deficient murine embryonic fibroblast (MEF) cells show slight centrosome amplification compared with complementary cells. Meanwhile, HR factors such as ATM, NBS1, BRCA1, BRCA2 and Rad51 localize centrosome and depletion of these factors show significant centrosome amplification [39]. The role of NBS1 and BRCA1 in centrosome maintenance is discussed in the following section. ATM phosphorylates centrosome protein CEP63 to regulate spindle assembly after DNA damage [40]. Inhibition of ATM in RAD51 deficient cells shows centrosome amplification which means that ATM and RAD51 interaction is important for centrosome proper duplication [41]. BRCA2 interacts with NPM to form BRCA2-NPM complex to maintain centrosome duplication and cell division [42]. CHK1 is one of key regulator for cell cycle checkpoint to activate G2/M checkpoint. Depletion of CHK1 expression leads to centrosome amplification [41, 43]. After DNA damage, ATM- and Rad3-related (ATR) phosphorylates CHK1 to move to the centrosome from nucleus. ATR-dependent CHK1 translocation is important for centrosome duplication after DNA damage [44, 45]. Recent reports unveiled importance and molecular mechanism of HR factors in centrosome maintenance. However, function and physical means of NHEJ factors in centrosome still remain unclear.

2. Ubiquitination of DNA repair proteins and development

2.1. E3 ubiquitin ligase BRCA1

Breast and ovarian cancer gene, BRCA1 have multiple function in cell metabolism, including DNA repair, chromatin remodeling, microtubule maintenance and centrosome duplication. About 10% of women patients with breast cancer have inherited mutations in BRCA1 or BRCA2. BRCA1 forms heterodimer with BRCA1-associated RING domain (BARD1) to act as E3 ubiquitin ligase, which has several substrates including H2A, H2AX, RNA pol III, THIIE, NPM1, CtIP, ER-α and claspin [46–51] (**Figure 2**). Since BRCA1 can mono-ubiquitinates H2A and H2AX in vivo, it is believed that BRCA1 is required for chromatin remodeling after DNA damage [52, 53].

2.2. BRCA1 and centrosomes

BRCA1-BARD complex mono-ubiquitinates γ-tubulin at Lysine 48 and Lysine 344, which is the main component of centrosome [54–57]. Previously, we reported that Nijmegen breakage syndrome (*NBS*) gene and *ATR* gene products, NBS1 and ATR are involved in BRCA1 dependent γ-tubulin mono-ubiquitination to regulate centrosome duplication [58, 59]. Deficient of BRCA1 leads to centrosome amplification. Furthermore, BRCA1 and NBS1 are required for suppression of low dose rate IR dependent centrosome amplification [60]. This result suggests that BRCA1 keep genome integrity through cell cycle. So far, it is not known de-ubiquitinating enzymes (DUBs) of γ-tubulin. CP110 is a centriolar protein that regulates centrosome duplication. The level of CP110 is regulated by ubiquitination and de-ubiquitination by ubiquitin ligase complex SCF$^{\text{cyclinF}}$ and DUB USP33, respectively [61]. Destabilized CP110 levels by ubiquitination status lead to centrosome amplification and genome instability. Thus, balance of ubiquitination status is important. To identify DUBs of BRCA1-dependent γ-tubulin ubiquitination will contribute therapeutic strategies.

2.3. Mouse model of BRCA1

Since BRCA1 is involved in multiple cellular functions, complete defect of that leads to embryonic lethality. To identify the role of BRCA1 in mammalian development, conditional knockout mice were generated. Deletion of BRCA1 in mammary gland result in a phenotype of human basal like breast cancer [62, 63]. Central nervous system (CNS) specific BRCA1 knockout using nestin promoter resulted in microcephaly [64, 65]. Apoptotic cells were increased in brain layer structure during embryonic stage in BRCA1 brain specific KO mice. As another possibility, since genetic background such as Plk4 overexpression or genotoxic stress such as IR induce

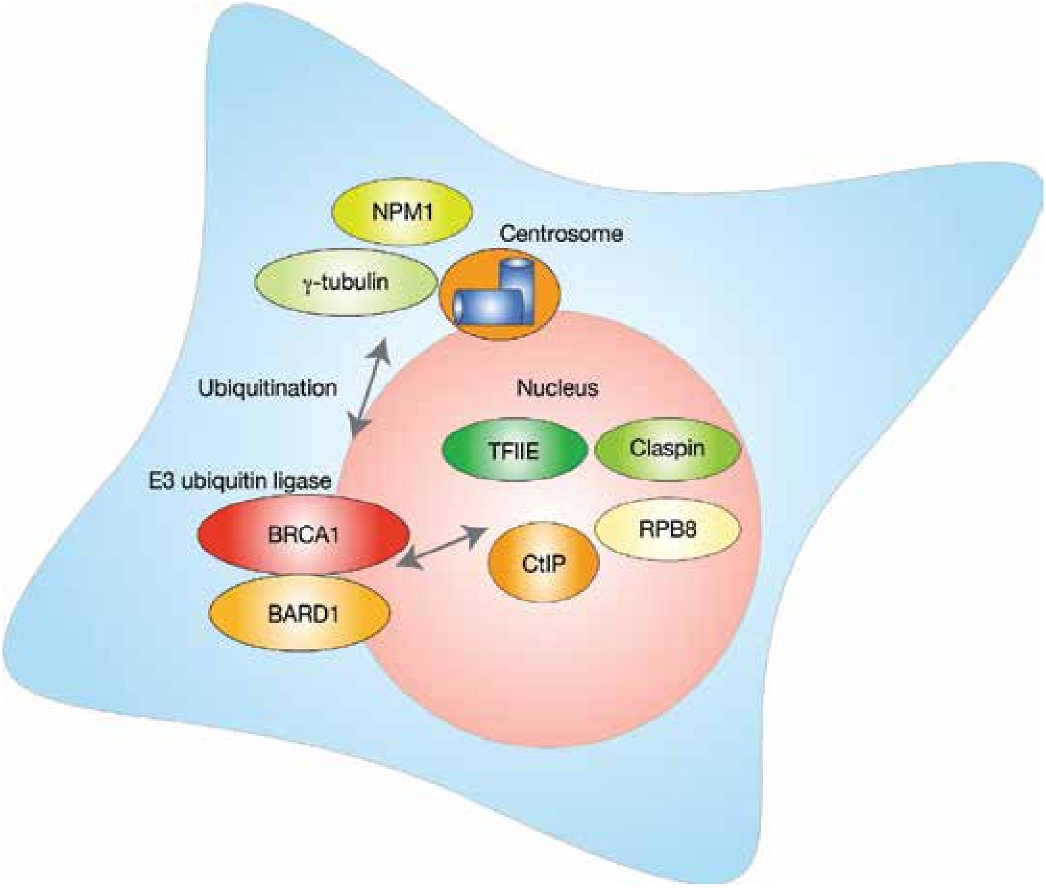

Figure 2. BRCA1 and its substrates.

centrosome amplification during CNS development result in microcephaly, centrosome amplification might be involved in microcephaly formation in BRCA1 deficient mouse brain [8, 66]. This result suggests that BRCA1 is important for genome maintenance in mammalian neural development.

2.4. Fanconi anemia (FA) pathway

Fanconi anemia (FA) is a hereditary disease clinically characterized as skeletal and visceral malformations, attrition of bone marrow stem cells [67, 68]. FA is firstly reported by Fanconi in 1927 and founded to be sensitive to DNA cross link damage by Sasaki et al. [69]. FA proteins pathway is important for inter cross link (ICL) DNA damage repair and HR repair [70, 71]. Currently, at least 21 FA proteins are reported. FANCD2 is a key player in FA pathway [72–75]. Mono-ubiquitination of FANCD2 at Lysine 561 by FA core complex is important event for activation of FA pathway. FA core complex consists by eight FA proteins (FANCA, B, C, E, F, G, L, M) and associated factors (FAAP100, FAAP24, FAAP20, MHF1 and MHF2). K561 mutated FANCD2 proteins cannot form DNA damage foci and localize to chromatin suggest that mono-ubiquitination of FANCD2 is essential event for DNA repair. FANCD2 forms heterodimer with FANCI, which is phosphorylated by ATR-ATRIP complex. Mono-ubiquitination of FANCD2 is de-ubiquitinated by USP1 after completion of DNA repair [76, 77]. Knockout mice of USP1 show FA like phenotype. This suggests that regulation of mono-ubiquitination level of FANCD2 is critical for DNA repair pathway [76, 77].

2.5. Mouse model of FA proteins

Knockout mice of FA genes show decreasing of fertility and chromosome breaks [78–80]. *Fancg* knockout mice show germ cell defects and decreasing of fertility. *Fancg*−/− cells display high sensitivity to the IR and DNA crosslink inducer mitomycin C (MMC). Fancd2−/− mice show more severe phenotype characterized by perinatal lethality microphthalmia and hypogonadism. Fancd2−/− mice are also prone to developing epithelial cancers than Fanca−/−, Fancc−/− and Fancg−/− mice [78, 79, 81–84].

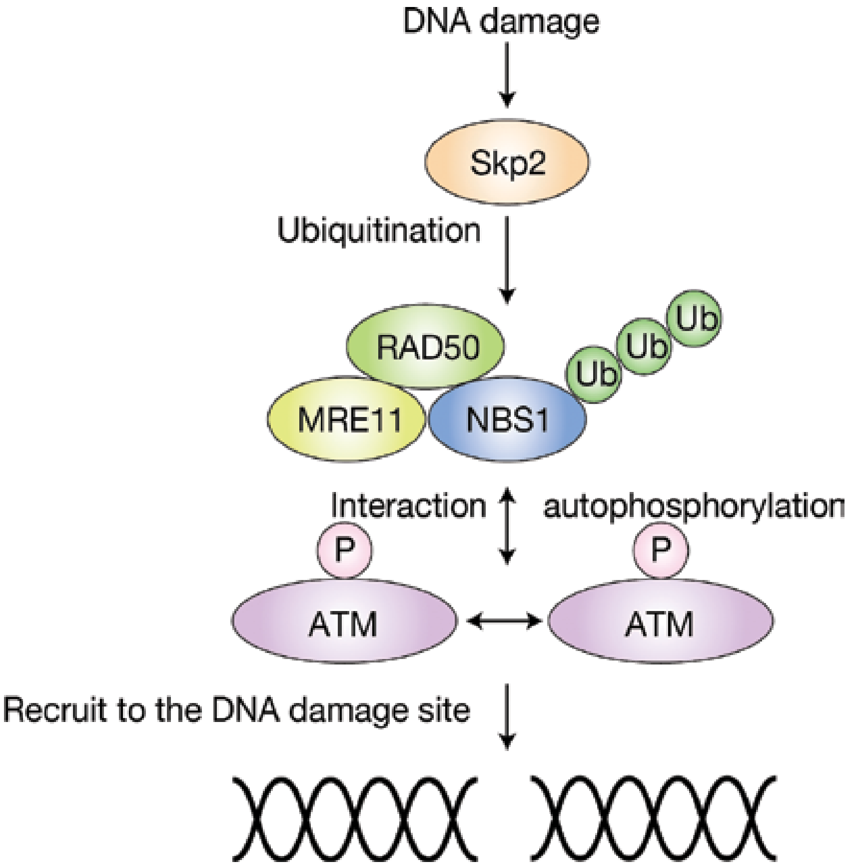

Figure 3. Ubiquitination of NBS1 by Skp2 is important for DNA repair pathway.

2.6. NBS1

Nijmegen breakage syndrome (NBS) is characterized by immunodeficiency, predisposition to the malignancy and IR hypersensitivity [85, 86]. Gene product NBS1 is 95 kDa protein and has several roles to maintain genome stability such as, HR repair, DNA replication initiation, cell cycle checkpoint, apoptosis, UV damage repair and centrosome duplication [58, 87–95]. NBS1 forms complex with MRE11 and RAD50 as MRN complex and act as DNA damage sensor and initiator [96]. Complete deletion of NBS1 proteins in mice leads to embryonic lethality. 70 kDa fragment of NBS1 protein expresses in NBS patient cells. NBS1 localizes to the nucleus and centrosomes. Depletion of NBS1 by siRNA in human osteosarcoma U2OS cells and murine embryonic fibroblast NIH3T3 cells show radio-sensitivity and centrosome amplification which suggest that NBS1 is required for DNA repair and centrosome duplication process. NBS1 is phosphorylated by ATM and ATR to activate G1/S checkpoint and G2/M checkpoint, respectively. NBS1 acts as DNA damage sensor and is important for ATM recruitment to the DNA damage sites. Ubiquitination of NBS1 by E3 ubiquitin ligase 3 Skp2 is required for interaction with ATM and activation [97] (**Figure 3**). Defect of Skp2 leads to decreasing of ATM foci formation at the DNA damage sites. Furthermore, NBS1 is involved in translesion DNA synthesis (TLS) [92]. After UV exposure, E3 ubiquitin ligase RAD18 recruited to the DNA damage sites and mono-ubiquitinates PCNA to initiate TLS. NBS1 controls RAD18 function because depletion of NBS1 results in decreasing of foci formation of pol eta and mono-ubiquitination of PCNA.

3. Concluding remarks

Genome DNA is attacked by several factors not only environmental stress but also metabolic stress to maintain cellular homeostasis. DNA repair and genome maintenance molecular mechanisms are strictly regulated by many enzymes. Since Goldstein and Ciechanover first reported about ubiquitin, the biological significance of this small peptide has been focused on several fields such as proteasome maintenance, translational signaling and DNA repair [98–100]. Ubiquitination of DNA repair factors are important for facilitates signaling cascade, because protein posttranslational modification is useful tool to diverse signaling pathway. DNA repair proteins defects cause several diseases such as immunodeficiency, neurodegeneration, growth defects and cancer progression. Furthermore, ubiquitination of DNA repair pathway is strong target for cancer therapy [101]. To understand of molecular pathway is necessary for clinical application.

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References

[1] Iyama T, Wilson 3rd DM DNA repair mechanisms in dividing and non-dividing cells. DNA Repair (Amst). 2013;**12**(8):620-636

[2] Ward JF. DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation, and reparability. Progress in Nucleic Acid Research and Molecular Biology. 1988;**35**:95-125

[3] Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annual Review of Biochemistry. 2010;**79**:181-211

[4] Kobayashi J, Iwabuchi K, Miyagawa K, Sonoda E, Suzuki K, Takata M, et al. Current topics in DNA double-strand break repair. Journal of Radiation Research. 2008;**49**(2):93-103

[5] Wood JW, Johnson KG, Omori Y. In utero exposure to the Hiroshima atomic bomb. An evaluation of head size and mental retardation: twenty years later. Pediatrics. 1967;**39**(3):385-392

[6] McKinnon PJ. Maintaining genome stability in the nervous system. Nature Neuroscience. 2013;**16**(11):1523-1529

[7] Wood JW, Johnson KG, Omori Y, Kawamoto S, Keehn RJ. Mental retardation in children exposed in utero to the atomic bombs in Hiroshima and Nagasaki. American Journal of Public Health Nations Health. 1967;**57**(8):1381-1389

[8] Shimada M, Matsuzaki F, Kato A, Kobayashi J, Matsumoto T, Komatsu K. Induction of excess centrosomes in neural progenitor cells during the development of radiation-induced microcephaly. PLoS One. 2016;**11**(7):e0158236

[9] Roque T, Haton C, Etienne O, Chicheportiche A, Rousseau L, Martin L, et al. Lack of a p21waf1/cip -dependent G1/S checkpoint in neural stem and progenitor cells after DNA damage in vivo. Stem Cells. 2012;**30**(3):537-547

[10] Lombardi PM, Matunis MJ, Wolberger C. RAP80, ubiquitin and SUMO in the DNA damage response. Journal of Molecular Medicine (Berl). 2017;**95**(8):799-807

[11] Taccioli GE, Gottlieb TM, Blunt T, Priestley A, Demengeot J, Mizuta R, et al. Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. Science. 1994;**265**(5177):1442-1445

[12] Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, et al. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO Journal. 1998;**17**(18):5497-5508

[13] Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 2002;**108**(6):781-794

[14] Koch CA, Agyei R, Galicia S, Metalnikov P, O'Donnell P, Starostine A, et al. Xrcc4 physically links DNA end processing by polynucleotide kinase to DNA ligation by DNA ligase IV. EMBO Journal. 2004;**23**(19):3874-3885

[15] Chappell C, Hanakahi LA, Karimi-Busheri F, Weinfeld M, West SC. Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. EMBO Journal. 2002;**21**(11):2827-2832

[16] Jilani A, Ramotar D, Slack C, Ong C, Yang XM, Scherer SW, et al. Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3′-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. Journal of Biological Chemistry. 1999;**274**(34):24176-24186

[17] Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Guyader G, Abramowski V, Revy P, et al. V(D)J and immunoglobulin class switch recombinations: A paradigm to study the regulation of DNA end-joining. Oncogene. 2007;**26**(56):7780-7791

[18] Woodbine L, Gennery AR, Jeggo PA. The clinical impact of deficiency in DNA non-homologous end-joining. DNA Repair (Amst). 2014;**16**:84-96

[19] Shimada M, Dumitrache LC, Russell HR, McKinnon PJ. Polynucleotide kinase-phosphatase enables neurogenesis via multiple DNA repair pathways to maintain genome stability. EMBO Journal. 2015;**34**(19):2465-2480

[20] Shiloh Y, Ziv Y. The ATM protein kinase: Regulating the cellular response to genotoxic stress, and more. Nature Reviews Molecular Cell Biology. 2013;**14**(4):197-210

[21] Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003;**421**(6922):499-506

[22] van Attikum H, Gasser SM. Crosstalk between histone modifications during the DNA damage response. Trends in Cell Biology. 2009;**19**(5):207-217

[23] Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature. 2015;**527**(7578):389-393

[24] Shema E, Tirosh I, Aylon Y, Huang J, Ye C, Moskovits N, et al. The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. Genes & Development. 2008;**22**(19):2664-2676

[25] Nakamura K, Kato A, Kobayashi J, Yanagihara H, Sakamoto S, Oliveira DV, et al. Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. Molecular Cell. 2011;**41**(5):515-528

[26] Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, et al. Human CtIP promotes DNA end resection. Nature. 2007;**450**(7169):509-514

[27] McIlwraith MJ, Van Dyck E, Masson JY, Stasiak AZ, Stasiak A, West SC. Reconstitution of the strand invasion step of double-strand break repair using human Rad51 Rad52 and RPA proteins. Journal of Molecular Biology. 2000;**304**(2):151-164

[28] McIlwraith MJ, West SC. DNA repair synthesis facilitates RAD52-mediated second-end capture during DSB repair. Molecular Cell. 2008;**29**(4):510-516

[29] Nishi R. Balancing act: To be, or not to be ubiquitylated. Mutation Research. 2017;**803-805**: 43-50

[30] McKinnon PJ. Genome integrity and disease prevention in the nervous system. Genes & Development. 2017;**31**(12):1180-1194

[31] Boveri T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. Journal of Cell Science. 2008;**121**(Suppl 1):1-84

[32] Nigg EA. Centrosome duplication: of rules and licenses. Trends in Cell Biology. 2007;**17**(5):215-221

[33] Doxsey S. Re-evaluating centrosome function. Nature Reviews Molecular Cell Biology. 2001;**2**(9):688-698

[34] Fukasawa K. Oncogenes and tumour suppressors take on centrosomes. Nature Reviews Cancer. 2007;**7**(12):911-924

[35] Ganem NJ, Godinho SA, Pellman D. A mechanism linking extra centrosomes to chromosomal instability. Nature. 2009;**460**(7252):278-282

[36] Godinho SA, Kwon M, Pellman D. Centrosomes and cancer: How cancer cells divide with too many centrosomes. Cancer and Metastasis Reviews. 2009;**28**(1-2):85-98

[37] Shimada M, Hirayama R, Komatsu K. High LET radiation amplifies centrosome overduplication through a pathway of gamma-tubulin monoubiquitination. International Journal of Radiation Oncology, Biology, Physics. 2013;**86**(2):358-365

[38] Zhang S, Hemmerich P, Grosse F. Werner syndrome helicase (WRN), nuclear DNA helicase II (NDH II) and histone gammaH2AX are localized to the centrosome. Cell Biology International. 2007;**31**(10):1109-1121

[39] Niwa T, Saito H, Imajoh-ohmi S, Kaminishi M, Seto Y, Miki Y, et al. BRCA2 interacts with the cytoskeletal linker protein plectin to form a complex controlling centrosome localization. Cancer Science. 2009;**100**(11):2115-2125

[40] Smith E, Dejsuphong D, Balestrini A, Hampel M, Lenz C, Takeda S, et al. An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63. Nature Cell Biology. 2009;**11**(3):278-285

[41] Dodson H, Bourke E, Jeffers LJ, Vagnarelli P, Sonoda E, Takeda S, et al. Centrosome amplification induced by DNA damage occurs during a prolonged G2 phase and involves ATM. EMBO Journal. 2004;**23**(19):3864-3873

[42] Wang HF, Takenaka K, Nakanishi A, Miki Y. BRCA2 and nucleophosmin coregulate centrosome amplification and form a complex with the Rho effector kinase ROCK2. Cancer Research. 2011;**71**(1):68-77

[43] Bourke E, Dodson H, Merdes A, Cuffe L, Zachos G, Walker M, et al. DNA damage induces Chk1-dependent centrosome amplification. EMBO Reports. 2007;**8**(6):603-609

[44] Griffith E, Walker S, Martin CA, Vagnarelli P, Stiff T, Vernay B, et al. Mutations in pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. Nature Genetics. 2008;**40**(2):232-236

[45] Rauch A, Thiel CT, Schindler D, Wick U, Crow YJ, Ekici AB, et al. Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. Science. 2008;**319**(5864):816-819

[46] Chen A, Kleiman FE, Manley JL, Ouchi T, Pan ZQ. Autoubiquitination of the BRCA1* BARD1 RING ubiquitin ligase. Journal of Biological Chemistry. 2002;**277**(24):22085-22092

[47] Kleiman FE, Wu-Baer F, Fonseca D, Kaneko S, Baer R, Manley JL. BRCA1/BARD1 inhibition of mRNA 3′ processing involves targeted degradation of RNA polymerase II. Genes & Development. 2005;**19**(10):1227-1237

[48] Starita LM, Horwitz AA, Keogh MC, Ishioka C, Parvin JD, Chiba N. BRCA1/BARD1 ubiquitinate phosphorylated RNA polymerase II. Journal of Biological Chemistry. 2005;**280**(26):24498-24505

[49] Sato K, Hayami R, Wu W, Nishikawa T, Nishikawa H, Okuda Y, et al. Nucleophosmin/B23 is a candidate substrate for the BRCA1-BARD1 ubiquitin ligase. Journal of Biological Chemistry. 2004;**279**(30):30919-30922

[50] Wu W, Nishikawa H, Hayami R, Sato K, Honda A, Aratani S, et al. BRCA1 ubiquitinates RPB8 in response to DNA damage. Cancer Research. 2007;**67**(3):951-958

[51] Eakin CM, Maccoss MJ, Finney GL, Klevit RE. Estrogen receptor alpha is a putative substrate for the BRCA1 ubiquitin ligase. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(14):5794-5799

[52] Wu W, Koike A, Takeshita T, Ohta T. The ubiquitin E3 ligase activity of BRCA1 and its biological functions. Cell division. 2008;**3**(1)

[53] Densham RM, Morris JR. The BRCA1 Ubiquitin ligase function sets a new trend for remodelling in DNA repair. Nucleus. 2017;**8**(2):116-125

[54] Parvin JD, Sankaran S. The BRCA1 E3 ubiquitin ligase controls centrosome dynamics. Cell Cycle. 2006;**5**(17):1946-1950

[55] Sankaran S, Parvin JD. Centrosome function in normal and tumor cells. Journal of Cellular Biochemistry. 2006;**99**(5):1240-1250

[56] Sankaran S, Starita LM, Simons AM, Parvin JD. Identification of domains of BRCA1 critical for the ubiquitin-dependent inhibition of centrosome function. Cancer Research. 2006;**66**(8):4100-4107

[57] Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP, et al. BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. Molecular and Cellular Biology. 2004;**24**(19):8457-8466

[58] Shimada M, Sagae R, Kobayashi J, Habu T, Komatsu K. Inactivation of the Nijmegen breakage syndrome gene leads to excess centrosome duplication via the ATR/BRCA1 pathway. Cancer Research. 2009;**69**(5):1768-1775

[59] Shimada M, Komatsu K. Emerging connection between centrosome and DNA repair machinery. Journal of Radiation Research. 2009;**50**(4):295-301

[60] Shimada M, Kobayashi J, Hirayama R, Komatsu K. Differential role of repair proteins, BRCA1/NBS1 and Ku70/DNA-PKcs, in radiation-induced centrosome overduplication. Cancer Science. 2010;**101**(12):2531-2537

[61] Li J, D'Angiolella V, Seeley ES, Kim S, Kobayashi T, Fu W, et al. USP33 regulates centrosome biogenesis via deubiquitination of the centriolar protein CP110. Nature. 2013;**495**(7440):255-259

[62] Liu X, Holstege H, van der Gulden H, Treur-Mulder M, Zevenhoven J, Velds A, et al. Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(29):12111-12116

[63] McCarthy A, Savage K, Gabriel A, Naceur C, Reis-Filho JS, Ashworth A. A mouse model of basal-like breast carcinoma with metaplastic elements. Journal of Pathology. 2007;**211**(4):389-398

[64] Pao GM, Zhu Q, Perez-Garcia CG, Chou SJ, Suh H, Gage FH, et al. Role of BRCA1 in brain development. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(13):E1240-E1248

[65] Pulvers JN, Huttner WB. Brca1 is required for embryonic development of the mouse cerebral cortex to normal size by preventing apoptosis of early neural progenitors. Development. 2009;**136**(11):1859-1868

[66] Marthiens V, Rujano MA, Pennetier C, Tessier S, Paul-Gilloteaux P, Basto R. Centrosome amplification causes microcephaly. Nature Cell Biology. 2013;**15**(7):731-740

[67] Ishiai M, Sato K, Tomida J, Kitao H, Kurumizaka H, Takata M. Activation of the FA pathway mediated by phosphorylation and ubiquitination. Mutation Research. 2017; **803-805**:89-95

[68] Pang Q, Andreassen PR. Fanconi anemia proteins and endogenous stresses. Mutation Research. 2009;**668**(1-2):42-53

[69] Sasaki MS, Tonomura A. A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. Cancer Research. 1973;**33**(8):1829-1836

[70] Stingele J, Bellelli R, Boulton SJ. Mechanisms of DNA-protein crosslink repair. Nature Reviews Molecular Cell Biology. 2017;**18**(9):563-573

[71] D'Andrea AD. Targeting DNA repair pathways in AML. Best Practice & Research Clinical Haematology. 2010;**23**(4):469-473

[72] Ceccaldi R, Sarangi P, D'Andrea AD. The Fanconi anaemia pathway: new players and new functions. Nature Reviews Molecular Cell Biology. 2016;**17**(6):337-349

[73] Kee Y, D'Andrea AD. Molecular pathogenesis and clinical management of Fanconi anemia. Journal of Clinical Investigation. 2012;**122**(11):3799-3806

[74] Kim H, D'Andrea AD. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes & Development. 2012;**26**(13):1393-1408

[75] D'Andrea AD. Susceptibility pathways in Fanconi's anemia and breast cancer. New England Journal of Medicine. 2010;**362**(20):1909-1919

[76] Nijman SM, Huang TT, Dirac AM, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**(3):331-339

[77] Parmar K, Kim J, Sykes SM, Shimamura A, Stuckert P, Zhu K, et al. Hematopoietic stem cell defects in mice with deficiency of Fancd2 or Usp1. Stem Cells. 2010;**28**(7):1186-1195

[78] Yang Y, Kuang Y, Montes De Oca R, Hays T, Moreau L, Lu N, et al. Targeted disruption of the murine Fanconi anemia gene, Fancg/Xrcc9. Blood. 2001;**98**(12):3435-3440

[79] Reliene R, Yamamoto ML, Rao PN, Schiestl RH. Genomic instability in mice is greater in Fanconi anemia caused by deficiency of Fancd2 than Fancg. Cancer Research. 2010;**70**(23):9703-9710

[80] Parmar K, D'Andrea A, Niedernhofer LJ. Mouse models of Fanconi anemia. Mutation Research. 2009;**668**(1-2):133-140

[81] Cheng NC, van de Vrugt HJ, van der Valk MA, Oostra AB, Krimpenfort P, de Vries Y, et al. Mice with a targeted disruption of the Fanconi anemia homolog Fanca. Human Molecular Genetics. 2000;**9**(12):1805-1811

[82] Wong JC, Alon N, McKerlie C, Huang JR, Meyn MS, Buchwald M. Targeted disruption of exons 1 to 6 of the Fanconi Anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. Human Molecular Genetics. 2003;**12**(16):2063-2076

[83] Koomen M, Cheng NC, van de Vrugt HJ, Godthelp BC, van der Valk MA, Oostra AB, et al. Reduced fertility and hypersensitivity to mitomycin C characterize Fancg/Xrcc9 null mice. Human Molecular Genetics. 2002;**11**(3):273-281

[84] Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Meyn MS, et al. Epithelial cancer in Fanconi anemia complementation group D2 (Fancd2) knockout mice. Genes & Development. 2003;**17**(16):2021-2035

[85] Matsuura S, Tauchi H, Nakamura A, Kondo N, Sakamoto S, Endo S, et al. Positional cloning of the gene for Nijmegen breakage syndrome. Nature Genetics. 1998;**19**(2):179-181

[86] Komatsu K. NBS1 and multiple regulations of DNA damage response. Journal of Radiation Research. 2016;**57**(Suppl 1):i11-i17

[87] Matsuura S, Kobayashi J, Tauchi H, Komatsu K. Nijmegen breakage syndrome and DNA double strand break repair by NBS1 complex. Advances in Biophysics. 2004;**38**(Complete):65-80

[88] Tauchi H, Kobayashi J, Morishima K, van Gent DC, Shiraishi T, Verkaik NS, et al. Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. Nature. 2002;**420**(6911):93-98

[89] Sakamoto S, Iijima K, Mochizuki D, Nakamura K, Teshigawara K, Kobayashi J, et al. Homologous recombination repair is regulated by domains at the N- and C-terminus of NBS1 and is dissociated with ATM functions. Oncogene. 2007;**26**(41):6002-6009

[90] Iijima K, Muranaka C, Kobayashi J, Sakamoto S, Komatsu K, Matsuura S, et al. NBS1 regulates a novel apoptotic pathway through Bax activation. DNA Repair (Amst). 2008;**7**(10):1705-1716

[91] Kobayashi J, Tauchi H, Chen B, Burma S, Tashiro S, Matsuura S, et al. Histone H2AX participates the DNA damage-induced ATM activation through interaction with NBS1. Biochemical and Biophysical Research Communications. 2009;**380**(4):752-757

[92] Yanagihara H, Kobayashi J, Tateishi S, Kato A, Matsuura S, Tauchi H, et al. NBS1 recruits RAD18 via a RAD6-like domain and regulates Pol eta-dependent translesion DNA synthesis. Molecular Cell. 2011;**43**(5):788-797

[93] Tauchi H, Matsuura S, Kobayashi J, Sakamoto S, Komatsu K. Nijmegen breakage syndrome gene, NBS1, and molecular links to factors for genome stability. Oncogene. 2002;**21**(58):8967-8980

[94] Kobayashi J, Antoccia A, Tauchi H, Matsuura S, Komatsu K. NBS1 and its functional role in the DNA damage response. DNA Repair (Amst). 2004;**3**(8-9):855-861

[95] Morishima K, Sakamoto S, Kobayashi J, Izumi H, Suda T, Matsumoto Y, et al. TopBP1 associates with NBS1 and is involved in homologous recombination repair. Biochemical and Biophysical Research Communications. 2007;**362**(4):872-879

[96] Stracker TH, Petrini JH. The MRE11 complex: starting from the ends. Nature Reviews Molecular Cell Biology. 2011;**12**(2):90-103

[97] Wu J, Zhang X, Zhang L, Wu CY, Rezaeian AH, Chan CH, et al. Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. Molecular Cell. 2012;**46**(3):351-361

[98] Goldstein G, Scheid M, Hammerling U, Schlesinger DH, Niall HD, Boyse EA. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proceedings of the National Academy of Sciences of the United States of America. 1975;**72**(1):11-15

[99] Schlesinger DH, Goldstein G, Niall HD. The complete amino acid sequence of ubiquitin, an adenylate cyclase stimulating polypeptide probably universal in living cells. Biochemistry. 1975;**14**(10):2214-2218

[100] Ciehanover A, Hod Y, Hershko A. A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. Biochemical and Biophysical Research Communications. 1978;**81**(4):1100-1105

[101] Hosoya N, Miyagawa K. Targeting DNA damage response in cancer therapy. Cancer Science. 2014;**105**(4):370-388

Chapter 9

Ubiquitination and DNA Repair in Multiple Myeloma

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Additional information is available at the end of the chapter

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Abstract

Multiple myeloma (MM) is a hematological neoplasm characterized by the clonal proliferation of malignant plasma cells in the bone marrow. MM cells are characterized by genomic abnormalities that arise during the pathogenesis of disease and accumulate during progression. DNA repair pathways are critical to repair the plethora of DNA lesions that occur in MM, and deregulation of these pathways is implicated in disease onset and survival. The ubiquitin proteasome system has emerged as a central player in the regulation of DNA damage response (DDR). In this chapter, we review defects within the ubiquitin proteasome system that are associated with abnormal DNA damage response in MM and discuss current and potential novel ways of targeting these aberrations in the clinic.

Keywords: multiple myeloma, ubiquitin proteasome system, DNA repair, proteasome inhibitors

1. Introduction

Multiple myeloma (MM) is a malignancy characterized by the abnormal proliferation of plasma cells in the bone marrow. Plasma cells are terminally differentiated B cells that provide protective immunity through the production and secretion of antibodies. During their maturation, plasma cells undergo physiological DNA rearrangements to generate a diverse range of antibodies. This process involves chromosomal breaks and subsequent DNA repair. In addition to the intrinsic genomic instability of plasma cells, clonal MM cells are exposed to enhanced exogenous stresses such as replication and proteotoxic stress. Defective DNA repair pathways are implicated in the pathogenesis and survival of MM cells. The importance of post-translational modification with ubiquitin in the regulation of DNA repair pathways is being increasingly recognized. The ubiquitin proteasome system is an important therapeutic target in MM. This chapter provides an overview of ubiquitin signaling, describes genomic instability in MM cells and defects in ubiquitin-mediated regulation of DNA repair pathways in MM, and discusses the impact of current and potentially novel therapeutic approaches in targeting these aberrations.

2. Ubiquitination

Ubiquitin is a highly conserved, 76-amino acid protein that is expressed in the cytoplasm and nucleus of all cells. Post-translational modification with ubiquitin, a process known as ubiquitination or ubiquitylation, is involved in the regulation of a wide range of cellular processes. Ubiquitin modification is an ATP-dependent process involving the sequential action of three classes of enzymes. An E1 (ubiquitin activating enzyme) activates ubiquitin through the formation of a thioester bond and transfers it to an E2 (ubiquitin conjugating enzyme), and an E3 (ubiquitin ligase) then mediates the transfer of the activated ubiquitin to a lysine (K) residue on a target protein, thereby forming an isopeptide bond between ubiquitin and the protein. There is one main E1 enzyme in eukaryotic cells, over 30 E2s and more than 600 E3s [1]. Ubiquitin can be attached to a target protein as a monomer or a polymer, resulting in different fates (**Figure 1**), determined largely by the pairing of E2s and E3s [2, 3].

At the simplest level, a single ubiquitin moiety is added to the ε-amino of a lysine residue on a substrate protein, in a process termed mono-ubiquitination. Mono-ubiquitination is typically involved in protein localization, complex formation, or altering the activity of the modified protein [4]. A single ubiquitin molecule can be conjugated to multiple lysine residues on a target protein, termed multi-monoubiquitination, and this modification is important for receptor endocytosis [5]. Proteins may also be modified by attachment of a chain of ubiquitin molecules in a process known as polyubiquitination. Ubiquitin contains seven lysine residues, at positions 6, 11, 27, 29, 33, 48, and 63, and a methionine group at position 1 (M1), that provide attachment sites for further ubiquitin molecules, thereby allowing the formation of polyubiquitin chains [6]. K48-linked polyubiquitination is the most widely studied ubiquitin chain and labels a protein for degradation through the proteasome. K63-linked polyubiquitination functions in mediating protein-protein interactions or conformational changes and plays an established role in regulating the DNA damage response (DDR) [7]. The other lysine linkages are less abundant and therefore less well characterized; however, most have been implicated to some extent with proteasome-mediated degradation or DNA repair [8, 9]. M1-linked ubiquitin chains, also referred to as linear ubiquitin chains, play a central role in inflammatory signaling cascades by regulating the activation of the transcription factor NFKB [10]. Mixed and branched ubiquitin chains have also been described; however, the function of these has not been fully delineated.

Like all post-translation modifications, the process of ubiquitination is reversible and ubiquitin removal is mediated by a large and diverse family of proteins known as deubiquitinating enzymes (DUBs). DUBs function predominantly to generate free ubiquitin from ubiquitin precursors and to edit or disassemble ubiquitin chains. The balance between ubiquitination and deubiquitination plays a critical role in regulating protein turnover and function.

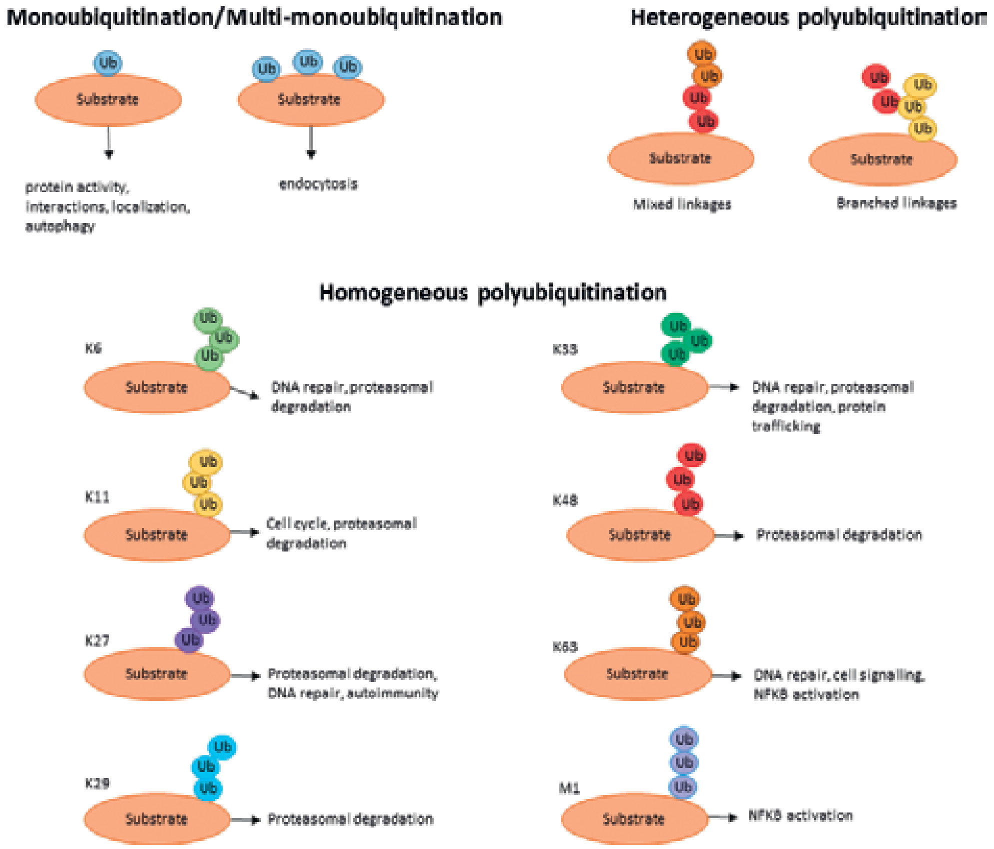

Figure 1. The ubiquitin code. An ubiquitin (Ub) molecule can be attached to a single site (monoubiquitination) or multiple sites (multi-monoubiquitination) on a substrate protein. In addition, ubiquitin contains seven lysine (K) residues and a methionine (M) residue that can support the assembly of homogenous or heterogeneous polyubiquitin chains.

2.1. The ubiquitin proteasome system

The ubiquitin proteasome system plays a central role in maintaining cellular protein homeostasis through the selective degradation of damaged, misfolded, and short-lived regulatory proteins that control essential cellular processes. The best-studied aspect of ubiquitination is the formation of a K48-linked polyubiquitin chain, which flags the target proteins for degradation through the 26S proteasome. The 26S or constitutive proteasome is a multicatalytic protease composed of two distinct subcomplexes: the 20S core particle and the 19S regulatory particle. The 19S regulatory particle is attached to one or both ends of the core particle and functions to recognize K48-linked polyubiquitin-tagged proteins, cleave and recycle ubiquitin, unfold the target protein, and feed it into the 20S proteolytic chamber for degradation. The 20S core particle is a barrel-shaped structure made up of 28 subunits arranged into four stacked rings. The two outer rings are composed of seven different α-subunits (α1–7), which serve as docking domains for the 19S regulatory caps.

Alpha subunits function as a gate controlling entry of substrates to the central chamber where proteolysis occurs. The two inner rings are composed of seven different β-subunits (β1–7), at least three of which contain catalytic sites, which are classified based upon preference to cleave after a particular amino acid residue. Chymotrypsin-like, trypsin-like, and caspase-like catalytic activities are associated with the β5, β2, and β1 subunits, respectively, due to their preference to cleave after hydrophobic, basic or acidic amino acid residues [11, 12]. An additional proteasome isoform known as the immunoproteasome also exists and is composed of an alternative set of catalytic beta subunits and regulatory cap. Constitutive subunits β5, β2, and β1 are replaced with subunits β5i (LMP7), β2i (MECL1), and β1i (LMP2), and the 19S regulatory cap is replaced with an 11S regulatory structure. These modifications allow the immunoproteasome to generate peptides for antigen presentation by major histocompatibility complex (MHC) class 1 molecules [13]. Immunoproteasomes are predominantly expressed in lymphoid tissues and hematopoietic cells, but can be formed in other cell types in response to stimuli such as interferon-γ and tumor necrosis factor-α.

3. Multiple myeloma

Multiple myeloma is a malignancy of plasma cells. It is almost always preceded by an asymptomatic premalignant stage called monoclonal gammopathy of undetermined significance (MGUS). An intermediate asymptomatic stage between MGUS and MM is referred to as smoldering MM (SMM). MM has historically been defined by four key features: hyper**c**alcemia, **r**enal failure, **a**nemia, and the presence of osteolytic **b**one lesions. These are commonly referred to as CRAB features. The International Myeloma Working Group revised these diagnostic criteria in 2014 to allow the inclusion of specific biomarkers and the addition of modern imaging tools to define MM bone disease [14]. The revised criteria made it possible to distinguish patients with SMM with high risk of progression to symptomatic MM. This facilitates the possibility of therapeutic intervention before end organ damage occurs and increases the possibility of success. Following diagnosis, patients can be further stratified into distinct prognostic subgroups using the revised international staging system (ISS) [15]. This algorithm builds on the original ISS (β2 microglobulin and serum albumin levels) and further includes cytogenetic abnormalities (outlined below) and serum lactate dehydrogenase.

Substantial progress has been made in the treatment of MM over the past decade. The approval of new types of biological agents, such as proteasome inhibitors and immunomodulatory drugs, has improved treatment options and led to improved overall survival [16]. The first-in-class proteasome inhibitor, bortezomib, was approved by the FDA for relapsed and refractory MM in 2003, and bortezomib-based combinations now form the backbone of many treatment regimens, across all stages of disease [17]. In addition, two second generation proteasome inhibitors, carfilzomib and ixazomib, have recently been approved highlighting the importance of the UPS as a therapeutic target for MM [18, 19].

4. Genomic instability in multiple myeloma

Genomic instability is a hallmark of MM and is associated with the evolution and progression of the disease [20]. Almost all patients display cytogenetic abnormalities including ploidy changes, deletions, amplifications, and chromosomal translocations. Based on initiating events, MM can be broadly categorized into hyperdiploid or nonhyperdiploid depending on the number of chromosomes present [21]. Hyperdiploid tumors, characterized by the presence of 48–75 chromosomes, make up approximately half of the MM cases and often have multiple trisomies of odd numbered chromosomes (3, 5, 7, 11, 15, 19, 21). Primary translocations involving the IGH locus on 14q32 with five recurrent partners are found in the majority (≥ 70%) of nonhyperdiploid MM cases and are associated with poorer overall survival. MM cells originate from postgerminal center B cells, which have undergone physiological DNA rearrangement during their maturation, including immunoglobulin (Ig) variable region rearrangement, somatic hypermutation, and Ig class switch recombination. Errors in these physiological processes can result in translocations that juxtapose one of five oncogenes [CCND1 (11q13), MMSET/FGFR3 (4p16), MAF (16q23), CCND3 (6p21), MAFB (20q11)] under the control of an immunoglobulin heavy (IGH) locus enhancer [22]. These initiating events are present in the majority of MGUS patients, and secondary genetic alterations, along with intraclonal heterogeneity, occur with increased incidence in disease progression from MGUS to MM [23]. These secondary events include further translocations, deletions, and chromosome gains, involving genes such as MYC, KRAS, NRAS, TP53, and NFKB-related genes (NIK, BIRC2/3, TRAF3), all of which are involved in DNA damage response and repair pathways. There is accumulating evidence eluding to deregulation of DNA repair pathways in MM as a mediator of the onset and progression of the disease, as well as survival. Changes in the expression of DNA repair genes have been suggested to play an important role in the pathogenesis of MM by leading to genomic instability and accumulation of genetic mutations [24]. In addition, ongoing DNA damage has been shown to intensify across the disease spectrum from MGUS to MM, thus providing a mechanism by which chromosomal abnormalities and tumor heterogeneity may be acquired in malignant plasma cells [25]. Furthermore, a number of studies report that alterations in DNA damage repair pathways are associated with poor prognosis in MM [26, 27].

5. DNA repair pathways

Cells are continuously challenged by DNA damage, induced through endogenous and exogenous sources. The ability to repair this damage is essential for the maintenance of genome integrity. Multiple proteins function together to detect and repair DNA damage, a process collectively termed the DNA damage response (DDR). There are six major DNA repair pathways involved in the DDR in mammalian cells. Base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) pathways repair nucleotide lesions on single strands. The BER pathway repairs small lesions induced by UV radiation, ionizing radiation, oxidative stressors, and alkylating agents [28]. The NER pathway removes bulky lesions induced by UV light, environmental mutagens, and some chemotherapeutic agents [29]. The MMR pathway corrects replication errors such as base mismatches and insertion/deletion loops that occur during replication [30]. DNA double-strand breaks (DSBs), which are a more serious form of DNA damage, are generated by exogenous agents such as ionizing radiation or chemicals or endogenously by reactive oxygen species, replication of single-strand breaks, replication stress, or class switch recombination. DSBs can be repaired by two main pathways, homologous recombination (HR) and nonhomologous end joining (NHEJ). HR takes place in late S- and G2-phases of the cell cycle and corrects DSBs in an error-free manner using an undamaged sister chromatid as a homologous DNA template [31]. NHEJ can take place in all phases of the cell cycle and repairs DSBs in an error-prone manner by direct ligation of the two broken ends [32]. Interstrand crosslinks (ICL), which are covalent links between two opposite strands of DNA, are induced by endogenous metabolites and exogenous chemicals such as alkylating agents [33]. The Fanconi anemia (FA) pathway detects ICL and repairs ICL lesions in co-operation with NER and HR pathways [34].

6. Ubiquitin-mediated DDR signaling in MM

All DDR pathways are tightly controlled by reversible post-translational modifications, including ubiquitination and de-ubiquitination, which act to regulate protein stability, localization, and activity. Deregulation of these DNA repair pathways can lead to genomic instability and promote tumorigenesis. Aberrant ubiquitin-mediated signaling of DNA damage repair in MM is summarized below.

6.1. DSB repair

Nonproteolytic ubiquitination of histones at the chromatin surrounding DSBs is a key step in DDR activation. Two E3 ubiquitin ligases, RING finger 8 (RNF8) and RNF168, are critical mediators of DSB repair. DSBs trigger activation of the ataxia-telengiectasia mutated (ATM) kinase, which phosphorylates histone H2AX (referred to as γ-H2AX) and mediator of DNA damage checkpoint protein 1 (MDC1). Phosphorylated MDC1 recruits RNF8, which promotes K63-linked polyubiquitination of H1 linker histones at DSB sites. This in turn recruits RNF168, leading to mono-ubiquitination of H2A-type histones at K13 and K15. These modifications provide a platform for binding of two essential effectors of the DDR to the DSB site, p53 binding protein 1 (53BP1), and breast cancer 1 (BRCA1). 53BP1 and BRCA1 have key roles in DSB repair pathway choice: BRCA1 promotes the HR pathway, whereas 53BP1 facilitates repair through the NHEJ pathway. A number of studies have reported deregulation of DSB repair in MM. Walters and colleagues demonstrated that H2AX is constitutively phosphorylated in MM, leading to constitutive activation of DSB repair pathways [25]. Consistent with this, elevated activity of both HR and NHEJ repair pathways has been observed in MM [35]. Furthermore, upregulation of NHEJ pathway-related gene expression is significantly associated with poor overall survival in MM [36]. This upregulation of DSB repair pathways likely contributes to the inherent genomic instability of malignant plasma cells with consequences for disease progression and acquisition of drug resistance.

Increased NHEJ, in particular, is associated with frequent chromosome aberrations and translocations that may contribute to tumor heterogeneity. RNF168 plays a crucial role in the recruitment of 53BP1 to sites of DNA damage by both recruiting 53BP1 and removing competing proteins from sites of DNA damage. Overexpression of RNF168 in tumor cells has been found to alter the DSB DNA repair response by shifting the balance from HR to NHEJ [37]. Proteotoxic stress, arising due to aneuploidy, copy-number variations, and transcriptional alterations, is an emerging hallmark of cancer cells. Previous studies have reported aberrant ubiquitin-mediated signaling of DNA damage under proteotoxic stress, whereby there is a depletion of free ubiquitin available for ubiquitin-dependent aspects of the DSB response, as a consequence of accumulating ubiquitinated substrates. However, a recent study identified a subset of cancer cell lines overexpressing RNF168 that could preferentially exploit the residual free ubiquitin to recruit 53BP1 and activate the NHEJ pathway [37]. Proteotoxic stress is particularly prominent in immunoglobulin-producing myeloma cells, and therefore, using MM cell lines as a model, this altered DSB response was found to be even more pronounced in MM cells, correlating with higher expression of RNF168. Furthermore, upregulation of RNF168 was found to influence the response of tumor cells to cancer therapies. RNF168-high tumors exhibit increased resistance to ionizing radiation under conditions of enhanced proteotoxic stress. However, this phenotype and associated alterations in DSB repair pathways render cells sensitive to topoisomerase and poly-ADP-ribose-polymerase (PARP) inhibitors.

6.2. The Fanconi anemia pathway

The FA pathway is a DNA damage activated pathway required for the repair of ICLs. ICLs are covalent bonds between two strands of DNA leading to a block in DNA replication and translation. Ubiquitination plays a pivotal role in the regulation of ICL repair by the FA pathway. In response to ICLs, a complex of eight FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) along with two associated proteins, FANCA-associated polypeptide 24 (FAAP24) and FAAP100, forms a multisubunit E3 ligase complex required for the monoubiquitination of FANCD2 on K561 and FANCI on K523. Monoubiquitinated FANCD2-FANCI is recruited to sites of DNA damage, where as a complex, they co-localize with BRCA1 and RAD51 to co-ordinate downstream reactions including nucleolytic incision, translesion synthesis (TLS), and DSB repair. Nucleolytic incisions unhook the ICL, creating a DSB and translesion synthesis that allows the bypass of unhooked crosslinked oligonucleotides. The DSB is then repaired by HR and remaining adducts are excised by the NER repair pathway [38, 39].

The FA pathway plays a key role in the cellular response to alkylating agents. The DNA alkylating agent, melphalan, is a commonly used chemotherapeutic agent in MM therapy. The majority of MM patients are initially sensitive to alkylating agents such as melphalan but inevitably acquire resistance, leading to disease progression. Studies into the mechanisms of resistance to melphalan in MM identified an increase in expression of genes coding for FA and HR pathways along with enhanced ICL repair and decreased DNA damage [40, 41] in melphalan-resistant cells. Further studies revealed a role of the NFKB pathway as a regulator of the FA pathway in response to melphalan-induced DNA damage [42]. The NFKB pathway is frequently dysregulated in MM and plays a central role in survival, proliferation, and resistance of MM cells to anticancer therapies. NFKB subunits RelB/p50 are transcriptional activators of the FA pathway through binding to the FANCD2 promoter. Chronic exposure of MM cells to melphalan resulted in an increased NFKB activity and associated FA pathway activity in MM cell lines and patient cells. Inhibition of the NFKB pathway, both using a proteasome inhibitor or a selective NFKB inhibitor, results in decreased expression of FA pathway genes in melphalan-sensitive and melphalan-resistant cell lines [42].

7. Targeting ubiquitin-mediated DDR signaling in MM

The UPS is recognized as an important therapeutic target in MM. Proteasome inhibitors are a principal component of current anti-MM therapy, and there is an increasing interest in targeting other parts of the UPS. An overview of current and novel UPS drugs that act directly on DNA repair pathways is given below and in **Figure 2**.

7.1. Proteasome inhibitors

Proteasome inhibition has emerged as a powerful strategy to treat MM. Since its introduction into the clinic in 2003, mechanisms underlying the therapeutic effect of the first-in-class proteasome inhibitor, bortezomib, have been widely investigated. One of the first mechanisms of action attributed to bortezomib was the inhibition of the inflammation-associated transcription factor nuclear factor kappa B (NFKB), a key signaling molecule in MM, through stabilization of its inhibitor IKB [43]. Initial studies also suggested that the unique sensitivity of MM cells to bortezomib was largely related to their high proteasome load, with inhibition of the proteasome resulting in an increased endoplasmic reticulum (ER) stress and a prolonged activation of the unfolded protein response (UPR) [44]. In addition, bortezomib was found to promote apoptosis through upregulation of proapoptotic proteins and induce cell cycle arrest through stabilization of cyclin-dependent kinase inhibitors [45]. Furthermore, bortezomib was found to exert an effect on the protective bone marrow microenvironment through inhibition of the angiogenic factor vascular endothelial growth factor (VEGF) and decreased binding of MM cells to bone marrow stromal cells [46]. Later studies found that proteasome inhibitors impair the DDR in MM in a number of ways [42, 47–50].

As mentioned previously, proteotoxic stress can impact ubiquitin-mediated DDR pathways through depletion of available nuclear ubiquitin. Inhibition of proteasome activity exacerbates endogenous proteotoxic stress in MM by preventing degradation of polyubiquitinated proteins, leading to a reduction in the amount of free ubiquitin in the cell [47, 48]. This results in a loss of ubiquitination at sites of DSBs and consequently impairs DSB repair. Bortezomib has been shown to alter HR by abrogating K63 polyubiquitination of H1 histones and subsequently impairing recruitment of BRCA1 and RAD51 [49]. Proteasome inhibition also leads to a reduction in FANCD2/FANCI monoubiquitination, thereby blocking a critical step in the FA pathway [42]. Along with altered ubiquitin signaling, proteasome nuclear activity is important for DSB repair. Proteasomes are recruited to sites of DSBs to degrade key regulatory proteins. Inhibition of proteasome activity blocks degradation of MDC1 at DSB sites, thereby inhibiting recruitment of BRCA1 in an RNF8 independent manner. Furthermore, proteasome inhibition blocks NFKB-mediated activation of the FA pathway [50].

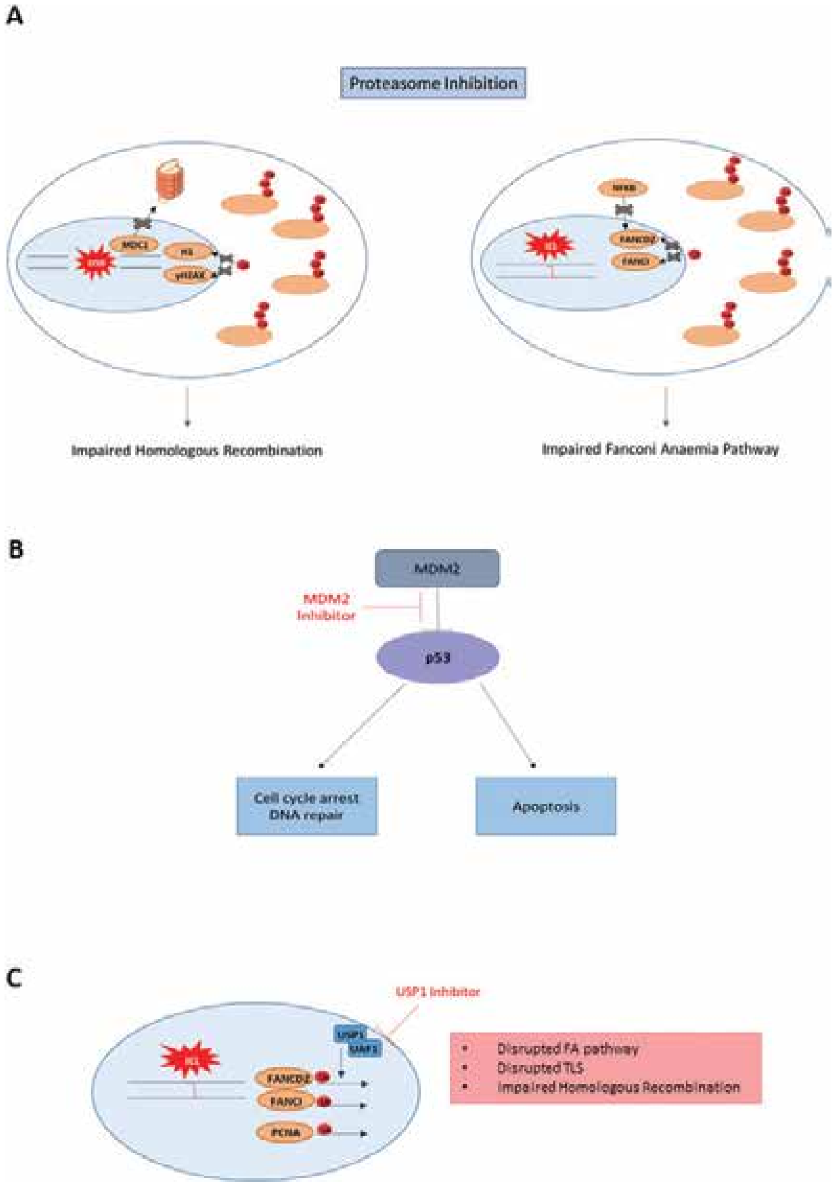

Figure 2. Targeting ubiquitin-mediated DNA damage repair signaling in MM. (A) Proteasome inhibitors prevent degradation of polyubiquitinated proteins leading to a depletion of available nuclear ubiquitin. Proteasome inhibition leads to reduced ubiquitination of H1 histones and γH2AX and reduced degradation of mediator of DNA damage checkpoint protein 1 (MDC1), resulting in impaired repair of double-strand breaks (DSBs) through homologous recombination. Proteasome inhibition disrupts repair of interstrand crosslinks (ICLs) by the Fanconi anemia (FA) pathway through reduced ubiquitination of FANCD2 and FANCI and a block in nuclear factor kappa B (NFKB)-mediated activation of the FA pathway. (B) In response to DNA damage, p53 is activated to induce cell cycle arrest to allow DNA repair or to induce apoptosis of a damaged cell. Mouse double minute 2 (MDM2) inhibitors block the interaction of p53 and its endogenous inhibitor MDM2 to stabilize p53 levels and increase its activity. (C) Ubiquitin-specific protease 1 (USP1) along with its binding partner USP1-associated factor (UAF1) regulate the FA pathway and translesion synthesis (TLS) through the de-ubiquitination of FAND2, FANCI, and PCNA. Inhibition of USP1 leads to a disruption of the FA pathway, TLS, and impaired homologous recombination.

Bortezomib was initially approved as a single agent; however, its predominant use is in combination therapies with steroids and/or standard chemotherapy drugs. Given its effect on DSB repair, it is not surprising that bortezomib is reported to sensitize MM cells to DNA damage inducing chemotherapeutics. In preclinical studies, bortezomib was found to both sensitize tumor cells to conventional DNA damaging agents, doxorubicin and melphalan, and to overcome resistance to these therapies [51]. Furthermore, bortezomib demonstrates clinical efficacy in combination with doxorubicin, melphalan, and cyclophosphamide [52]. Finally, bortezomib has been shown to act in synergy with PARP inhibitors by blocking HR, resulting in marked cell death [49].

7.2. p53 as a therapeutic target

The tumor suppressor p53 plays an important role in maintaining genomic stability. Under normal conditions, p53 levels are low due to rapid degradation through the UPS. In response to DNA damage, p53 is stabilized and acts to halt cell division and allows repair of DNA lesions prior to DNA synthesis. The E3 ubiquitin ligase mouse double minute 2 (MDM2) homolog is the most prominent E3 ligase involved in the negative regulation of p53 [53]. MDM2 can regulate p53 with both mono- and polyubiquitination to regulate both its stability and cellular location. Monoubiquitination of p53 triggers nuclear export, whereas K48-linked polyubiquitination targets p53 for proteasomal degradation. Overexpression of MDM2 has been reported in a number of malignancies, including MM, and can act to suppress p53 levels, even under stress conditions [54]. Numerous pharmacological approaches have been developed to disrupt MDM2-p53 binding, thus stabilizing p53 levels and increasing p35 activity. The nutlins were the first small molecule inhibitors of MDM2 to be developed and have been demonstrated to stabilize p53 and its substrates, resulting in increased apoptosis and cell cycle arrest in MM cell lines and primary cells [55]. Similar effects have been reported with a number of other MDM2 inhibitors. In addition to targeting MDM2 directly, another promising approach is to inhibit the deubiquitinating enzyme ubiquitin-specific protein 7 (USP7). USP7 deubiquitinates and stabilizes levels of MDM2. Expression of USP7 is elevated in MM and small molecule inhibition of this DUB leads to decreased levels of MDM2 and accumulation of p53 [56]. Early phase clinical trials are in preparation for two MDM2 inhibitors in MM (AMG-232, NCT01723020; DS-3032b, NCT02579824), highlighting the potential of disrupting the DDR through inhibition of this E3 ligase in MM.

7.3. USP1 as a therapeutic target

USP1 is the most widely characterized DUB known to be involved in the DNA damage response. USP1, along with its binding partner USP1-associated factor (UAF1), is important for the regulation of the FA pathway and translesion synthesis (TLS) [57, 58]. Monoubiquitination of FANCD2-FANCI directs this complex to DNA damage foci to activate the FA repair pathway. USP1 in conjunction with UAF1 is the DUB responsible for deubiquitinating FANCD2 and FANCI. USP1-UAF1 is also responsible for the deubiquitination of proliferating cell nuclear antigen (PCNA), a central regulator of TLS. Elevated expression of USP1 has been reported in MM and is associated with poor prognosis. A recent study demonstrated that both siRNA knockdown and small molecule inhibition of USP1 in MM cells results in increased levels of ubiquitinated FANCD2, FANCI and PCNA and decreased RAD51 formation, ultimately leading to inhibition of the FA pathway and HR [59]. Inhibition of USP1 was also found to trigger synergistic cytotoxicity with a number of MM therapies highlighting its potential as a therapeutic agent in MM.

8. Concluding remarks

Chromosomal translocations and genetic abnormalities are a hallmark of MM, contributing to the initiation and progression of the disease. Genomic instability is largely beneficial to MM cells by providing a growth advantage or contributing to drug resistance; however, an understanding of the mechanisms driving this can create therapeutic opportunities to exploit vulnerabilities within the malignant cells. Alterations in DNA damage repair pathways are implicated as one mechanism contributing to genomic instability. The UPS plays a central role in the regulation of DNA damage repair through ubiquitination and degradation of key proteins. The UPS is already recognized as an important therapeutic target in MM, through the clinical success of proteasome inhibitors. Proteasome inhibitors have recently been demonstrated to impair HR and FA DNA repair pathways, leading to increased sensitivity of MM cells to a number of DNA damage-inducing agents. In addition to the proteasome, there is mounting interest in the therapeutic potential of targeting ubiquitination and deubiquitination enzymes. A number of these enzymes involved in regulating DDR are also deregulated in MM. Expression of the E3 ligase RNF168 in MM has implications for treatment response to DNA damage-inducing agents in MM, whereas the E3 ligase MDM2 and DUBs USP7 and USP1 are under investigation as therapeutic targets. As our knowledge of the role of the UPS in regulating DNA damage repair increases, it is likely that further opportunities for targeted therapies will emerge.

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References

[1] Heride C, Urbe S, Clague MJ. Ubiquitin code assembly and disassembly. Current Biology. 2014;**24**(6):R215-R220. DOI: 10.1016/j.cub.2014.02.002

[2] Swatek KN, Komander D. Ubiquitin modifications. Cell Research. 2016;**26**(4):399-422. DOI: 10.1038/cr.2016.39

[3] Yau R, Rape M. The increasing complexity of the ubiquitin code. Nature Cell Biology. 2016;**18**(6):579-586. DOI: 10.1038/ncb3358

[4] Hicke L. Protein regulation by monoubiquitin. Nature Reviews Molecular Cell Biology. 2001;**2**(3):195-201. DOI: 10.1038/35056583

[5] Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. Nature Cell Biology. 2003;**5**(5):461-466. DOI: 10.1038/ncb983

[6] Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. Recognition of the polyubiquitin proteolytic signal. The EMBO Journal. 2000;**19**(1):94-102. DOI: 10.1093/emboj/19.1.94

[7] Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature. 2015;**527**(7578):389-393. DOI: 10.1038/nature15401

[8] Elia AE, Boardman AP, Wang DC, Huttlin EL, Everley RA, Dephoure N, et al. Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. Molecular Cell. 2015;**59**(5):867-881. DOI: 10.1016/j.molcel.2015.05.006

[9] Grice GL, Nathan JA. The recognition of ubiquitinated proteins by the proteasome. Cellular and Molecular Life Sciences. 2016;**73**(18):3497-3506. DOI: 10.1007/s00018-016-2255-5

[10] Tokunaga F. Linear ubiquitination-mediated NF-kappaB regulation and its related disorders. Journal of Biochemistry. 2013;**154**(4):313-323. DOI: 10.1093/jb/mvt079

[11] Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Bartunik HD, et al. Structure of 20S proteasome from yeast at 2.4 a resolution. Nature. 1997;**386**(6624):463-471. DOI: 10.1038/386463a0

[12] Groll M, Bajorek M, Kohler A, Moroder L, Rubin DM, Huber R, et al. A gated channel into the proteasome core particle. Nature Structural Biology. 2000;**7**(11):1062-1067. DOI: 10.1038/80992

[13] Sijts EJ, Kloetzel PM. The role of the proteasome in the generation of MHC class I ligands and immune responses. Cellular and Molecular Life Sciences. 2011;**68**(9):1491-1502. DOI: 10.1007/s00018-011-0657-y

[14] Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. The Lancet Oncology. 2014;**15**(12):e538-e548. DOI: 10.1016/S1470-2045(14)70442-5

[15] Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised international staging system for multiple myeloma: A report from International Myeloma Working Group. Journal of Clinical Oncology. 2015;**33**(26):2863-2869. DOI: 10.1200/JCO.2015.61.2267

[16] Kazandjian D. Multiple myeloma epidemiology and survival: A unique malignancy. Seminars in Oncology. 2016;**43**(6):676-681. DOI: 10.1053/j.seminoncol.2016.11.004

[17] Mohan M, Matin A, Davies FE. Update on the optimal use of bortezomib in the treatment of multiple myeloma. Cancer Management and Research. 2017;**9**:51-63. DOI: 10.2147/CMAR.S105163

[18] Herndon TM, Deisseroth A, Kaminskas E, Kane RC, Koti KM, et al. U.S. Food and Drug Administration approval: Carfilzomib for the treatment of multiple myeloma. Clinical Cancer Research. 2013;**19**(17):4559-4563. DOI: 10.1158/1078-0432.CCR-13-0755

[19] Shirley M. Ixazomib: First global approval. Drugs. 2016;**76**(3):405-411. DOI: 10.1007/s40265-016-0548-5

[20] Neri P, Bahlis NJ. Genomic instability in multiple myeloma: Mechanisms and therapeutic implications. Expert Opinion on Biological Therapy. 2013;**13**(Suppl 1):S69-S82. DOI: 10.1517/14712598.2013.814637

[21] Kuehl WM, Bergsagel PL. Early genetic events provide the basis for a clinical classification of multiple myeloma. Hematology. American Society of Hematology. Education Program. 2005;346-352. DOI: 10.1182/asheducation-2005.1.346.

[22] Bergsagel PL, Kuehl WM. Chromosome translocations in multiple myeloma. Oncogene. 2001;**20**(40):5611-5622. DOI: 10.1038/sj.onc.1204641

[23] Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nature Reviews Cancer. 2012;**12**(5):335-348. DOI: 10.1038/nrc3257

[24] Sharma A, Heuck CJ, Fazzari MJ, Mehta J, Singhal S, Greally JM, et al. DNA methylation alterations in multiple myeloma as a model for epigenetic changes in cancer. Wiley Interdisciplinary Reviews. Systems Biology and Medicine. 2010;**2**(6):654-669. DOI: 10.1002/wsbm.89

[25] Walters DK, Wu X, Tschumper RC, Arendt BK, Huddleston PM, Henderson KJ, et al. Evidence for ongoing DNA damage in multiple myeloma cells as revealed by constitutive phosphorylation of H2AX. Leukemia. 2011;**25**(8):1344-1353. DOI: 10.1038/leu.2011.94

[26] Kassambara A, Gourzones-Dmitriev C, Sahota S, Reme T, Moreaux J, Goldschmidt H, et al. A DNA repair pathway score predicts survival in human multiple myeloma: The potential for therapeutic strategy. Oncotarget. 2014;**5**(9):2487-2498. DOI: 10.18632/oncotarget.1740

[27] Cottini F, Hideshima T, Suzuki R, Tai YT, Bianchini G, Richardson PG, et al. Synthetic lethal approaches exploiting DNA damage in aggressive myeloma. Cancer Discovery. 2015;**5**(9):972-987. DOI: 10.1158/2159-8290.CD-14-0943

[28] Krokan HE, Bjoras M. Base excision repair. Cold Spring Harbor Perspectives in Biology. 2013;**5**(4):a012583. DOI: 10.1101/cshperspect.a012583

[29] Scharer OD. Nucleotide excision repair in eukaryotes. Cold Spring Harbor Perspectives in Biology. 2013;**5**(10):a012609. DOI: 10.1101/cshperspect.a012609

[30] Aquilina G, Bignami M. Mismatch repair in correction of replication errors and processing of DNA damage. Journal of Cellular Physiology. 2001;**187**(2):145-154. DOI: 10.1002/jcp.1067

[31] Dudas A, Chovanec MDNA. Double-strand break repair by homologous recombination. Mutation Research. 2004;**566**(2):131-167. DOI: 10.1016/jmrrev.2003.07.001.

[32] Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annual Review of Biochemistry. 2010;**79**:181-211. DOI: 10.1146/annurev.biochem.052308.093131

[33] Noll DM, Mason TM, Miller PS. Formation and repair of interstrand cross-links in DNA. Chemical Reviews. 2006;**106**(2):277-301. DOI: 10.1021/cr040478b

[34] Walden H, Deans AJ. The Fanconi anemia DNA repair pathway: Structural and functional insights into a complex disorder. Annual Review of Biophysics. 2014;**43**:257-278. DOI: 10.1146/annurev-biophys-051013-022737

[35] Herrero AB, San Miguel J, Gutierrez NC. Deregulation of DNA double-strand break repair in multiple myeloma: Implications for genome stability. PloS One. 2015;**10**(3):e0121581. DOI: 10.1371/journal.pone.0121581

[36] Calimeri T, Caracciolo D, Amodio N, Samur MK, Leotta M, Fulciniti M, et al. Targeting aberrant non-homologous end joining in multiple myeloma: Role of the classical and alternative pathways in genomic instability. Blood. 2014;**124**:3417

[37] Chroma K, Mistrik M, Moudry P, Gursky J, Liptay M, Strauss R, et al. Tumors overexpressing RNF168 show altered DNA repair and responses to genotoxic treatments, genomic instability and resistance to proteotoxic stress. Oncogene. 2016;**36**(17):2405-2402. DOI: 10.1038/onc.2016.392

[38] Kim H, D'Andrea AD. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes & Development. 2012;**26**(13):1393-1408. DOI: 10.1101/gad.195248.112

[39] Palovcak A, Liu W, Yuan F, Zhang Y. Maintenance of genome stability by Fanconi anemia proteins. Cell & Bioscience. 2017;**7**(13):8-016-0134-2. DOI: 10.1186/s13578-016-0134-2

[40] Hazlehurst LA, Enkemann SA, Beam CA, Argilagos RF, Painter J, Shain KH, et al. Genotypic and phenotypic comparisons of de novo and acquired melphalan resistance in an isogenic multiple myeloma cell line model. Cancer Research. 2003;**63**(22):7900-7906

[41] Chen Q, Van der Sluis PC, Boulware D, Hazlehurst LA, Dalton WS. The FA/BRCA pathway is involved in melphalan-induced DNA interstrand cross-link repair and accounts for melphalan resistance in multiple myeloma cells. Blood. 2005;**106**(2):698-705. DOI: 10.1182/blood-2004-11-4286

[42] Yarde DN, Oliveira V, Mathews L, Wang X, Villagra A, Boulware D, et al. Targeting the Fanconi anemia/BRCA pathway circumvents drug resistance in multiple myeloma. Cancer Research. 2009;**69**(24):9367-9375. DOI: 10.1158/0008-5472.CAN-09-2616

[43] Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Research. 2001;**61**(7):3071-3076

[44] Lee AH, Iwakoshi NN, Anderson KC, Glimcher LH. Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. Proceedings of the National Academy of Sciences. 2003;**100**(17):9946-9951. DOI: 10.1073/pnas.1334037100

[45] Crawford LJ, Walker B, Irvine AE. Proteasome inhibitors in cancer therapy. Journal of Cell Communication and Signaling. 2011;**5**(2):101-110. DOI: 10.1007/s12079-011-0121-7

[46] Roccaro AM, Hideshima T, Raje N, Kumar S, Ishitsuka K, Yasui H, et al. Bortezomib mediates antiangiogenesis in multiple myeloma via direct and indirect effects on endothelial cells. Cancer Research. 2006;**66**(1):184-191. DOI: 10.1158/0008-5472.CAN-05-1195

[47] Xu Q, Farah M, Webster JM, Wojcikiewicz RJ. Bortezomib rapidly suppresses ubiquitin thiolesterification to ubiquitin-conjugating enzymes and inhibits ubiquitination of histones and type I inositol 1,4,5-trisphosphate receptor. Molecular Cancer Therapeutics. 2004;**3**(10):1263-1269

[48] Meister S, Schubert U, Neubert K, Herrmann K, Burger R, Gramatzki M, et al. Extensive immunoglobulin production sensitizes myeloma cells for proteasome inhibition. Cancer Research. 2007;**67**(4):1783-1792. DOI: 10.1158/0008-5472.CAN-06-2258

[49] Neri P, Ren L, Gratton K, Stebner E, Johnson J, Klimowicz A, et al. Bortezomib-induced "BRCAness" sensitizes multiple myeloma cells to PARP inhibitors. Blood. 2011;**118**(24):6368-6379. DOI: 10.1182/blood-2011-06-363911

[50] Jacquemont C, Taniguchi T. Proteasome function is required for DNA damage response and Fanconi anemia pathway activation. Cancer Research. 2007;**67**(15):7395-7405. DOI: 10.1158/0008-5472.CAN-07-1015

[51] Mitsiades N, Mitsiades CS, Richardson PG, Poulaki V, Tai YT, Chauhan D, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: Therapeutic applications. Blood. 2003;**101**(6):2377-2380. DOI: 10.1182/blood-2002-06-1768

[52] D'Arena G, Valentini CG, Pietrantuono G, Guariglia R, Martorelli MC, Mansueto G, et al. Frontline chemotherapy with bortezomib-containing combinations improves response rate and survival in primary plasma cell leukemia: A retrospective study from GIMEMA multiple myeloma working party. Annals of Oncology. 2012;**23**(6):1499-1502. DOI: 10.1093/annonc/mdr480

[53] Pant V, Lozano G. Limiting the power of p53 through the ubiquitin proteasome pathway. Genes & Development. 2014;**28**(16):1739-1751. DOI: 10.1101/gad.247452.114

[54] Oliner JD, Saiki AY, Caenepeel S. The role of MDM2 amplification and overexpression in tumorigenesis. Cold Spring Harbor Perspectives in Medicine. 2016;**6**(6):a026336. DOI: 10.1101/cshperspect.a026336

[55] Saha MN, Jiang H, Chang H. Molecular mechanisms of nutlin-induced apoptosis in multiple myeloma: Evidence for p53-transcription-dependent and -independent pathways. Cancer Biology & Therapy. 2010;**10**(6):567-578. DOI: 10.4161/cbt.10.6.12535

[56] Chauhan D, Tian Z, Nicholson B, Kumar KG, Zhou B, Carrasco R, et al. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell. 2012;**22**(3):345-358. DOI: 10.1016/j.ccr.2012.08.007

[57] Huang TT, Nijman SM, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, et al. Regulation of monoubiquitinated PCNA by DUB autocleavage. Nature Cell Biology. 2006;**8**(4):339-347. DOI: 10.1038/ncb1378.

[58] Nijman SM, Huang TT, Dirac AM, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**(3):331-339. DOI: 10.1016/j.molcel.2005.01.008

[59] Das DS, Das A, Ray A, Song Y, Samur MK, Munshi NC, et al. Blockade of deubiquitylating enzyme USP1 inhibits DNA repair and triggers apoptosis in multiple myeloma cells. Clinical Cancer Research. 2017; Epub ahead of print. DOI: 10.1158/1078-0432.CCR-16-2692

Chapter 10

Ubiquitin and Fanconi Anemia

Ioannis Drikos and Alexandros Sachinidis

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77123

Abstract

Fanconi anemia (FA) is an inherited disease distinct from the failure of bone marrow, growth disturbance, predisposition to cancer and concomitant chromosomal abnormalities. FA is associated with genes involved in DNA replication and DNA repair processes. More than 20 proteins have been identified to be related with FANC pathway operation. Necessary prerequisite for activation and regulation of FA pathway is the monoubiquitination of heterodimer FANCD2-FANCI by core proteins of Fanc complex. The monoubiquitination of FANCD2-FANCI is crucial for nuclear localization of heterodimer, binding to chromatin and regulation of DNA repair procedure. Mutations of genes of FANC complex proteins associated with deficiency of DNA repair pathways affected cellular and genome instability. The interaction between proteins and ubiquitination affected genomic integrity and stability.

Keywords: Fanconi anemia, DNA repair, ubiquitination, FANC proteins, FANCL, FANCD2

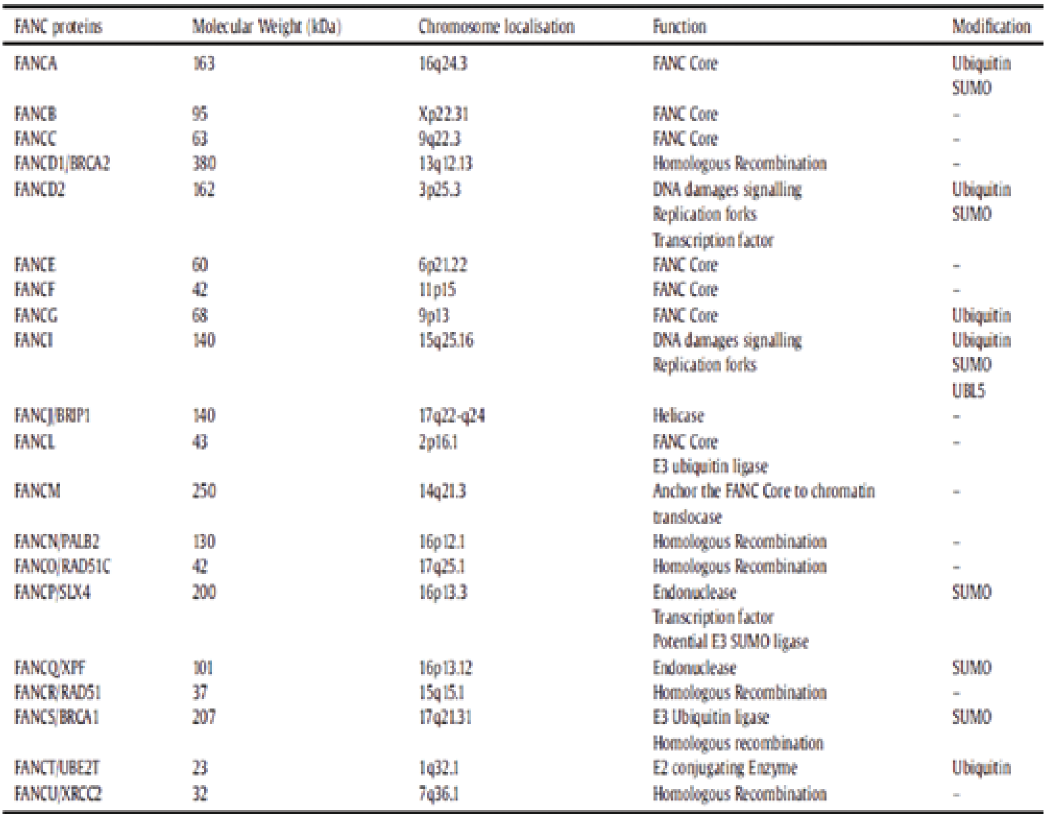
1. Introduction

Fanconi anemia is a rare recessive human genetic disorder first described by pediatrician Guido Fanconi in 1927. Patients with Fanconi anemia characterized by insufficient bone marrow regulation, developmental abnormalities and predisposition of cancer. Abnormal cell cycle progression, production of inflammatory cytokines and chromosomal instability also considered to be characteristics of the syndrome. Developmental abnormalities occur in 70% of patients, 40% develop defects of the skin and rare disorders such as renal deficiencies have been reported in less than 10% [1, 2].

The development of FA associated with DNA repair processes and regulation of cell cycle control. Nowadays, more than 20 genes and proteins have already been identified which are crucial for FA development (**Figure 1**). Except these genes, patients with mutations in RAD51C hitherto display bone marrow failure even if RAD51C seems to have a crucial role in FA development. In the majority of patients appeared, FA biallelic mutations inherited from each parent. FANC proteins interact through intracellular signaling pathway during cell cycle progression and FANC/BRCA complex demonstrate the genome stability [3–5].

Intracellular FA's evolution pathway involved eight key proteins such as FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM forming a complex which monoubiquitylates the FANCD2/FANCI. The monoubiquitylated ID complex interacts with FAN1 (anemia Fanconi Associated Nuclease 1) appearing endonuclease and exonuclease activity in vitro. According to several studies, FA intracellular pathway involved FANCD1 and BRCA2 has been already revealed. These molecules are necessary for homologous recombination process via interaction with helicase BACH1 [5–7].

The FANCJ interacts with BRCA1 and protein complex of FANCP/SLX4. The SLX4 connecting with multiple proteins such as XPF, MUS81, and SLX1 responsible for Holliday intersections. The ubiquitylation of FANCD2 and FANCI is absent in cells with mutations in crucial proteins of the FA pathway. FANCL considered to be an essential part of the FA complex



FANC proteins	Molecular Weight (kDa)	Chromosome localisation	Function	Modification
FANCA	163	16q24.3	FANC Core	Ubiquitin SUMO
FANCB	95	Xp22.31	FANC Core	-
FANCC	63	9q22.3	FANC Core	-
FANCD1/BRCA2	380	13q12.13	Homologous Recombination	-
FANCD2	162	3p25.3	DNA damages signalling Replication forks Transcription factor	Ubiquitin SUMO
FANCE	60	6p21.22	FANC Core	-
FANCF	42	11p15	FANC Core	-
FANCG	68	9p13	FANC Core	Ubiquitin
FANCI	140	15q25.16	DNA damages signalling Replication forks	Ubiquitin SUMO UBL5
FANCJ/BRIP1	140	17q22-q24	Helicase	-
FANCL	43	2p16.1	FANC Core E3 ubiquitin ligase	-
FANCM	250	14q21.3	Anchor the FANC Core to chromatin translocase	-
FANCN/PALB2	130	16p12.1	Homologous Recombination	-
FANCO/RAD51C	42	17q25.1	Homologous Recombination	-
FANCP/SLX4	200	16p13.3	Endonuclease Transcription factor Potential E3 SUMO ligase	SUMO
FANCQ/XPF	101	16p13.12	Endonuclease	SUMO
FANCR/RAD51	37	15q15.1	Homologous Recombination	-
FANCS/BRCA1	207	17q21.31	E3 Ubiquitin ligase Homologous recombination	SUMO
FANCT/UBE2T	23	1q32.1	E2 conjugating Enzyme	Ubiquitin
FANCU/XRCC2	32	7q36.1	Homologous Recombination	-

Figure 1. The FANC proteins according to chromosome location in the human genome and main cellular function [6].

and represents an E3 ligase activity, which is required for coupling ubiquitin to lysine 561 of human FANCD2 and lysine 523 of FANCI [8, 9].

FANCL has three distinct structural regions such as region DRWD and RING domain. Functionally, DRWD domain coordinates the substrate binding and is essential for the interaction with E2 protein. The C-terminal region (RING) is required for adequate binding of the FANCL to UBE2T. The importance of RING domain reflected in the evolutionary conservation of FANCL homologous proteins. The UBE2T considered as crucial enzyme in ubiquitin E2 ligase function and is essential for FANCL-mediated ubiquitylation of FANCD2 in vivo [9–12].

2. Ubiquitylation and deubiquitylation of FANC pathway

Ubiquitylation is an important event for activation of ID complex and ICLs repair. Except ubiquitylation are also important molecules deubiquitylation. This process is crucial for recycling of the ID molecules while the ubiquitylated and nonubiquitylated forms are necessary for normal cellular function. USP1 seems to be enzyme involved in deubiquitylating procedure [13, 14].

FANCD2 and FANCI have been identified as targets of FANCL monoubiquitylation. In vitro studies of FANCD2 have already increased the understanding of the operation of FANC complex and the regulation of signaling pathway. As already mentioned, FANCD2 is a 160 kDa protein which can be monoubiquitinated at lysine 561 (K561) [15, 16].

Recent studies revealed that ubiquitylation of FANCD2/FANCI regulated by the FANCL, FANCB, FAAP100, and FANCT/UBE2T which participate in reconstitution of E2-E3 ligase complex. Mutants of proteins involved in FANC complex lead to destabilization and significant reduction of ubiquitin action of FANCD2/FANCI [17].

In DT40, cell line mutations of FANC complex proteins lead to inactivating of FANCC and USP1 without affecting FANCD2 ubiquitylation. These results suggesting FANC complex proteins may have a significant role in the DNA damage response and other cellular functions interpedently of FANCD2/FANCI ubiquitylation [13, 14].

Proper monoubiquitination of FANCD2 and FANCI also requires several other proteins such as PTMs, ATM, ATR, and CHK1, and other basic proteins of FANC complex important for optimal monoubiquitylation of FANCD2 and FANCI [15, 16].

It is also crucial that the phase of cell cycle monoubiquitylation of FANCD2/FANCI occurred. The complex of FANC proteins is considered active only during S phase assessed by the monoubiquitylation of FANCD2. Additionally, FANCD2 foci located on chromatids during G2 and M phases.

FANCD2 and FANCI are known as direct targets of ubiquitin ligase FANC complex [2]. Proteins of the complex demonstrated ligase E3 ubiquitin action. Additionally, UBE2T may act as E2 ligase while in vitro experiments UBE2T undergo monoubiquitylation in K91 lysine. Ubiquitylation is required for binding of the dimer to the chromatin. In order to be effective the process of DNA repair both proteins must undergo ubiquitination/deubiquitination.

USP1 is crucial for deubiquitination demonstrated complex with UAF1. The USP1-UAF1 cluster locates FANCI into the SIM area and causes deubiquitination of both FANCI and FANCD2 [18]. The deubiquitination signal allows the precision and track replication [12, 18].

Another important checkpoint of complex FANCD2/FANCI related to structure of chromatin. In vitro studies revealed that the chromatin structure affects the FANCI dependent FANCD2 ubiquitination. It has been reported that phosphorylation of histone H2AX is necessary to connect FANCD2 in chromatin after MMC or UVC damage responses. In cases of monoubiquitination, deficiencies of FANCD2 may be activated two other protein molecules with E3 ligase activity such as BRCA1 and RAD18 interacting with FANCD2 in order to detect FANCD2 in proper position [19].

Recent studies have shown that FANCD2 may act independently of the ubiquitination as a modulator of the NF-kB transcriptional activity. D'Andrea et al. proposed this activity be amended under stress and expression of the regulated genes such as FANCD2 activated after DNA damage responses.

It is widely accepted that the function of proteins of FA pathway involved in DNA repair by promoting homologous recombination (HR) rather than nonhomologous (NHEJ). Molecular failure during the operation of FANC pathway leads to deficiency of HR processes increasing the NHEJ [19, 20].

Cloning of the FANCD2 gene increased our understanding of the operation of FANC complex proteins [20]. The FANCL is a protein consisting of a characteristic region with ubiquitin-ligase E3 action. Mutations in ubiquitination region of FANCL affected the ligase E3 action on FANCD2 without affecting the interaction with other proteins of the complex. Recent studies have shown that it is necessary to connect the FANCD2/FANCI ubiquitination with FANCT/UBE2T for reconstitution of the E2-E3 ligase activation [21, 22] (**Figure 2**). This activation is unaffected by the FANCL, FANCB, and FAAP100.

Disturbance of any core proteins of FANC complex leads to destabilization and significant reduction of ubiquitination of FANCD2/FANCI and FANC inactivation [19, 21, 22].

The FANC complex monoubiquitinates protein FANCI at lysine 523 (K523) [10]. The monoubiquitination of FANCD2 and FANCI also requires the activity of several other proteins. ATM, ATR, and CHK1 cause phosphorylation of FANCD2 and FANCI and several core proteins of FANC complex crucial for the monoubiquitination of FANCD2 and FANCI [23].

Additionally, FANCD2/FANCI monoubiquitination is also dependent for the activity of Rad18. Rad18 and phosphorylated H2AX (Z-H2AX) lead FANCD2 and FANCI in subcellular regions [23].

Apart from the breakdown processes, the phase of the cell cycle occurred FANCD2/FANCI monoubiquitination is also important. The FANC complex proteins are active during the S

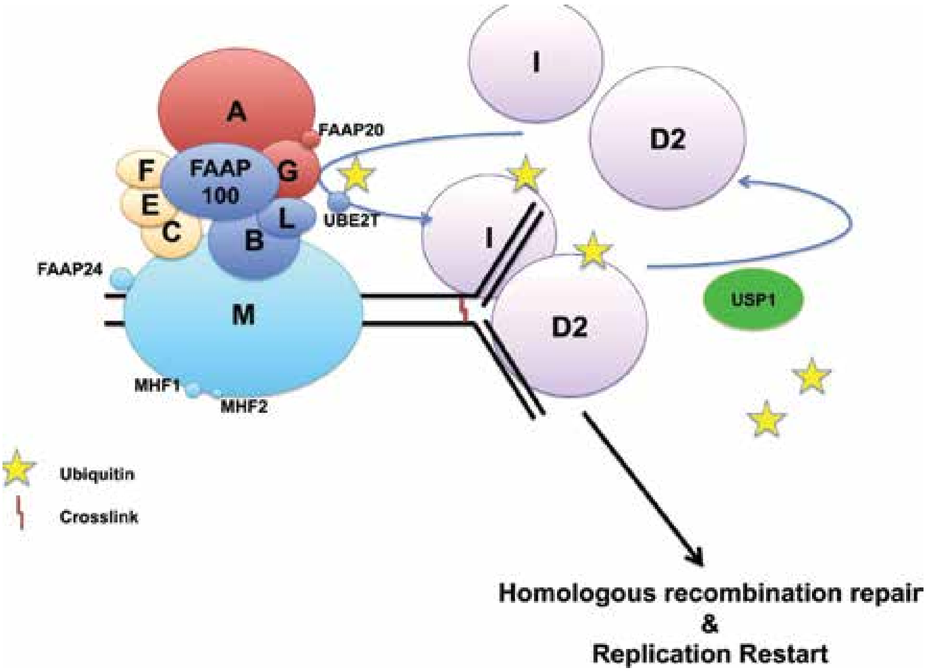


Figure 2. The FANC core complex is an E3 ubiquitin ligase targeting FANCD2 and FANCI to coordinate DNA repair. In response to stalled replication forks by DNA lesions (here a crosslink), the FANC core complex is recruited by FANCM-FAAP24 and MHF1 and MHF2 [6].

phase according to FANCD2 monoubiquitination [23, 24], while the presence of FANCD2 foci in sister chromatids also referred during G2 and M phases [25].

Therefore, when the cell stress processes occur and the cells are in S/G2 phase monoubiquitinated FANCD2 and FANCI identified in DNA repair area. These two monoubiquitinated proteins form a heterodimer complex [13]. The binding of FANCD2 with histone H2B reveals that ubiquitination is crucial of complex identification to chromatin and DNA repair process [26, 27].

The UBE2T protein is constitutively present in the core of the complex of FANC proteins, in contrast with the other proteins detected to cytoplasm. Mutations of UBE2T display FA considered as an important member of FANC complex proteins [28, 29]. Except the UBE2T, UBE2W appears to have ligase E2 activity regulated monoubiquitination of FANCD2/FANCI after UV exposure [30–32] (**Figure 2**).

Additionally, FANCD2/FANCI complex ubiquitination is required for chromatin regulation. As already mentioned, the effective regulation of these proteins induced after DNA damage not only undergoes ubiquitination but also regulates deubiquitination effectively [33–35].

FANCD2 can act independently of ubiquitination as a regulator of NF-kB activity, particularly through the action of the TNF-a promoter [35, 36]. D'Andrea et al. demonstrated the expression of the FANCD2 gene may be altered by DNA damage caused by UV exposure via SLX4/

FANCP ubiquitination of FANCD2. Besides, the FANCD2, FANCI, FANCA, and FANCG are also ubiquitinated. The FANCA is ubiquitinated and regulates the activity of proteasome [34].

The FANCG is not necessary for ubiquitination of FANCD2 and FANCI but is required for the interaction with RAP80-BRCA1 complex [35]. It seems that the interaction of FANCG-RAP80-BRCA1 affects the regulation of FANC complex proteins on HR/NHEJ procedures [36–38].

Recent studies have shown RAD18 E3 ubiquitin ligase activity, being crucial of monoubiquitylation of PCNA on replication forks [39, 40]. Monoubiquitylation of PCNA in the lysine-164 by the RAD18 and RAD6 activates the polymerase switching [40]. Apart from its role in the mechanism of regulation of the polymerase, the RAD18 associated with monoubiquitylation of molecules of DNA repair procedure such as 53BP1 and also interact with the DNA repair protein WRNIP1 [41].

RAD18 protein is also important in the process of homologous recombination independent of ubiquitylation [41]. Also in recent studies, it appears to be decisive in the RAD6 ubiquitylation of FANCD2. Experimental data by immunoprecipitation reveal that RAD18-FANCD2 binding takes place both in the presence or in absence of damage DNA. The RAD18 affected monoubiquitylation of FANCD2 and FANCI after treatment with various factors of DNA cross without depending on PCNA modification [40, 41]. The limited response of RAD18 leads to hypersensitivity after MMC and cisplatin treatment [40, 41].

The data indicate an essential role of E3 ligase for RAD18 identification FANCD2 and FANCI into chromatin, while the ubiquitylation process observed in phase S. The RAD18 regulates monoubiquitylation of FANCD2 and FANCI in FANCL independent manner [42–44].

Other recent reports indicated that RAD18 may coordinate events of homologous recombination. Huang et al. have shown that the RAD18 bound to DNA damage sites attracting other proteins in DNA repair process. This function is independent of its role as an E3 ubiquitin ligase [45].

Huang et al. demonstrated that RAD18 is important for attracting RAD51C. Cells with RAD51C deficiency revealed increased sensitivity and radial configuration in response to treatment with ICL [45]. Geng et al. reported that RAD18 participates in FANCD2 deubiquitylation and ubiquitylation of ID complex in ICLs repair process. Although the significance of ubiquitylation is equally important, deubiquitylation process may affect the normal cell cycle progression [42–44].

USP1-UAF1 complex causes deubiquitination both FANCI and FANCD2. This procedure is important for the proper function of DNA repair such as different procedure revealed [33]. Deubiquitination seems to regulate the proper DNA repair and replication procedure.

USP1 is regulated at a transcriptional level during phase S such as the molecule activity must be increased. USP1 levels during S phase are quite stable. There are unknown regulatory processes of chromatin related with deubiquitylation of FANCD2 [42–44]. USP1 activity is greatly enhanced by WD40 and UAF1. USP1 reveals to act as deubiquitylating enzyme for PCNA [45, 46].

3. The role of sort proteins in FANC complex operation

Considering both biochemical and functional criteria, FANC proteins are divided into three main groups [47]. According to immunoprecipitation experiments, major proteins of the first group constitute the main core of FANC complex [48, 49]. Proteins such as FANCA, B, C, E, F, G and L together with the FA-associated protein such as FAAP20 and FAAP100 detected during DNA repair processes on chromatin through FANCM complex. The complex binds FAAP24 and FANCM interacting histones through MHF1 and MHF2 [50].

Mutations in FAAP20, FAAP24, FAAP100 or MHF1, MHF2 deactivate DNA repair mechanisms through FANC proteins. The core proteins of FANC interact with UBE2T [51].

The UBE2T reveals E2 ubiquitin ligase activity monoubiquitinated FANCD2 and FANCI. The FANCD2 and FANCI, group II proteins are known targets of the ubiquitin ligase in the core of FANC complex. Experimental data have shown lack or improper function of FANC complex affects monoubiquitination of FANCD2 and FANCI [19, 52].

Ubiquitination of FANCD2 and FANCI is necessary for transfer of these proteins into the chromatin region interacting with proteins involved in homologous recombination process of DNA. Experimental results in mice revealed USP1 association in PCNA de-ubiquitination. USP-1 monoubiquitinate FANCD2/FANCI affecting the deterioration of DNA repair proteins [43, 44].

Proteins of the third group (peptides III) do not cause serious problems in ubiquitination of FANCD2 and FANCI. In this group, proteins related to the homologous recombination (HR) such as FANCD1/BRCA2, FANCJ/BRIP1, FANCN/PALB2, FANCO/RAD51C, FANCR/RAD51, FANCS/BRCA1, FANCU/XRCC2 (Sawyer et al. 2014), and proteins with endonuclease action including FANCP/SLX4 (interacts with SLX1) and FANCQ/XPF (interacts with ERCC1) [53].

The main proteins constituting core complex localized in the cytoplasm during cell cycle and contribute into nucleus after DNA damage display. Thus, proteins such as FANCA and FANCG are interacting with FAAP20, FANCB, FANCL, FAAP100, and FANCC. FANCL, FAAP100, and FANCB represent the catalytic subunit of the FANC complex [53].

Recent studies revealed the role of proteins of FANC complex in DNA damage control. FANCA, FANCC, and FANCD2 seem to have a crucial role in evolution of mitophagy that allows the degradation of damaged mitochondria [54, 55].

4. Evolutionary conservation of proteins in the Fanconi anemia pathway

Among the FA pathway, proteins such as FANCM, SLX4, and BRCA2 are most conserved in mammalians [56–61]. This evolutionary conservation indicates the importance of these proteins in cell cycle progression. Vertebrates, flies, worms, plants revealed recognizable orthologs of some of the components of the FANCL complex [62–64]. All organisms with FANCL also have FANCD2 and FANCI orthologs proven to be monoubiquitylated [62–64].

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References

[1] Sims AE et al. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. Nature Structural & Molecular Biology. 2007;**14**(6):564-567

[2] Sobeck A et al. The Fanconi anemia protein FANCM is controlled by FANCD2 and the ATR/ATM pathways. The Journal of Biological Chemistry. 2009;**282**:25560-25568

[3] Svendsen JM et al. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell. 2009;**138**(1):63-77

[4] Levran O et al. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. Nature Genetics. 2005;**37**(9):931-933

[5] Liu T et al. FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. Science. 2010;**329**(5992):693-696

[6] Renaudin X et al. The ubiquitin family meets the Fanconi anemia proteins. Mutation Research. 2016;**796**:36-46

[7] Garcia-Higuera I et al. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Molecular Cell. 2001;**7**(2):249-262

[8] Auerbach AD. Fanconi anemia and its diagnosis. Mutation Research. 2009;**668**(1-2):4-10

[9] Simpson LJ, Sale JE. Rev1 is essential for DNA damage tolerance and non-templated immunoglobulin gene mutation in a vertebrate cell line. The EMBO Journal. 2003; **22**(7):1654-1664

[10] Smogorzewska A et al. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. Cell. 2007;**129**:289-301

[11] Rickman K et al. Deficiency of UBE2T, the E2 ubiquitin ligase necessary for FANCD2 and FANCI ubiquitination causes FA-T subtype of Fanconi anemia. Cell Reports. 2015;**13**:35-41

[12] Singh TR et al. Impaired FANCD2 monoubiquitination and hypersensitivity to camptothecin uniquely characterize Fanconi anemia complementation group M. Blood. 2009;**114**(1):174-180

[13] Renaudin X et al. Proteomic analysis reveals a FANCA-modulated neddylation pathway involved in CXCR5 membrane targeting and cell mobility. Journal of Cell Science. 2014;**127**:3546-3554

[14] Lobitz E et al. (1892-1979): A jack of all trades. Nature Reviews. Cancer. 2006;**6**:893-898

[15] Bogliolo M, Surrallés J. Fanconi anemia: A model disease for studies on human genetics and advanced therapeutics. Current Opinion in Genetics & Development. 2015;**33**:32-40

[16] Bogliolo M et al. Histone H2AX and Fanconi anemia FANCD2 function in the same pathway to maintain chromosome stability. The EMBO Journal. 2007;**26**:1340-1351

[17] Soulier J. Fanconi anemia, Hematol. American Society of Hematology Education Program. 2011:492-497

[18] Nijman S et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**:331-339

[19] Rajendra E et al. The genetic and biochemical basis of FANCD2 monoubiquitination. Molecular Cell. 2014;**23**:858-869

[20] Timmers C et al. Positional cloning of a novel Fanconi anemia gene, FANCD2. Molecular Cell. 2001;**7**:241-248

[21] Renaud E, Rosselli F. FANCpathway promotes UV-induced stalled replication forks recovery by acting both upstream and downstream Polh and Rev1. PLoS One. 2013;**8**:536-593

[22] Renaud E et al. Impaired TIP60-mediated H4K16 acetylation accounts for the aberrant chromatin accumulation of 53BP1 and RAP80 in Fanconi anemia pathway deficient cells. Nucleic Acids Research. 2016;**44**:648-656

[23] Pichierri P, Rosselli F. The DNA crosslink-induced S-phase checkpoint depends on ATR-CHK1 and ATR-NBS1-FANCD2 pathways. The EMBO Journal. 2004;**23**:1178-1187

[24] Chan K et al. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. Nature Cell Biology. 2009;**11**:753-760

[25] Joo W et al. Structure of the FANCI-FANCD2 complex: Insights into the Fanconi anemia DNA repair pathway. Science. 2011;**333**:312-316

[26] Matsushita N et al. Direct inhibition of TNF-a promoter activity by Fanconi anemia protein FANCD2. PLoS One. 2011;**6**:233-245

[27] Matsushita N et al. A FancD2-monoubiquitin fusion reveals hidden functions of Fanconi anemia core complex in DNA repair. Molecular Cell. 2005;**19**:841-847

[28] Alpi A et al. Monoubiquitylation in the Fanconi anemia DNA damage response pathway. DNA Repair. 2009;**8**:430-435

[29] Virts E et al. AluY-mediated germline deletion, duplication and somatic stem cell reversion in UBE2T defines a new subtype of Fanconi anemia. Human Molecular Genetics. 2015;**24**:5093-5108

[30] Zhang J et al. FANCD2 monoubiquitination provides a link between the HHR6 and FA-BRCA pathways. Cell Cycle. 2008;**7**(3):407-413

[31] Zhang Y et al. UBE2W interacts with FANCL and regulates the monoubiquitination of Fanconi anemia protein FANCD2. Molecules and Cells. 2011;**31**:113-122

[32] Alpi AF et al. Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. Molecular Cell. 2008;**32**(6):767-777

[33] Oestergaard V et al. Deubiquitination of FANCD2 is required for DNA crosslink repair. Molecular Cell. 2007;**18**:798-809

[34] Xie J et al. RNF4-mediated polyubiquitination regulates the Fanconi anemia/ BRCA pathway. The Journal of Clinical Investigation. 2015;**115**:1523-1532

[35] Zhu B et al. K63- linked ubiquitination of FANCG is required for its association with the Rap80-BRCA1 complex to modulate homologous recombination repair of DNA interstand crosslinks. Oncogene. 2015;**43**:2867-2878

[36] Du W et al. Hyper-active non-homologous end joining selects for synthetic lethality resistant and pathological Fanconi anemia hematopoietic stem and progenitor cells. Scientific Reports. 2016;**6**:22167

[37] Naim V et al. ERCC1 and MUS81-EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis. Nature Cell Biology. 2013;**15**:1008-1015

[38] Naim V, Rosselli F. The FANCpathway and BLM collaborate during mitosis to prevent micro-nucleation and chromosome abnormalities. Nature Cell Biology. 2009;**11**:761-768

[39] Xin H et al. The human RAD18 gene product interacts with HHR6A and HHR6B. Nucleic Acids Research. 2000;**28**(14):2847-2854

[40] Watanabe K et al. Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. The EMBO Journal. 2004;**23**(19):3886-3896

[41] Huang J et al. RAD18 transmits DNA damage signalling to elicit homologous recombination repair. Nature Cell Biology. 2009;**11**(5):592-603

[42] Crossan G et al. Patel, disruption of mouse Slx4 a regulator of structure-specific nucleases, phenocopies Fanconi anemia. Nature Genetics. 2011;**43**:147-152

[43] Kim JM et al. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. Developmental Cell. 2009;**16**(2):314-320

[44] Kim Y et al. Mutations of the SLX4 gene in Fanconi anemia. Nature Genetics. 2011; **43**:142-146

[45] Vaz F, Hanenberg H, Schuster B, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nature Genetics. 2010;**42**(5):406-409

[46] Manandhar M et al. The ERCC1 and ERCC4 (XPF) genes and gene products. Gene. 2015;**569**:153-161

[47] Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nature Reviews. Genetics. 2007;**8**:735-748

[48] Meetei AR et al. A multiprotein nuclear complex connects Fanconi anemia and bloom syndrome. Molecular and Cellular Biology. 2003;**23**:3417-3426

[49] Meetei AR et al. A novel ubiquitin ligase is deficient in Fanconi anemia. Nature Genetics. 2003;**35**(2):165-170

[50] Zhao Q et al. The MHF complex senses branched DNA by binding a pair of crossover DNA duplexes. Nature Communications. 2014;**8**:2987-2992

[51] Hira A et al. Mutations in the gene encoding the E2 conjugating enzyme UBE2T cause Fanconi anemia. American Journal of Human Genetics. 2015;**96**:1001-1007

[52] Machida Y et al. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Molecular Cell. 2006;**23**:589-596

[53] Shamseldin H et al. Exome sequencing reveals a novel Fanconi group defined by XRCC2 mutation. Journal of Medical Genetics. 2012;**49**:184-186

[54] Orvedahl A et al. Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature. 2011;**480**:113-117

[55] Sumpter R et al. Fanconi anemia proteins function in mitophagy and immunity. Cell. 2016;**165**:867-881

[56] Cohn M et al. UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. Mol. Cell. 2007;**18**:786-797

[57] Cole AR et al. The structure of the catalytic subunit FANCL of the Fanconi anemia core complex. Nature Structural & Molecular Biology. 2010;**17**(3):294-298

[58] Guervilly J et al. Loss of CHK1 function impedes DNA damage-induced FANCD2 monoubiquitination but normalizes the abnormal G2 arrest in Fanconi anemia. Human Molecular Genetics. 2008;**17**:679-689

[59] Litman R et al. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell. 2005;**8**(3):255-265

[60] Munoz IM et al. Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. Molecular Cell. 2009;**35**(1):116-127

[61] Sato K et al. DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. Nucleic Acids Research. 2012;**40**:4553-4561

[62] Sawyer S et al. Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. Cancer Discovery. 2014;**5**:135-142

[63] Haynes B et al. Crosstalk between translesion synthesis, Fanconi anemia network, and homologous recombination repair pathways in interstrand DNA crosslink repair and development of chemoresistance. Mutation Research. 2016;**736**:258-266

[64] Sonoda E et al. Multiple roles of Rev3, the catalytic subunit of polzeta in maintaining genome stability in vertebrates. The EMBO Journal. 2003;**22**(12):3188-3197

Chapter 11

Review of the Ubiquitin Role in DNA Repair and Tumorigenesis, with Emphasis in Breast Cancer Treatment; Current Data and Future Options

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Additional information is available at the end of the chapter

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Abstract

Breast carcinoma remains the commonest carcinoma among women worldwide. Despite the fact that impressive progression has been achieved so far regarding pathophysiology, histopathology and treatment of this cancer, there are still undiscovered fields on molecular and therapeutic levels. The need of resolving problems such as chemoresistance, recurrence and metastasis has led in revealing key molecules in the development and progression of malignancies, including breast tumors. In this review, we will briefly describe the functions of ubiquitin and post-translational modifications (PTMs) focusing specially in DNA repair and then discuss about the implication of ubiquitin and related molecules in tumorigenesis and specifically in breast carcinoma. So far there are only few drugs approved by FDA that target the ubiquitin system. There will be an analysis regarding the current and potential anti-cancer therapeutic strategies based on targeting specific ubiquitin-related molecules.

Keywords: ubiquitin, DNA repair, deubiquitinating enzymes, breast carcinoma, target therapy

1. Introduction

Breast cancer is the most common carcinoma and the second cause of death among women worldwide. Incidence rates vary greatly worldwide from 19.3/100,000 women in Eastern Africa to 89.7/100,000 women in Western Europe. Although age-adjusted breast cancer mortality rates have declined by 36% from 1989 to 2012, the incidence of this carcinoma is increasing due to increase life expectancy and adoption of western lifestyle [1]. In US, during 2015 there were 231,840 new cases of invasive carcinoma, 60,290 new cases of in situ carcinoma and 40,290 deaths due to breast cancer, for women of all age groups [2, 3]. As of March 2017, there are more than 3.1 million women with a history of breast cancer in the US; this includes women currently being treated and women who have finished treatment [4].

This increase explains the interest for research in depth for mechanisms of tumor progression and subsequently for potential therapeutic drugs. During the last years, there has been progress in understanding the histopathology and molecular biology of breast carcinoma, but still there are many unanswered questions to be solved. The complexity of interaction between internal and external factors that lead to the evolution of breast cancer cells is evident via the variety of the clinical, morphological, histopathological and molecular characteristics of this malignancy. Currently, there are many therapeutic tools that in combination with surgery offer better survival rates and quality of life for breast carcinoma patients, including hormonotherapy, radiotherapy, chemotherapy and targeted monoclonal antibody therapy (Trastuzumab and Herceptin), depending on the tumor stage and receptors expression in each patient [4]. Despite improved treatments that have been achieved, many breast tumors are not eradicated effectively due to their resistance—intrinsic or acquired—or relapse following initial response, resulting in metastasis at later stages and subsequently to patient death. This outcome suggests that further understanding of the molecular mechanisms underlying the development of breast cancer is essential to identify new therapeutic agents and achieve better treatment. In recent years, there is an increasing interest and investigation regarding potential targets for molecular therapy. One of the most promising field concerns the different levels of ubiquitin system, however, so far there are only a handful of drugs targeting this system that have been approved by the FDA.

Mammalian cells maintain their homeostasis regarding cell cycle control through proliferation and apoptosis signals that lead to specific protein expression. Ubiquitination is one of the PTMs that are involved in a variety of complex cellular processes such as endocytosis, cell cycle progression and activating to inactivating substrates. The ubiquitin system regulates the majority of cellular activities via proteolysis and/or signal transduction—modulating protein-protein interactions and participates in DNA damage repair. DNA repair not only has a crucial role regarding cell cycle control, proliferation and apoptosis but also in diseases, tumor progression and even metastasis of malignant neoplasms, including breast carcinoma [5].

2. General features and functions of ubiquitin

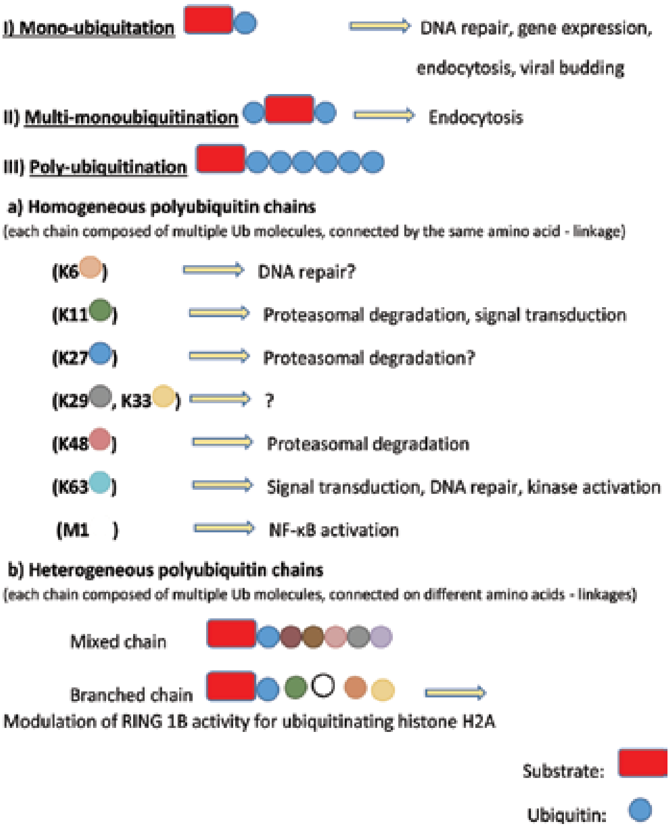
The genome of the organisms can be modified by epigenetic alterations, except for the DNA sequence of genes. Epigenetic modifications include DNA methylation, histone modifications (methylation, acetylation, phosphorylation, sumoylation and ubiquitination) and non-coding RNAs that lead to changes in gene expression [6]; in addition, PTMs regulate the cellular functions of most proteins—the best studied of which include phosphorylation, acetylation and ubiquitination—with constant interplay among them. All the aforementioned changes can influence both normal and disease states of an organism and are reversible. There is an interplay between PTMs on target proteins and the attachment or removal of PTMs can determine the substrate fate [7].

Ubiquitin is a 76-amino acid protein that is expressed in eukaryotic cells and is involved in the cell cycle regulation by proteolysis and/or signal transduction, while the majority of cellular activities are regulated with the involvement of the ubiquitin system [8].

The ligation of ubiquitin molecules to substrate proteins leads either to their degradation or can also change the substrate activity, localization and affinity to binding partners or even lead to other non-proteolytic processes [9]. Three enzymes catalyze the attachment of ubiquitin to a target protein: E1 activating enzyme, E2 conjugating enzyme and E3 ligase [8–10].

In general, the target protein is subjected to degradation with the involvement of 26S proteasome, a large multi-enzyme complex [8, 10].

The progress in novel technologies has provided information about the structure and function of the ubiquitin and thanks to the latest mass spectrometric techniques our understanding of the complexity and diversity of the polyubiquitin chains has increased; proteins can be modified at one or more lysine (K) residues with either a single ubiquitin molecule—mono- and multi-monoubiquitination, respectively—or with ubiquitin polymers—polyubiquitilation. Ubiquitin moieties can be conjugated through one of their lysine residues (K6, K11, K27, K29, K33, K48 and K63) or the N-terminal methionine residue (M1). There can be homotypic and



Scheme 1. Variety of Ub roles in cellular processes, regarding chains and linkage types.

heterotypic chains, by single or mixed linkage types, respectively, and chains can be branched; also, ubiquitin molecules can be modified by PTMs (e.g. acetylation and phosphorylation) [9].

The process of ubiquitination is reversible thanks to deubiquitinating enzymes (DUBs) that count to approximately 100 in the human genome and are classified into at least 6 families (Ub-specific proteases, Ub carboxy-terminal hydrolases, ovarian tumor proteases, Machado-Joseph disease protein domain proteases, metalloenzymes and monocyte chemotactic protein-induced proteases) [11, 12]. The ubiquitin code through all these complex steps are involved not only in many biological processes such as DNA damage repair, apoptosis, cell cycle control, differentiation, but also in diseases, including carcinogenesis [12]. A lot have been achieved so far in our understanding but there are still many to be solved in the analysis of the numerous different roles of the ubiquitin code (**Scheme 1**, [13-15]).

3. Ubiquitin and DNA repair

3.1. Ubiquitin and cell cycle control

Ubiquitin is an essential, highly conserved protein expressed in various cells. It can be found in either free form or covalently attached to a target protein [16–18]. Ub by acting as cellular signal controls a wide range of biological processes including protein degradation, DNA repair, endocytosis, autophagy, degradation, immunity and inflammation.

Ub, E1, E2 and E3 enzymes are successively required to target certain substrate for degradation. Ub is attached to specific substrates in a three-step mechanism, with distinct enzymes catalyzing each step. During first activating step, Ub becomes covalently conjugated to the side chain of an E1-cysteine via its carboxy-terminal (C-terminal) glycine in an ATP-dependent reaction. Activated Ub is transferred via E2-enzyme (ubiquitin-conjugating enzyme) at C-terminal glycine residue of Ub at an internal cysteine. Finally, Ub bound E2 interacts with an E3 Ub ligase that catalyzes Ub transfer from E2 to a specific target protein [19, 20].

The specificity of Ub signaling is accomplished due to association of the molecule with different substrates (mono- and poly-ubiquitination) [21]. The activity of protein molecules depends on the interacting region with Ub (UBD – ubiquitin binding domain) affecting intracellular signaling pathways [22, 23]. Monoubiquitination plays an important role in the recognition of double-strand breaks (DSBs). K63 linked chains of Ub are involved in the production of signaling processes during DNA repair [24, 25]. The ubiquitin/proteasome system (UPS) is a main regulator of protein stability and plays an important role in execution of DNA damage response (DDR). Several studies using proteasome inhibitors validated UPS as a valuable therapeutic target in cancer [26, 27].

Recognition of DNA damage sites by Ub accumulation can be detected immediately during DNA damage [28]. Ubiquitination of H2A, H2B and H2AX is crucial in order to promote destabilization of nucleosomes [29, 30]. Monoubiquitination of H2A from RNF2-BM1 cluster seems to be essential for transcriptional repression of RNA-PoI II [31]. RNF2-BM1 is also involved in monoubiquitination of H2AX at K119 and K120 (E2: UbcH5C), which initiates recruitment of the apical PI3K-related kinase ATM (ataxia telangiectasia mutated) [32, 33].

DNA repair process induces cell cycle arrest by non-homologous recombination (NHEJ) and phosphorylated p53. The p53 cooperates with the ATM/ATR and induces cell cycle arrest by activating apoptosis [34]. The ubiquitin selective Cdc48/p97/VCP affects Ddr protein and participates in ubiquitination and activation of degradation via proteasome [35–37]. Consequently, the interaction of ubiquitination and phosphorylation mechanism modulates the activity of many proteins such as p53.

Cell cycle procedures and DNA repair mechanisms are crucial for the genetic stability in all eukaryotic cells [38]. The NHEJ process reunites free ends of the DNA with relatively restricted homology [39]. The NHEJ process can be considered as less robust procedure according to reconnection of non-complementary ends of the chaperone activity such as DNA-PK, which remove the mismatched nucleotides [40].

The cell cycle control procedure and the reliability of the progress of NHEJ depend on the presence of NHEJ DNA repair proteins such as Ku70/80 and ligase XRCC4-IV [41, 42]. Besides NHEJ, the process of homologous recombination (HR) is also crucial for the genetic stability during predominantly S and G2 cell phases demonstrated the recombination of sister chromatids or intact homologous chromosomes [43].

Protein ubiquitination plays an important role during DNA repair process, as several different molecules of the cell cycle are ubiquitinated such as 53BP1. The 53BP1 protein promotes NHEJ and HR activation and the protein interaction with BRCA1 during HR [44]. The binding of the protein to the double-strand break (DSB) region is associated with activity of the RNF8 protein. K63 acts as E3 ubiquitin ligase and catalyzes the ubiquitination of substrate proteins via RNF8 [45].

During DNA damage, RNF8 binds to the damage site interacting with MDC1 and catalyzes monoubiquitination of H2A and H2AX through action of K63. The monoubiquitination of histones promotes RNF8-dependent recruitment of a second E3 ligase RNF168 at the DSB [46, 47]. The polyubiquitination of H2AX further promotes the recruitment of RNF168 which amplifies the RNF8 dependent histone ubiquitination by K63 [47, 48]. The interaction of complex RNF8/RNF168/K63 affects the accumulation of DNA repair proteins such as BRCA1 and 53BP1.

53BP1 cannot directly bind to K63 according to absence of any relevant binding site [49]. For accumulation of 53BP1, p97 activation is necessary in order to remove L3MBTL1 protein to the DSBs sites. The p97 binds to the ubiquitinated L3MBTL1 in chromatin, affecting the binding of 53BP1 [35].

According to these, RNF8 ubiquitinates K48-dependent substrates such as JMJD [50], protein of NHEJ KU 80 [28] and polymerase DNA (PCNA), which are crucial for DNA repair procedure [51]. These proteins may be removed from chromatin by the action of the proteasome.

Polyubiquitination of PCNA may change cellular signaling of significant molecules [52]. This regulator leads to waterfall ubiquitination during the DDR. During this phase, PCNA mono- or poly-ubiquitinated at K164 residue regulates DNA repair process [53, 54]. BRCA1 plays a key role through BRCA1—BRCT domain for the cell cycle progression [55]. BRCA1 reveals E3 ubiquitin ligase activity which is vital for genomic integrity [56, 57].

p53 has already been identified as an important molecule for cell cycle progression. The RITA (enabler of p53 and induction of tumor apoptosis) is a small molecule that blocks p53 action [58].

However, it appears to be dependent by ubiquitin even if in pro-apoptotic stage has been described as p53 independent [59, 60]. Nowadays, small molecules are designed to prevent the interaction between the p53 and MDM2, affecting in this way cell cycle control or apoptosis [61].

3.2. The importance of ubiquitin to cellular apoptosis

Besides the activation of DNA repair, the apoptotic response determines cellular integrity [62]. DNA damage responses depend on operation of p53 as a key factor of cell function. p53 is an important component of the mitochondrial apoptosis and plays a crucial role in the coordinated cell death by DNA damage processes.

The expression of pro- and anti-apoptotic proteins such as members of Bcl2 family is decisive for DDR signaling. The ability of apoptosis of immune cells is crucial for cellular differentiation. Almost 90% of pre B and T cells reveal apoptosis during maturation [63].

In this process, UBE2C regulates the correct alignment of chromosomes during mitosis. Overexpression of UBE2C has been detected in many cancers including breast, colon, prostate, ovary, thymus, uterus and lung. Expression varies during the cell cycle and peaks at pro-metaphase and is reduced into anaphase [64, 65]. Suppression of expression of UBE2C contributes in tumor progression and regulates chromosome segregation during mitosis in various malignancies (cancer tissues). Overexpression of UBE2S and HIF1a is detected in various tumors such as liver, colon cancer, breast cancer and metastatic cholangiocarcinoma [66, 67].

There are approximately 600 E3 ubiquitin ligases encoded by human genome and the mechanism of ubiquitination depends on conserved catalytic domains [68, 69]. E2 and E3 ubiquitin ligase activity contribute to aberrant oncogenic signaling, metastasis and resistance to chemotherapy. STUB1 molecule demonstrated E3 ubiquitin ligase activity in cancer cells enhanced by limited expression of STIP1. The STUB1 operates as a complex with other proteins and promotes control of different regulatory molecules such as c-myc and SRC-3 through ubiquitination. The restricted expression of STUB1 regulates the signaling of NFkB and the anti-apoptotic proteins Bcl2 and AKT, enhancing the process of inflammation and cellular metastasis. STUB1 is E3 ubiquitin ligase which modulates the stability of the p65 subunit of NFkB in cell colon cancer lines [70, 71].

Negative regulation of expression of STUB1 affects the activity of the p65 subunit and increases VEGF, cyclin D1, c-Myc, IL-8 and MMP-2 by the action of NFkV involved in angiogenesis and cancer metastasis. According to cell lines of pancreatic cancer, STUB1 has tumor suppressor activity and regulates the stability of EGFR via proteasomal degradation mediated by tyrosine kinase receptor (RTK). The STUB1 regulates phosphorylation of EGFR at Tyr845 and Tyr1068 positions, activating signaling pathways of PI3K/AKT and Src/FAK. Downregulation of STUB1 increases oncogene signaling of EGFR and enhances action of RTK inhibitor, erlotinib, leading to apoptosis *in vivo*. STUB1 modulates proteasomal degradation of NFkB and the EGFR in several volumes [72–74].

The ultimate central goal of conventional cancer therapy is the effective elimination of tumors by DNA damage-induced apoptosis (DDIA) since the balance of protein abundance and functionality are decisive for DDR. Deregulation of ubiquitin-signaling pathways is intimately associated with tumorigenesis and therapy resistance [60].

Accumulating recent evidence conclusively identified ubiquitin signaling as a valuable target in DDR and cancer chemoresistance. The majority of these efforts focused on the regulation of p53 as one of the central determinants of DDR outcomes. Accordingly, an increasing number of specific regulators of p53 have been identified and evaluated as therapeutic target.

RITA (reactivation of p53 and induction of tumor cell apoptosis) is a small molecule that blocks p53 [58]. p53 regulates cell cycle control in different tissues including myelomas [60]. MI-63 and MI-219 molecules can block the interaction between p53 and MDM2, demonstrating that p53 mediates the disruption process of the cell cycle or apoptosis in tumor cells [61].

3.3. The importance of ubiquitination in the evolution of cell proliferation

Ubiquitination is a crucial post-translational modification that regulates cell cycle progression. Proteins that show E3 ubiquitin ligase activity such as SCFSKP2, SCFFBW7 and APC/C are responsible for coupling ubiquitin in different specific proteins affecting proteasome and regulation of degradation.

Many proteins with E3 ligase activity may operate either as oncoproteins or as tumor suppressors. SKP2 is an oncoprotein involved in cell cycle progression through interaction with cyclins, p27kip1 inhibitors and p21WAF/Cip1 complex [75, 76]. The identification of related E3 ligases and their substrates may be important therapeutic options for cancer treatment. Molecules such as RNF115 and BRCA2 reveal ubiquitin E3 ligase activity overexpressed in estrogen receptor (ER)-positive tumors [77].

A recent study showed that BRCA2 directly interacts with RCL [78]. The BRCA1 and BRCA2 are key molecules for the cell cycle control interacting with a large number of other factors including Rab7, UBC9 and hHR23a 14-3-3s [79, 80]. Several studies have also shown that protein p21 show ligase E3 activity interacting with SCFSkp2, CRL4Cdt2 [81], the APC/CCdc20, MKRN1 and RNF126; BRCA2 promotes proliferation of ER-positive breast cancer cells through its interaction with p21 [82, 83].

In eukaryotic cells, CDKs activation is controlled by the availability of other cyclins and molecules, for example, p21, p27 and p57. Deregulation procedures of cyclins or CDKs determine cell cycle control and proliferation, affecting cancer progression. The process and effect of ubiquitin through regulation of proteasomal activity also plays a critical role in cell cycle control [84].

BRCA2 has E3 ubiquitin ligase activity inducing ubiquitination of p21 protein. Alterations of BRCA2 activity affect and promote proliferation of breast cancer cells. BRCA2 activity is influenced by estrogen through ER receptor activity in MCF-7 cells [78]. It has also been found that BRCA2 is expressed at high levels in ER-positive breast cancers in MCF-7 and T47D cell lines.

BRCA2 reveals 46% homology to SEQ RNF126, affecting the activity of p21 [83]. Both BRCA2 and RNF126 interact with the p21. This complex may promote ubiquitination of the protein [82, 83]. BRCA2, RNF126 and other E3 ligases coordinately regulate the expression levels of p21. Except for p21, many other proteins interact with BRCA2 including Rab7, tetherin, UBC9, hHR23a and 14–3-3 s [82, 83]. However, BRCA2 does not appear to affect proteins degradation via ubiquitin mediation. BRCA2, by the action of an Ub ligase E3, induces proliferation of breast cancer cells via the ubiquitination of p21 and its degradation via proteasome.

3.4. Ubiquitin and tumor progression: Metastasis

Overexpression of E2 molecules in tumor metastasis plays an active role in regulating cell cycle progression and inflammation processes. In cases of defective expression of E3 ubiquitin ligases, the oncogene signaling, metastasis and resistance in chemotherapy are elevated.

Downregulation of ubiquitin E3 ligase in cancer is associated with STUB1 action. STIP1 is an escort molecule which regulates the cell cycle control and promotes ubiquitination of regulators of the cell cycle such as c-Myc and SRC-3. Reduced expression and downregulation of expression of STUB1 and NFkB activates signaling of anti-apoptotic proteins, for example, Bcl-2- and AKT-promoting inflammation, cell survival, cellular infiltration and metastasis [70, 71].

The reduced expression of STUB1 in colon cancer is associated with reduced degradation of the subunit p65 and increased expression of NFkB and molecules of cyclin D1, c-myc, IL-8 and MMP-2 involved in angiogenesis and procedure of metastasis [85]. In pancreatic cancer, STUB1 is a tumor suppressor and regulates the stability of EGFR via proteasomal degradation. In addition, decreased expression of STUB1 increases oncogenic signaling of EGFR in pancreatic cancer cells by limiting the response of RTK, erlotinib, affecting tumorigenesis [86].

Interaction of UBE2N/UBE2V1 affects metastasis in breast cancer cells. UBE2N shows ubiquitin ligase activity interacting with the cofactor UBE2V1 and induces ubiquitination in Lys63 residue activating NFkB during inflammation. UBE2N is overexpressed in many tumors such as breast, pancreatic, colon, prostate, ovarian and lymphoma. UBE2N is important molecule in the process of metastasis of breast cancer cells to the lung by activating TGFb mediated by TAK1 and p38 [85].

It has been shown in several studies that UBE2N E2 ubiquitin ligase (UBE2V1) is overexpressed in breast cancer cells and increases penetration and migration. Breast cancer cells are characterized by increased expression of metalloproteinase-1 (MMP1) via activation of NFkB [87, 88].

UBE2V1 inhibition via shRNAs reduced breast tumor progression and metastasis *in vivo*. Molecules with E3 ubiquitin ligase activity have been determined in pluripotent cancer stem cells. Pluripotent cancer stem cells are characterized by overexpression of the embryonic stem cell markers SOX2 (SRY-2), Oct4 (Octamer-4) and Nanog. These transcription factors are key regulators of pluripotency and of the inhibition of cellular differentiation. Specific indicators in the surface of cancer cells CD133 and CD44 are associated with increased resistance to chemotherapy. E3 ligases control cell differentiation by regulation of expression of these specific proteins in different cancer types [89].

4. Ubiquitin-related molecules and breast cancer; target therapy

4.1. p53/MDM2

In about 50% of human malignancies there is mutation of TP53, making this "genome guardian" the most frequently mutated gene in cancer [90, 91]. In addition, for most cancers lacking mutation, the wild-type p53 is inactivated by interaction with cellular (MDM2/MDM4) or viral proteins that lead to its degradation. For these reasons, there is a great interest regarding targeting these molecules for the cancer treatment and during the last years several compounds have become available that can restore wild-type properties of p53 for TP53-mutated malignancies or prevent the binding of MDM2/MDM4 to wild-type p53, thereby blocking its degradation in a variety of malignancies; the disparity provides distinct therapeutic opportunities for targeting cancers with p53 wild-type than those with p53 mutant cancers. Several preclinical studies have demonstrated that reconfiguration of mutant, to its normal, active WT p53 conformation, restores apoptosis and promotes tumor regression (88). Although the overall frequency of p53 mutation in breast cancer is approximately 20–30% (70–80% are TP53 wild-type), certain types of it are associated with higher frequencies, for example, in carriers of germline BRCA1 and BRCA2 mutations, strikingly, in typical medullary breast carcinomas, p53 mutation occurs in 100% of cases [92, 93]. In addition, TP53 mutation distribution is highly linked to molecular breast tumor subtypes found in 26% of luminal tumors (17% of luminal A and 41% of luminal B), in 50% of HER2-amplified tumors, in 69% of molecular apocrine breast carcinomas and in 88% of basal-like carcinomas [93].

In general, patients carrying a *TP53* missense mutation, leading to expression of a mutant p53 protein in the germline, have a significantly earlier cancer onset than patients with mutations in *TP53* that result in loss of p53 protein expression. Mutant p53 has been shown to play a role in many different cellular processes, for example, proliferation, invasion, increased migration, genomic instability, cell survival, angiogenesis, EMT, stem cell dedifferentiation and drug resistance. The tumor suppressor role of wild-type p53 is undoubtable, but studies showed that mutated p53 can result in both loss of wild-type activity and gain of a novel functions, promoting tumorigenesis and a more aggressive tumor profile [94]. The strategies that are currently being explored to target mutant p53 include small molecule compounds that specifically restore wild-type conformation and transcriptional activity of mutant p53, induce depletion of mutant p53, inhibit the downstream pathways of oncogenic mutant p53 and induce synthetic lethality to mutant p53 [95, 96]. The fact that most mutant p53s are expressed at very high levels in cancer cells, leading to their immunohistochemical detection, makes these proteins very attractive therapeutic targets.

4.1.1. *Hsp90 inhibitors: geldanamycin, 17-AAG and ganetespib*

Blocking the function of heat shock protein 90 (Hsp90) leads to depletion of several oncogenic proteins such as ErbB2 and mutant p53, because Hsp90 contributes to the accumulation of mutant p53 by inactivating p53 ubiquitin ligases, MDM2 and CHIP [95]. Geldanamycin is one of the most potent and effective hsp90 alpha inhibitor and is used to target breast cancer; however, it failed to move into the clinics due to the toxicity associated with its solubility. Geldanamycin was modified chemically to develop 17-AAG and later 17-DMAG, which have higher solubility and lesser toxicity [97]. Nonetheless, in order to achieve better efficacy against breast cancer, a more potent, soluble and least toxic analogues need to be developed such as an analogue and geldanamycin-based polymeric magnetite nanocomposite; the latter plays a vital role in efficacious therapy, showing selective cell kill of cancerous breast cells, while vanquishing normal cells and hepatic toxicity [98]. Another Hsp90 inhibitor, ganestespib, has proved to have little effect on wild-type p53 levels and induces mutant p53 depletion with increased apoptosis in tumors *in vivo* in both p53^{R248Q} Hupki (human p53 knock-in) and p53^{R172H} knock-in mouse models. Ganetespib is under evaluation in clinical trials, supported by preclinical evidence of its potent anti-tumor activity in different breast cancer subtypes; a phase II trial of single agent ganetespib was conducted in patients with unselected metastatic breast cancer. The clinical activity was notable in patients with trastuzumab-refractory HER2+ and triple negative breast cancer. In addition there was evidence of tumor shrinkage, specifically in patients with lung metastases. In that trial ganetespib was well tolerated, thus responses in more targeted populations harboring specific HSP90-dependent oncoproteins justify its further study [99].

4.1.2. Histone deacetylase inhibitors (vorinostat/SAHA, romidepsin/depsipeptide, belinostat)

Histone deacetylases (HDACs) are a group of enzymes that remove acetyl groups from histones and regulate expression of tumor suppressor genes such as p53. HDAC inhibitors (HDACi) have the potential to disrupt multiple signaling pathways to inhibit tumor growth and induce apoptosis. HDAC inhibitors are differentiated by their structure and further characterized into different subgroups [100]. Specifically, suberoylanilide hydroxamic acid (SAHA, also known as vorinostat), a FDA-approved HDACi that inhibits class I, II and IV HDACs, induces degradation of mutant p53 by inhibiting HDAC6 activity, an essential positive regulator of Hsp90 and subsequent disruption of the HDAC6/Hsp90/mutant p53 complex, leading to mutant p53 ubiquitination by MDM2 and CHIP. SAHA shows higher cytotoxic effects on cancer cells carrying mutant p53 than those having wild type or null for p53. SAHA also sensitizes cancer cells to a topoisomerase inhibitor camptothecin in a mutant p53-dependent manner. Currently, all three drugs approved by FDA (vorinostat (SAHA), romidepsin/depsipeptide (Istodax)) and belinostat (Beleodaq) are being further evaluated for other than hematological malignancies and for solid tumors, either as a single agent or in combination with other drugs. Specifically, investigation of romidepsin for the treatment of inflammatory breast cancer revealed that it potentially induced destruction of IBC tumor emboli and lymphatic vascular architecture. Also, a combination of depsipeptide and gemcitabine was tested in patients with advanced solid tumors including pancreatic, breast, NSCLC and ovarian and the study identified a dose level of 12 mg/m^2 romidepsin and 88 mg/m^2 gemcitabine for phase II trial [101]. Clinical trials studying the combination of chemotherapy or hormone therapy and HDAC inhibitors show promising efficacy. [100].

4.1.3. Beclin1

Autophagy is an important intracellular catabolic mechanism that mediates the degradation of cytoplasmic proteins and organelles. We report a potent small molecule inhibitor of autophagy named “spautin-1” for specific and potent autophagy inhibitor-1. Spautin-1 promotes the degradation of Vps34 PI3 kinase complexes by inhibiting two ubiquitin-specific peptidases, USP10 and USP13 that target the Beclin1 subunit of Vps34 complexes. Beclin1 is a tumor suppressor and frequently monoallelically lost in human cancers. Interestingly, Beclin1 also controls the protein stabilities of USP10 and USP13 by regulating their deubiquitinating activities. Since USP10 mediates the deubiquitination of p53, regulating deubiquitination activity of USP10 and USP13 by Beclin1 provides a mechanism for Beclin1 to control the levels of p53 [102]. One study provided a molecular mechanism involving protein deubiquitination that connects two important tumor suppressors, p53 and Beclin1, and a potent small molecule inhibitor of autophagy as a possible lead compound for developing anti-cancer drugs. Actually, since Beclin 1 is expressed in breast cancer cells, it could be a unique effective drug target for the prevention and treatment of breast cancer. However, the expression of Beclin 1 varies according to cancer molecular subtypes, and Beclin 1 is involved in both breast cancer suppression and tumor progression; therefore, the decision of using a Beclin 1 inducer or inhibitor should be made based on breast cancer stage and subtype [103].

4.1.4. Nutlin-3a

In 2004, a small molecule antagonist of Mdm2 was discovered, known as Nutlin-3a or Nutlin. The discovery of nutlin-3a, the first in a class of small molecule MDM2 inhibitors that binds to MDM2, preventing its association with and degradation of p53, has led to an extensive list of related compounds. Preclinical modeling with nutlin-3a showed improved anti-cancer activity in combination with cytotoxic- and molecular-targeted therapies, in many tumor types; the high rate of MDM2 overexpression in ER-positive breast cancer, and the ability of MDM2 inhibitors to ubiquitinate steroid hormone receptors, has led to the evaluation of this class of drugs in combination with endocrine therapies [104]. It has also been shown that p53 activation with nutlin in combination with fulvestrant, a selective ER degrader, leads to a greater degree of apoptosis *in vitro*. However, the subsequent toxicity of the combination partner plays critical role for the success of such an approach clinically.

Also, there has been shown the potential of Nutlin-3 or similar drugs as treatment options to overcome chemoresistance due to cancer stem cells (CSCs) [105]. In a recent study, patient-derived xenografts were used, as a clinically relevant model of numb-deficient breast cancers; the unlimited self-renewal and high tumorigenic/metastatic potential of cancer stem cells in numb-deficient carcinomas could be selectively reverted by re-expression of the tumor suppressor numb, or pharmacological restoration of p53 function with the Nutlin-3. Targeting the numb/p53 dysfunction selectively interferes with the CSC compartment of numb-deficient BCs, with only modest effects at the level of the bulk tumor population. The combined use of Nutlin-3 with standard chemotherapy (paclitaxel), increases the response to therapy of numb-deficient breast carcinomas and prevents tumor relapse after therapy discontinuation [106].

4.1.5. RING finger protein 31 inhibitors

The atypical E3 ubiquitin ligase RNF31 (other names HOIP and ZIBRA) is highly expressed in breast cancer, decreasing p53 stability, whereas depletion of RNF31 in breast cancer cells causes cell cycle arrest and cisplatin-induced apoptosis in a p53-dependent manner. Furthermore, RNF31 is associated with the p53/MDM2 complex and facilitates p53 polyubiquitination and thus its degradation, suggesting that RNF31 regulates cell death. As p53 wild type tends to appear in ERα-positive breast cancers (Luminal A and B), there is still little known about RNF31 in HER2 type or triple negative breast cancers. Analysis of publically available clinical data sets displayed a negative correlation between RNF31 and p53 target genes, including *IGFBP3* and *BTG1*, consistent with the fact that RNF31 regulates p53 function *in vivo* as well, findings suggesting RNF31 as a potential therapeutic target to restore p53 function in breast cancer [107, 108]. A lot need to be investigated by future studies on the development of drug targets (RNF31 inhibitors) and their clinical application.

4.2. CDKs – CDIs

Cell cycle regulation has been identified as an attractive target for targeted drug therapy of cancer. Several compounds of CDK inhibitors entered preclinical and early clinical trials including first- and second-generation agents that are more specific to certain CDKs.

4.2.1. Palbociclib and related molecules

The results with broadly acting CDK inhibitors (first-generation inhibitors such as flavopiridol) so far were largely disappointing. The second-generation compounds have shown more potent activity against their targets and a more favorable safety profile. Recent preclinical and clinical studies using a novel (oral) reversible CDK4/6 inhibitor, palbociclib, have shown the role of CDK4/6 as a potential target in estrogen receptor-positive (ER+) breast carcinoma. Specifically palbociclib (PD-0332991) has recently received accelerated Food and Drug Administration approval for the treatment of hormone receptor-positive (HR+) metastatic breast cancer in combination with letrozole, while further data suggest improved outcome when combined with fulvestrant (hormonotherapy). In addition to palbociclib, two other small molecule CDK4/6 inhibitors are currently in clinical development, ribociclib (LEE011) and abemaciclib (LY2835219); actually the FDA has approved the CDK 4/6 inhibitor ribociclib (Kisqali) for use in combination with an aromatase inhibitor for the frontline treatment of postmenopausal women with HR+, HER2-negative advanced breast cancer [109, 110]. Finally, treatment with the CDK4/6 inhibitor abemaciclib has been approved by FDA in 2017, for HR + and HER2 – advanced or metastatic breast carcinoma [111].

4.3. SCF complex – SKP2/p27

4.3.1. SKP2 inhibitors

It has been proved that Skp2 is frequently overexpressed in a variety of human cancers including breast carcinoma; actually both Skp2 mRNA and protein display elevated levels in breast cancer cell lines and primary breast tumors. Therefore, inhibition of Skp2 may be a novel strategy for the prevention and/or treatment of this malignancy. Specific drugs that inactivate Skp2 in breast cancer are unavailable so far, although there is renewed interest in developing Skp2 inhibitors for breast cancer treatment.

It is noteworthy that several natural compounds have been found to downregulate Skp2 expression in human cancers—including breast cancer—such as curcumin, lycopene and quercetin. All-trans retinoic acid (ATRA) promoted the ubiquitination of Skp2 in breast cancer cell lines, leading to cell cycle arrest. Gallic acid markedly reduces cell growth of human breast cancer cells and induces cell cycle arrest by inhibiting Skp2, attenuating Skp2–p27 association and reducing p27 ubiquitination. Moreover, Huang et al. (2008) reported that epigallocatechin-3-gallate (EGCG), the main constituent of green tea, inhibits human breast cancer cell growth via downregulation of Skp2 expression and accumulation of p27 among others. Both tamoxifen and paclitaxel significantly and synergistically enhanced cell growth inhibition by EGCG mediated through the downregulation of Skp2 expression in breast cancer cells [112].

In addition, siRNA gene silencing—an important gene function analysis method widely used in molecular studies—could be useful tool in inhibiting Skp2 activity. At the cell level, siRNA interference technology is characterized by a shorter action cycle and fewer side effects, and can silence multiple genes specifically with minimized side effects. In a recent study, two pairs of SKP2-specific siRNA, siRNA1 and siRNA2, were designed and synthesized and detected the endogenous SKP2 expression inhibitory effect in breast cancer cells MDA-MB-231. Western blotting showed that SKP2-specific siRNA1 can effectively inhibit endogenous SKP2 expression in these cells. Moreover, SKP2 silence significantly reduced breast cancer cell MDA-MB-231 proliferation. This study could be the experimental basis for further investigation and potential clinical application [113].

4.4. Histone ubiquitination

Monoubiquitination is one of the largest histone PTMs, alongside smaller and better studied modifications such as methylation, acetylation and phosphorylation. Monoubiquitination of histone H2B at lysine 120 (H2Bub1) has key roles in transcription, the DNA damage response and stem cell differentiation. Global levels of monoubiquinated histone H2B (H2Bub1) are low to absent in advanced cancers including breast, colorectal, lung and parathyroid, marking H2Bub1 and the enzymes that regulate it as key molecules in new therapeutic strategies for the treatment of cancer. More specifically, H2Bub1 levels were found to be significantly reduced in malignant and metastatic breast cancer cells, proving its tumor suppressor role [114].

4.4.1. USP44 inhibitors

USP44 is a subunit of the N-CoR complex and deubiquitinates H2B *in vitro* and *in vivo*. ChIP experiments confirmed that USP44 recruitment reduces H2Bub1 levels at N-CoR target loci; USP44 high expression correlates with reduced levels of H2Bub1 in the breast cancer cell line MDA-MB-231 and is required for efficient invasiveness of triple negative breast cancer cells—highly expressed in aggressive breast cancer MDA-MB-231 cells, leading to low global H2Bub1 levels and contributing to the invasiveness of these cells. On the contrary, depletion of USP44 impairs the invasiveness of MDA-MB-231 cells *in vitro* and causes an increase of global H2Bub1 levels [115]. In recent years, studies are looking for negative regulators of DUBs such as USP44 and USP22, to be used as therapeutic agents for malignancies, including mammary carcinoma, but there are still a lot to be discovered in this field for an anti-cancer regiment to be suggested [116].

4.5. Estrogen receptor

On a molecular basis, for example, gene expression profile, the breast carcinomas are classified into five major subtypes: luminal A and B, HER2-enriched, triple negative (basal-like and non-basal-like). Each of these tumors has different risk factors, response to treatment and disease progression/preferential metastasis sites. In addition, the etiology, pathogenesis and prognosis of breast cancer are significantly influenced by intrinsic molecular breast cancer subtypes across the different ethnicities globally [117].

More than two-thirds of the breast carcinomas (approximately 75%) are of luminal subtype and initially responsive to anti-estrogens such as tamoxifen. Anti-estrogen resistance is of two types, *de novo* and acquired, and is likely to develop over time. The absence of both ERα and PR expression represents the prevailing mechanism of *de novo* resistance. The acquired resistance is defined by loss of anti-estrogen responsiveness by initially responsive tumors [117]. About 50–60% of ER-positive tumors respond to the first-line endocrine therapy, while about 40% of ER-positive tumors show resistance; the latter correlated with loss of ER expression, changes in ERα post-translational modifications, loss of ER-dependent growth, and activation of cross-talking pathways. About one-third of patients treated with tamoxifen for 5 years will have recurrent disease within 15 years [118]. It has been proved that approximately 25% of ER+/PR+, 66% of ER+/PR− and 55% of ER−/PR+ breast carcinomas do not respond to anti-estrogens. Several studies suggest that loss of ERα can be due to long-term activation of growth factor signaling pathways; approximately 30% of the patients show loss of ERα where EGFR/Her-2 activity is elevated. Accumulating evidence suggests that several mechanisms act at cellular/molecular levels and are likely to be responsible for the endocrine resistance, comprising a major challenge today in treating significant percentage of breast cancers by hormone therapy [118].

One contributing factor for the phenomenon of endocrine resistance is the ERα-specific ubiquitin ligases. Re-expression of ERα in ER-negative breast cancer cells can restore sensitivity to tamoxifen; restoring the ERα expression by inhibiting ERα-specific ubiquitin ligases provides potential novel strategies for restoring tamoxifen sensitivity and thus potential therapeutic drugs. Small molecule inhibitors specific to these ubiquitin ligases may overcome tamoxifen resistance in breast cancers. In particular, whether ER negativity is a cause or a consequence of the disease progression is a million dollar question in this field, but the current understanding of this phenomenon is still at premature stage [118].

4.5.1. GSK3 inhibitors

Glycogen synthase kinase-3 (GSK-3) protects ERα from proteasomal degradation and plays a crucial role in ERα protein stabilization and turnover and GSK-3 may be involved in ERα-mediated transcriptional activation without ERα degradation. Silencing of GSK-3 results in a reduction of ERα levels in breast cancer cells due to increased proteasomal degradation, so its inhibitors could consist an emerging therapeutic target in the treatment of human breast cancer [119]. In a recent study, the pharmacological inhibition of GSK-3 by two novel small molecules, 9-ING-41 and 9-ING-87, reduced the viability of breast cancer cells; in addition, treatment with 9-ING-41 enhanced the anti-tumor effect of irinotecan (CPT-11) against breast cancer cells *in vitro*. The same study established two patient-derived xenograft tumor models—from metastatic pleural effusions obtained from patients with chemo-refractory breast cancer—and demonstrated that 9-ING-41 also potentiated the effect of the chemotherapeutic drug CPT-11 *in vivo*, leading to regression of established tumors in mice. These results make GSK-3 inhibitor 9-ING-41 a promising candidate targeted agent for metastatic breast cancer therapy [120].

4.5.2. RNF31 inhibitors

The atypical E3 ubiquitin ligase RNF31 has an oncogenic role in breast cancer growth through facilitating ERα signaling and suppressing P53 signaling, although its ultimate role is not thoroughly studied yet. Modulation of ERα levels is one feasible approach to inhibit estrogen signaling and subsequently cellular proliferation and possible therapeutic inhibitors targeting

RNF31 could constitute a valuable drug for breast cancers. The development of RNF31 inhibitors is ongoing, for example, targeting its function on RBR domain [108].

4.6. BRCA1

The breast and ovarian cancer susceptibility gene *BRCA1* is implicated not only in familial breast cancers but also in sporadic breast cancers. Approximately 15% of sporadic breast cancers belong to the basal-like subtype that expresses basal/myoepithelial cell markers, but do not express estrogen receptor, progesterone receptor or HER2 and is associated with poor prognosis. Furthermore, the majority of hereditary breast cancers with BRCA1 mutations display a basal-like phenotype, thus implying the importance of BRCA1 not only in familial breast cancers but also in sporadic cancers. BRCA1 is a protein that coordinates a diverse range of cellular pathways to maintain genomic stability and participates in multiple cellular supercomplexes, in most of which it exists as a RING heterodimer with BARD1 to provide ubiquitin E3 ligase activity that is required for a tumor suppressor function. Reduced expression of BRCA1, due to mutations or epigenetic inactivation, leads to increased risk of breast cancer development. Although BRCA1 is present in all cells, it is still not clear why mutations in the BRCA1 gene predispose to breast and ovarian, but not to other types of cancer [121].

4.6.1. PARP inhibitors

Poly-ADP-ribose polymerases (PARP) are enzymes that are involved in DNA damage repair and their inhibition is a promising strategy for targeting cancers with defective DNA damage repair, including BRCA1 and BRCA2 mutation-associated breast and ovarian cancers. Several PARP inhibitors are currently in clinical trials in the adjuvant, neoadjuvant and metastatic settings for the treatment of BRCA-mutated breast carcinoma (phase 2–3) such as Olaparib (AZD2281), Veliparib (ABT-888) and Talazoparib (BMN-673), as single agents or in combination schemes with chemotherapy and the results are very promising. Also, PARP inhibitor activity could be applied to breast cancers without *BRCA1/2* mutations and several preclinical experiments support this possibility [122]. FDA has approved Lynparza (olaparib) to treat ovarian cancers in women who carry mutations in BRCA1 or BRCA2, and who have received chemotherapy treatments, but PARP inhibitors are not currently FDA approved for treating breast cancer; results from phase 3 using Lynparza has been promising. Nevertheless, more investigation needs to be done in order to have more data regarding possible long-term side effects [122, 123].

4.7. ErbB2/HER2/neu

Her-2/neu overexpression (gene amplification) is observed in about 20–30% of breast cancer patients and is directly linked to deregulated activation of intracellular mitogenic signaling, and thus leads to aggressive tumor behavior and resistance to cancer chemotherapy. HER-2 gene amplification in breast cancer has been associated with tumor invasiveness, accelerated angiogenesis, reduced apoptosis, and more progressive regional and distant metastases. So, carcinomas that present with a higher-than-average/'*positive*'HER-2 status will almost certainly be more aggressive [124].

The first approved monoclonal antibody treatment (immune targeted therapy) for breast cancer was trastuzumab (Herceptin), which results in significant improvement in patient survival when used in combination with chemotherapy in patients with (metastatic) Her-2/*neu*-positive carcinoma, both before and after surgery. Specifically trastuzumab blocks HER2 receptors from receiving growth signals so it inhibits signaling of MAPK and PI3K pathways, promotes cell cycle arrest and induces apoptosis. Herceptin can also help fight breast cancer by alerting the immune system to destroy cancer cells onto which it is attached and by mediating the internalization and degradation of the Her-2/*neu* receptor and consequently diminishing its intracellular signaling [125].

Treatments that specifically target HER2 are very effective and the prognosis for HER2-positive breast cancer is actually quite good. Other treatments that target HER2/neu receptor include Lapatinib (Tykerb), Pertuzumab (Perjeta) and/or Ado-trastuzumab emtansine (Kadcyla). Nevertheless, the primary or acquired resistance as well as the side effect toxicity of trastuzumab, for example, cardiac dysfunction have led to the investigation of other molecules that could downregulate HER2/neu. Several new medications are being developed are under clinical trials [126].

4.7.1. Quercetin

The natural product quercetin (3,5,7,3′,4′-pentahydroxyflavone) is orally bioavailable and is a flavonoid found in many fruits and vegetables. Previous research has shown that quercetin has anti-tumor, anti-inflammatory, anti-allergic and anti-viral activities and has been shown to be protective against breast cancer in animal model. A recent study showed that quercetin decreased the level of Her-2/neu protein in time- and dose-dependent manner and also inhibited the downstream survival PI3K-Akt signaling pathway in Her-2/neu-overexpressing breast cancer SK-Br3 cells. In addition, this product-induced polyubiquitination of Her-2/neu. The carboxyl terminus of Hsc70-interacting protein (CHIP), a chaperone-dependent E3 ubiquitin ligase, was found to play a crucial role in the quercetin-induced ubiquitination of Her-2/neu. Inhibition of tyrosine kinase activity of Her-2/neu by quercetin could indicate a lateration in the Her-2/neu structure which promotes CHIP recruitments and downregulation of Her-2/neu. So this agent could be one more promising drug to treat Her-2/neu-overexpressing cancers [127].

4.7.2. Afatinib

The novel next-generation tyrosine kinase inhibitors (TKIs), afatinib and neratinib, were designed to overcome the resistance by targeting multiple HER family members and irreversibly binding the targets. Despite the encouraging results of the afatinib monotherapies, multicenter international trials are still ongoing for their evaluation. Neratinib has been shown to be an effective treatment in the metastatic, neoadjuvant and adjuvant setting, both as a single agent or in combination with cytotoxic agents [128]. Recently, in July 2017, the US FDA approved neratinib for the extended adjuvant treatment of early stage HER2- overexpressed/amplified breast cancer, to follow adjuvant trastuzumab-based therapy [111].

4.8. TRAIL receptors

4.8.1. rhTRAIL

Drugs based on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is anticipated to be one of the most effective cancer treatments, however, resistance to TRAIL therapy remains a challenge and TRAIL combinations have been experimented with for over 10 years to induce synergism or sensitize resistant cancer cells. TRAIL is characterized by its ability to induce apoptosis in tumor cells but not in normal cells, thus qualifying as a potential drug specific for different types of cancer, including breast, bladder, lung and liver; the development of recombinant human TRAIL (rhTRAIL) as a promising therapy for different types of human cancer. However, a majority of breast cancer cell lines exhibit resistance to TRAIL treatment due to upregulation of pro-apoptotic proteins, downregulation of anti-apoptotic proteins and/or upregulation of death receptors 4 and 5. To overcome TRAIL resistance, a co-treatment option has been studied using the natural compound Quercetin [129]. One recent studies findings suggest that the co-treatment of Quercetin and rhTRAIL has the potential to be an anti-breast cancer therapeutic strategy, by enhancing pro-apoptotic and anti-proliferative effects in hormone dependent and triple negative breast cancer cells [130].

4.9. NEDD4

NEDD4 (neural precursor cell expressed, developmentally downregulated 4) is an E3 ubiquitin protein ligase that has been found to target numerous substrates for its biological functions and plays a pivotal role in the development and progression of human cancers; it is observed that NEDD4 expression was increased in breast cancer [131]. Additionally, it acts as an E3 ligase of PTEN—a tumor suppressor gene—catalyzing poly-ubiquitination of PTEN protein in cells and leading to its proteolysis. It has been reported to negatively regulate PTEN protein levels in carcinomas of the prostate, lung and bladder, but recent studies showed either no association or a positive correlation at the protein and transcript levels, regarding NEDD4's effect on PTEN in the breast cancer; this suggests that NEDD4 is not responsible for the frequent downregulation of PTEN protein in human breast carcinoma [132]. In general, NEDD4 could be a legitimate target for designing new drugs against human malignancies, although surprisingly, NEDD4 inhibitors have not been discovered so far. More investigation on this pathway could lead to the development of this E3 ubiquitin ligase inhibitors such as beta-TRCP that could contribute to the decrease of abundant NEDD4 oncoprotein levels by its destruction [133].

4.10. DUBs

Ubiquitination is reversible, like most regulatory cellular processes, and the enzymes that reverse protein ubiquitination are collectively known as deubiquitinases (DUBs). The mammalian genome encodes around 100 DUBs that are categorized into five classes, four of which are thiol proteases including ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs), ovarian tumor domain DUBs and machado Joseph domain DUBs. The fifth class comprises JAB1/MPN metalloenzyme, which functions as a zinc finger metalloprotease [134].

4.10.1. USP inhibitors

Generally, a number of regulate processes associated with cell proliferation and apoptosis, and as such represent candidate targets for cancer therapeutics. The majority of DUBs is cysteine proteases and is likely to be more "druggable" than E3 ligases. Ubiquitin-specific proteases (USPs) are one class of DUBs implicated in breast cancer by transforming growth factor beta (TGF-β) signaling; the latter has a well-documented role in mediating epithelial-to-mesenchymal transition (EMT), tumor progression and metastasis in breast cancer. Specifically, the deubiquitinases USP4, USP9X, USP9Y, USP10, USP11, USP15, USP25 and USP32, but also DUB3 are upregulated/overexpressed in human breast cancer [134].

Several inhibitors have been developed against USPs such as HBX 41,108, b-AP15 and pimozide. WP1130 is a partially selective DUB inhibitor that induces apoptosis in cells through rapid accumulation of poly-ubiquitinated proteins that targets USP5, USP9X and USP14, among others. USP9X inhibition by WP1130 in tumorigenic human breast cancer cell lines inhibits their growth and USP9X knockdown in all triple negative breast cancer cell lines caused apoptosis induction, so WP1130 and its derivatives could be a significant therapeutic agents in the treatment of breast cancer [134]. Moreover, the specific inhibitor WP1130 binds to DUB3 and inhibits the DUB3-mediating Snail1 stabilization *in vitro* and *in vivo*, as it blocked tumor cell migration, invasion and suppressed CSC-like properties, providing a proof for therapeutic development of small molecules to inhibit the activity of DUB3 in metastatic breast cancer. These data strengthen the view that DUB3 is an ideal candidate for the development of inhibitors for cancer treatment based on its dual role in regulating cell growth and metastasis [135].

b-AP15, an inhibitor for both UCH37 and USP14, was able to accumulate ubiquitinated substrates and had excellent efficacy in different *in vivo* solid tumor models, as well as an acute myeloid leukemia model. On the contrary, a more selective USP14 inhibitor, IU1, had an opposite effect by enhancing degradation of target proteins, leading to a dose-dependent reduction in overexpressed proteins including Tau, showing that selective inhibition of different proteasomal DUBs may have different outcomes; nevertheless inhibition of proteasomal DUBs is worthy of investigation as a potential anti-cancer therapy [136].

4.11. Antiestrogens (Tamoxifen, Raloxifene, Fulvestrant, Letrozole)

Antiestrogens are classified as selective estrogen receptor modulators (SERMs), for example, tamoxifen and raloxifene—that have antagonist activity in breast and partial agonist activity in uterus and bones—and as pure antiestrogens, for example, fulvestrant; the latter accelerate ERa proteasomal degradation so they represent selective estrogen receptor downregulators (SERDs). Tamoxifen is the first clinically approved antiestrogen and remains the standard adjuvant treatment for all stages of primary breast carcinoma [137]. It has been shown that ubiquitin ligase c-Cbl is involved (enhances) in tamoxifen-induced apoptosis of MCF-7 cells [138]. Nevertheless, significant number of these patients develop resistance to tamoxifen, which in addition shows negative side effects, such as thromboembolic events and endometrial cancer. Raloxifene has 76% of effectiveness of tamoxifen at reducing incidence of invasive breast cancer with less incidence of endometrial cancer. Fulvestrant acts via different molecular mechanism consisting in increased ERa turnover through the ubiquitin—proteasome pathway in ERa+ breast cancer cells [137]. Finally, it has been shown that inhibition of ubiquitin conjugating enzyme UBE2C reduces proliferation and sensitizes breast cancer cells to letrozole—an aromatase inhibitor—tamoxifen etc. [139]. All the above-mentioned antiestrogen drugs are FDA-approved and have been used in HR+ (advanced/metastatic) breast carcinomas, including for reducing cancer risk (tamoxifen, raloxifene) [111].

5. Conclusion

Ubiquitination involves the attachment of ubiquitin to numerous target proteins leading to regulation of their half-life, localization, activity and conformation. Recent years' analysis suggests that ubiquitin plays a very important role in several signaling and cell regulatory events in malignancies, including breast cancer, which remain the commonest carcinoma and second cause of death among women worldwide. Targeting molecules of the ubiquitination system is very promising for the treatment of breast cancer, as well as for other neoplasms. Although many potential targets that belong to this category are under trials, only few have already taken FDA approval and are used as therapeutic drugs so far such as ribociclib (Kisqali) and palbociclib (PD-0332991). Suberoylanilide hydroxamic acid (SAHA) also known as vorinostat, romidepsin (Istodax) and depsipeptide are HDAC inhibitors that are being used among others in clinical trials for treating breast malignancies, but also other molecules such as ganetespib and olaparib; yet, more targets are studied as possible treatment candidates, for example, nutlin-3 and quercetin (**Table 1**). In spite of the fact that drug targets belonging to the spectrum of ubiquitin system are very promising, a lot need to be done by future studies on their development and clinical application, either as monotherapy or as combination therapy for breast cancer.

Drug-related molecule	**Current phase**			
	Molecule selection/design production	**Preclinical modeling—in vitro/in vivo (cell lines, xenografts)**	**Clinical trials**	**FDA approved**
P53/MDM2	Beclin 1 inducer or inhibitor	Nutlin-3	Hsp90 Inhibitors (*Geldanamycin, 17-AAG, Ganetespib*)	
	RING finger protein 31 inhibitors (RNF31i)		Histone Deacetylase Inhibitors (*Vorinostat/SAHA, Romidepsin/Depsipeptide, Belinostat*)	
CDKs–CDIs				CDK4/6 inhibitors (*Palbociclib, Ribociclib, Abemaciclib*)
SCF complex –SKP2/p27		Curcumin, lycopene, pentagalloylglucose, quercetin, ATRA, garlic acid, EGCG, SKP2 siRNA		
Histone H2B	USP44 inhibitors	USP44 inhibitors		

Drug-related molecule	**Current phase**			
	Molecule selection/design production	**Preclinical modeling—in vitro/in vivo (cell lines, xenografts)**	**Clinical trials**	**FDA approved**
ER	RNF31 inhibitors	GSK-3 inhibitors (*9-ING-41, 9-ING-87*)		
BRCA1			PARP inhibitors (*Olaparib, Veliparib, Talazoparib*)	
ErbB2/HER2/neu		Quercetin	Tyrosine kinase inhibitors (*Afatinib*)	Tyrosine kinase inhibitors (Lapatinib, *Neratinib*) Monoclonal antibodies (*Trastuzumab, Ado-trastuzumabemtansine, Pertuzumab*)
TRAIL receptors		rhTRAIL (co-treatment of Quercetin)		
NEDD4	NEDD4 inhibitors (*beta-TRCP*)			
DUBs	DUB3 inhibitors	USP inhibitors (*WP1130, b-AP15*)		
Antiestrogens				*Tamoxifen, Raloxifene, Fulvestrant, Letrozole*

Table 1. Drugs/therapeutic agents for breast cancer related to ubiquitin system.

Abbreviations

CKIs	Cdk kinase inhibitors
DNA	Deoxyribonucleic acid
DUBs	Deubiquitinating enzymes
FDA	Food and drug administration
HR	Homologous recombination
Hsp90	Heat shock protein 90
MDM2	Murine double minute 2 homolog/oncoprotein
NEDD4	Neuronally expressed developmentally downregulated 4
NHEJ	Non-homologous recombination

PCNA	Polymerase DNA
PTM	Post-translational modifications
RING	Really interesting new gene
RNF31	RING finger protein 31
RTK	Tyrosine kinase receptor
SCF	SKP1-Cullin 1-F-box
SKP1	S-phase kinase-associated protein 1
Ub	Ubiquitin
UPP	Ubiquitin proteasome pathway
UPS	Ubiquitin/proteasome system
HR+	Hormone receptor-positive
H2Bub1	Monoubiquinated histone H2B
USP44	Ubiquitin-specific protease 44
TNBC	Triple negative breast cancer
CSC	Cancer stem cells
ATP	Adenosine triphosphate
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor2

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References

[1] Breast Cancer: Prevention and Control, Breast Cancer Burden. World Health Organiation (WHO)

[2] Alteri R, Bertaut T, Brinton LA, Fedewa S, Freedman RA, Gansler T, Gaudet MM, Kramer J, Lin CC, McCullough M, Miller K, Newman LA, Niemeyer D, Piercy A, Richards C, Suer AG, Simpson S, Smith R, Wagner D , Xu J. Breast Cancer Facts & Figures 2015-2016 is a publication of the American Cancer Society, Atlanta, Georgia

[3] U.S. Breast Cancer Statistics, breastcancer.org, March 2017

[4] Pathologyoutlines.com, October 2016

[5] Gallo LH, Ko J, Donoghue DJ. The importance of regulatory ubiquitination in cancer and metastasis. Cell Cycle. 2017 Apr 3;**16**(7):634-648. DOI: 10.1080/15384101.2017.1288326. Epub 2017 Feb 6. PubMed PMID: 28166483; PubMed Central PMCID: PMC5397262

[6] Rasool M, Malik A, Naseer MI, Manan A, Ansari S, Begum I, Qazi MH, Pushparaj P, Abuzenadah AM, Al-Qahtani MH, Kamal MA, Gan S. The role of epigenetics in personalized medicine: Challenges and opportunities. BMC Medical Genomics. 2015;**8**(Suppl 1): S5. DOI: 10.1186/1755-8794-8-S1-S5. Epub 2015 Jan 15. Review. PubMed PMID: 25951941; PubMed Central PMCID: PMC4315318

[7] Herhaus L, Dikic I. Expanding the ubiquitin code through post-translational modification. EMBO Reports. 2015 Sep;**16**(9):1071-1083. DOI: 10.15252/embr.201540891. Epub 2015 Aug 12. Review. PubMed PMID: 26268526; PubMed Central PMCID: PMC4576978

[8] Tu Y, Chen C, Pan J, Xu J, Zhou ZG, Wang CY. The ubiquitin proteasome pathway (UPP) in the regulation of cell cycle control and DNA damage repair and its implication in tumorigenesis. International Journal of Clinical and Experimental Pathology. 2012;**5**(8):726-738. Epub 2012 Oct 1 PubMed PMID: 23071855; PubMed Central PMCID: PMC3466981Review

[9] Akutsu M, Dikic I, Bremm A. Ubiquitin chain diversity at a glance. Journal of Cell Science. 2016 Mar 1;**129**(5):875-880. DOI: 10.1242/jcs.183954. Epub 2016 Feb 15. Review. PubMed PMID: 26906419

[10] Johnson DE. The ubiquitin-proteasome system: Opportunities for therapeutic intervention in solid tumors. Endocrine-Related Cancer. 2015 Feb;**22**(1):T1-17. DOI: 10.1530/ERC-14-0005. Epub 2014 Mar 21. Review. PubMed PMID: 24659480; PubMed Central PMCID: PMC4170053

[11] Lim KH, Song MH, Baek KH. Decision for cell fate: Deubiquitinating enzymes in cell cycle checkpoint. Cellular and Molecular Life Sciences. 2016 Apr;**73**(7):1439-1455. DOI: 10.1007/s00018-015-2129-2. Epub 2016 Jan 13. Review. PubMed PMID: 26762302

[12] Pinto-Fernandez A, Kessler BM. DUBbing cancer: Deubiquitylating enzymes involved in epigenetics, DNA damage and the cell cycle as therapeutic targets. Frontiers in Genetics. 2016 Jul 28;**7**:133. DOI: 10.3389/fgene.2016.00133. eCollection 2016. Review. PubMed PMID: 27516771; PubMed Central PMCID: PMC4963401

[13] Klein T, Viner RI, Overall CM. Quantitative proteomics and terminomics to elucidate the role of ubiquitination and proteolysis in adaptive immunity. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences. 2016 Oct 28;**374**(2079). Pii:20150372. DOI: 10.1098/rsta.2015.0372

[14] Dantuma NP, Bott LC. The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Frontiers in Molecular Neuroscience. 2014 Jul 31;**7**:70. DOI: 10.3389/fnmol.2014.00070.eCollection 2014

[15] Suryadinata R, Roesley SN, Yang G, Sarčević B. Mechanisms of generating polyubiquitin chains of different topology. Cells. 2014 Jul 1;**3**(3):674-89. DOI: 10.3390/cells3030674. PubMed PMID: 24987835; PubMed Central PMCID: PMC4197637

[16] Schlesinger DH, Goldstein G, Niall HD. The complete amino acid sequence of ubiquitin, an adenylate cyclase stimulating polypeptide probably universal in living cells. Biochemistry. 1975;**14**:2214-2218

[17] Hershko A, Ciechanover A. The ubiquitin system. Annual Review of Biochemistry. 1998; **67**:425-479

[18] Ciechanover A, Finley D, Varshavsky A. Mammalian cell cycle mutant defective in intracellular protein degradation and ubiquitin-protein conjugation. Progress in Clinical and Biological Research. 1985;**180**:17-31

[19] Ciechanover A, Finley D, Varshavsky A. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutants. Cell. 1984;**37**:57-66

[20] Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature. 1995;**373**:81-83

[21] Haglund K, Dikic I. Ubiquitylation and cell signaling. The EMBO Journal. 2005;**24**: 3353-3359

[22] Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, et al. A proteomics approach tounder standing protein ubiquitination. Nature Biotechnology. 2003;**21**:921-926

[23] Komander D, Rape M. The ubiquitin code. Annual Review of Biochemistry. 2012;**81**:203-229

[24] Chen S, Davies AA, Sagan D, Ulrich HD. The RING fin ger ATPase Rad5 pof *Saccharomyces cerevisiae* contributes to DNA double- strand break repair in a ubiquitin-independent manner. Nucleic Acids Research. 2005;**33**:5878-5886

[25] Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. Recognition of the polyubiquitin proteolytic signal. The EMBO Journal. 2000;**19**:94-102

[26] Voges D, Zwickl P, Baumeister W. The26Sproteasome: A molecular machine designed for controlled proteolysis. Annual Review of Biochemistry. 1999;**68**:1015-1068

[27] Orlowski RZ, Kuhn DJ. Proteasome inhibitors in cancer therapy: Lessons from the first decade. Clinical Cancer Research. 2008;**14**:1649-1657

[28] Feng L, Chen J. The E3 ligase RNF8 regulates KU80 removal and NHEJ repair. Nature Structural & Molecular Biology. 2012;**19**:201-206

[29] Li L, Tao Q, Jin H, van Hasselt A, Poon FF, Wang X, et al. The tumor suppressor UCHL1 forms a complex with p53/MDM2/ARF to promote p53 signaling and is frequently silenced in nasopharyngeal carcinoma. Clinical Cancer Research. 2010;**16**:2949-2958

[30] Biswas M, Voltz K, Smith JC, Langowski J. Role of histone tails instructural stability of the nucleosome. PLoS Computational Biology. 2011;**7**:e1002279

[31] Zhou W, Zhu P, Wang J, Pascual G, Ohgi KA, Lozach J, et al. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. Molecular Cell. 2008;**29**:69-80

[32] Pan MR, Peng G, Hung WC, Lin SY. Monoubiquitination of H2AX protein regulates DNA damage response signaling. The Journal of Biological Chemistry. 2011;**286**:28599-28607

[33] Wu H, Leng RP. UBE4B, aubiquitin chain assembly factor is required for MDM2-mediated p53 polyubiquitination and degradation. Cell Cycle. 2011;**10**:1912-1915

[34] Kim ST, Lim DS, Canman CE, Kastan MB. Substrate specificities and identification of putative substrates of ATM kinase family members. The Journal of Biological Chemistry. 1999;**274**:37538-37543

[35] Acs K, Luijsterburg MS, Ackermann L, Salomons FA, Hoppe T, Dan-tuma NP. The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strandbreaks. Nature Structural & Molecular Biology. 2011;**18**:1345-1350

[36] Dantuma NP, Hoppe T. Growing sphere of influence: Cdc48/p97 orchestrates ubiquitin-dependent extraction from chromatin. Trends in Cell Biology. 2012;**22**:483-491

[37] Jiang N, Shen Y, Fei X, Sheng K, Sun P, et al. Valos in containing protein regulates the proteasome – Mediated degradation of DNA-PKcs in glioma cells. Cell Death & Disease. 2013;**4**:e647

[38] Chapman JR, Taylor MR, Boulton SJ. Playing the endgame: DNA double-strand break repair pathway choice. Molecular Cell. 2012;**47**:497-510

[39] Lemmens BB, Tijsterman M. DNA double – Strand break repair in *Caenorhabditis elegans*. Chromosoma. 2010;**120**:1-21

[40] Bunting SF, Nussenzweig A. End-joining, translocations and cancer. Nature Reviews. Cancer. 2013;**13**:443-454

[41] Dahm-Daphi J, Hubbe P, Horvath F, El-Awady RA, Bouffard KE, Pow-ell SN, et al. Nonhomologous end-joining of site-specific but not of radiation-induced DNA double-strand breaks is reduced in the presence of wild-type p53. Oncogene. 2005;**24**:1663-1672

[42] Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. Nature Reviews. Molecular Cell Biology. 2010;**11**:196-207

[43] Clejan I, Boerckel J, Ahmed S. Developmental modulation of non- homologous end joining in Caenorhabditis Elegans. Genetics. 2006;**173**:1301-1317

[44] Yun MH, Hiom K. Understanding the functions of BRCA1 in the DNA-damage response. Biochemical Society Transactions. 2009 Jun;**37**(Pt 3):597-604. DOI: 10.1042/BST0370597. PubMed PMID: 19442256

[45] Lok GT, Sy SM, Dong SS, Ching YP, Tsao SW, Thomson TM, et al. Differential regulation of RNF8 mediated Lys48 and Lys63-based poly-ubiquitylation. Nucleic Acids Research. 2012;**40**:196-205

[46] Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**:887-900

[47] Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446

[48] Ramadan K, Meerang M. Degradation – Linked ubiquitin signal and proteasome are integral component so fDNA double strand break repair: New perspectives for anticancer therapy. FEBS Letters. 2011;**585**:2868-2875

[49] Al-Hakim A, Escribano-Diaz C, Landry MC, O'Donnell L, Panier S, Szilard RK, et al. The ubiquitous role of ubiquitin in the DNA damage response. DNA Repair (Amst). 2010;**9**:1229-1240

[50] Mallette FA, Mattiroli F, Cui G, Young LC, Hendzel MJ, Mer G, et al. RNF8 and RNF168 dependent degradation of KDM4A/JMJD2. A trig gers 53BP1 recruitment to DNA damage sites. The EMBO Journal. 2012;**31**:1865-1878

[51] Zhang S, Chea J, Meng X, Zhou Y, Lee EY, Lee MY. PCNA is ubiquitinated by RNF8. Cell Cycle. 2008;**7**:3399-3404

[52] Hoppe T. Multiubiquitylation by E4 enzymes: 'one size' doesn't fit all. Trends in Biochemical Sciences. April 2005;**30**(4)

[53] Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6 – Dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature. 2002; **419**:135-141

[54] Daigaku Y, Davies AA, Ulrich HD. Ubiquitin – Dependent DNA damage by pass is separable from genome replication. Nature. 2010;**465**:951-955

[55] Drikos I, Nounesis G, Vorgias C. Characterization of cancer linked BRCA1-BRCT missense variants and interaction with phosphoptotein targets. Proteins Journal. (Proteins Structure-Function and Bioinformatics Journal). 2009;**77**(2):464-476

[56] Wu-Baer F, Lagrazon K, Yuan W, Baer R. The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. The Journal of Biological Chemistry. 2003;**278**:34743-34746

[57] Irminger Finger I, Jefford CE. Is there more to BARD1 than BRCA1? Nature Reviews. Cancer. 2006;**6**:382-391

[58] Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, et al. Small molecule RITA binds to p53, blocks p53 – HDM - 2inter – Action and activates p53 function in tumors. Nature Medicine. 2004;**10**:1321-1328

[59] Surget S, Descamps G, Brosseau C, Normant V, Maiga S, Gomez-Bougie P, et al. RITA (reactivating p53 and inducing tumor apoptosis) is efficient against TP53 abnormal myeloma cells independently of the p53 pathway. BMC Cancer. 2014;**14**:437

[60] Krajewski M, Ozdowy P, D'Silva L, Rothweiler U, Holak TA. NMR indicates that the small molecule RITA does not block p53 – MDM2 binding in vitro. Nature Medicine. 2005;**11**:1135-1136

[61] Shangary S, Qin D, McEachern D, Liu M, Miller RS, Qiu S, et al. Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**:3933-3938

[62] Levine JS, Koh JS, Triaca V, Lieberthal W. Lysophosphatidic acid: A novel growth and survival factor for renal proximal tubular cells. The American Journal of Physiology. 1997; **273**:F575-F585

[63] Brinkmann K, Kashkar H. Targeting the mitochondrial apoptotic pathway: A preferred approach in hematology malignancies? Cell Death & Disease. 2014;**5**:e1098

[64] Fujita T, Ikeda H, Kawasaki K, Taira N, Ogasawara Y, Nakagawara A, Doihara H.Clinicopathological relevance of UbcH10 in breast cancer. Cancer Science. 2009;**100**:238-248

[65] van Ree JH, Jeganathan KB, Malureanu L, van Deursen JM. Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. The Journal of Cell Biology. 2010;**188**:83-100

[66] Narayan G, Bourdon V, Chaganti S, Arias-Pulido H, Nandula SV, Rao PH, Gissmann L, Durst M, Schneider A, Pothuri B, et al. Gene dosage alterations revealed by cDNA microarray analysis in cervical cancer: Identification of candidate amplified and overexpressed genes. Genes, Chromosomes & Cancer. 2007;**46**:373-384

[67] Tzelepi V, Zhang J, JF L, Kleb B, Wu G, Wan X, Hoang A, Efstathiou E, Sircar K, Navone NM, et al. Modeling a lethal prostate cancer variant with small-cell carcinoma features. Clinical Cancer Research. 2012;**18**:666-677

[68] Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. Annual Review of Biochemistry. 2009;**78**:399-434

[69] Berndsen CE, Wolberger C. New insights into ubiquitin E3 ligase mechanism. Nature Structural & Molecular Biology. 2014;**21**:301-307

[70] Kajiro M, Hirota R, Nakajima Y, Kawanowa K, So-ma K, Ito I, Yamaguchi Y, Ohie SH, Kobayashi Y, Seino Y, et al. The ubiquitin ligase CHIP acts as an upstream regulator of oncogenic pathways. Nature Cell Biology. 2009;**11**:312-319

[71] Jang KW, Lee KH, Kim SH, Jin T, Choi EY, Jeon HJ, Kim E, Han YS, Chung JH. Ubiquitin ligase CHIP induces TRAF2 proteasomal degradation and NF-kappaB inactivation to regulate breast cancer cell invasion. Journal of Cellular Biochemistry. 2011;**112**:3612-3620; PMID:21793045

[72] Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, Gelmon K, Chia S, Mar C, Wan A, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;**518**:422-426

[73] Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nature Communications. 2015;**6**:674

[74] Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, Marotz C, Giannopoulou E, Chakravarthi BV, Varambally S, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nature Medicine. 2016;**22**:298-305

[75] ZK Y, Gervais JL, Zhang H. Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21CIP1/WAF1 and cyclin D proteins. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:11324-11329

[76] Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nature Cell Biology. 1999;**1**:193-199

[77] Burger AM, Gao Y, Amemiya Y, Kahn HJ, Kitching R, Yang Y, Sun P, Narod SA, Hanna WM, Seth AK. A novel RING-type ubiquitin ligase breast cancer-associated gene 2 correlates with outcome in invasive breast cancer. Cancer Research. 2005;**65**:10401-10412

[78] Kona FR, Stark K, Bisoski L, Buac D, Cui Q, Dou QP. Transcriptional activation of breast cancer-associated gene 2 by estrogen receptor. Breast Cancer Research and Treatment. 2012;**135**:495-503

[79] Miyakawa K, Ryo A, Murakami T, Ohba K, Yamaoka S, Fukuda M, Guatelli J, Yamamoto N. BCA2/Rabring7 promotes tetherin-dependent HIV-1 restriction. PLoS Pathogens. 2009;**5**:e1000700

[80] Bacopulos S, Amemiya Y, Yang W, Zubovits J, Burger A, Yaffe M, Seth AK. Effects of partner proteins on BCA2 RING ligase activity. BMC Cancer. 2012;**12**:63

[81] Abbas T, Sivaprasad U, Terai K, Amador V, Pagano M, Dutta A. PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. Genes & Development. 2008;**22**:2496-2506

[82] Burger A, Amemiya Y, Kitching R, Seth AK. Novel RING E3 ubiquitin ligases in breast cancer. Neoplasia. 2006;**8**:689-695

[83] Zhi X, Zhao D, Wang Z, Zhou Z, Wang C, Chen W, Liu R, Chen C. E3 ubiquitin ligase RNF126 promotes cancer cell proliferation by targeting the tumor suppressor p21 for ubiquitin-mediated degradation. Cancer Research. 2013;**73**:385-394

[84] Nakayama KI, Nakayama K. Ubiquitin ligases: Cell-cycle control and cancer. Nature Reviews. Cancer. 2006;**6**:369-381

[85] Wang Y, Ren F, Wang Y, Feng Y, Wang D, Jia B, Qiu Y, Wang S, Yu J, Sung JJ, et al. CHIP/Stub1 functions as a tumor suppressor and represses NF-kappaB-mediated signaling in colorectal cancer. Carcinogenesis. 2014;**35**:983-991

[86] Wang T, Yang J, Xu J, Li J, Cao Z, Zhou L, You L, Shu H, Lu Z, Li H, et al. CHIP is a novel tumor suppressor in pancreatic cancer through targeting EGFR. Oncotarget. 2014;**5**:1969-1986

[87] Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Reviews. 2006;**25**:9-34

[88] Wu Z, Shen S, Zhang Z, Zhang W, Xiao W. Ubiquitin-conjugating enzyme complex Uev1A-Ubc13 promotes breast cancer metastasis through nuclear factor-small ka, CyrillicB mediated matrix metalloproteinase- 1 gene regulation. Breast Cancer Research. 2014;**16**:R75

[89] Morrison BJ, Morris JC, Steel JC. Lung cancer-initiating cells: A novel target for cancer therapy. Targeted Oncology. 2013;**8**:159-172

[90] Brooks CL, Gu W. p53 ubiquitination: Mdm2 and beyond. Molecular Cell. 2006 Feb 3;**21**(3):307-315 Review. PubMed PMID: 16455486; PubMed Central PMCID: PMC3737769

[91] Duffy MJ, Synnott NC, McGowan PM, Crown J, O'Connor D, Gallagher WM. p53 as a target for the treatment of cancer. Cancer Treatment Reviews. 2014 Dec;**40**(10):1153-1160. DOI: 10.1016/j.ctrv.2014.10.004 Review. PubMed PMID: 25455730

[92] Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. Breast Cancer Research. 2002;**4**:70. DOI: 10.1186/bcr426, BioMed Central Ltd 2002

[93] Bertheau P, Lehmann-Che J, Varna M, Dumay A, Poirot B, Porcher R, Turpin E, Plassa LF, de Roquancourt A, Bourstyn E, de Cremoux P, Janin A, Giacchetti S, Espié M, de Thé H, et al. Breast. 2013 Aug;**22**(Suppl 2):S27-S29. DOI: 10.1016/j.breast.2013.07.005. Review. PubMed PMID: 24074787

[94] Muller PA, Vousden KH. Mutant p53 in cancer: New functions and therapeutic opportunities. Cancer Cell, Volucella. 2014 Mar;**25**(3):304-317

[95] Parrales A, Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. Frontiers in Oncology. 2015;**5**:288. Published online 2015 Dec 21. doi: 10.3389/fonc.2015.00288 PMCID: PMC4685147

[96] Wang Z, Sun Y. Targeting p53 for novel anticancer therapy. Translational Oncology. 2010 Feb;**3**(1):1-12. PubMed PMID: 20165689; PubMed Central PMCID: PMC2822448

[97] Mahanta S, Pilla S, Paul S. Design of novel Geldanamycin analogue hsp90 alpha-inhibitor in silico for breast cancer therapy. Medical Hypotheses. 2013 Sep;**81**(3):463-469. DOI: 10.1016/j.mehy.2013.06.012. Epub 2013 Jul 13. PubMed PMID: 23860250

[98] Prabhu S, Ananthanarayanan P, Aziz SK, Rai S, Mutalik S, Sadashiva SR. Enhanced effect of geldanamycin nanocomposite against breast cancer cells growing in vitro and as xenograft with vanquished normal cell toxicity. Toxicology and Applied Pharmacology. 2017 Apr 1;**320**:60-72. DOI: 10.1016/j.taap.2017.02.012. Epub 2017 Feb 16. PubMed PMID: 28213093

[99] Jhaveri K, Modi S. Ganetespib: Research and clinical development. OncoTargets and Therapy. 2015 Jul 24;**8**:1849-1858. DOI: 10.2147/OTT.S65804. eCollection 2015. Review. PubMed PMID: 26244021; PubMed Central PMCID: PMC4521669

[100] Truong T-G, Munster P. Histone deacetylase inhibitors for the treatment of breast cancer: Recent trial data. The Clinical Investigator. 2013;**3**(6):557-569

[101] Mottamal M, Zheng S, Huang TL, Wang G. Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. Molecules. 2015 Mar 2;**20**(3):3898-3941. DOI: 10.3390/molecules20033898

[102] Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, Cai Y, Norberg HV, Zhang T, Furuya T, Jin M, Zhu Z, Wang H, Yu J, Li Y, Hao Y, Choi A, Ke H, Ma D, Yuan J. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011 Sep 30;**147**(1):223-234. DOI: 10.1016/j.cell.2011.08.037. PubMed PMID: 21962518; PubMed Central PMCID: PMC3441147

[103] Jung YY, Lee YK, Koo JS. The potential of Beclin 1 as a therapeutic target for the treatment of breast cancer. Expert Opinion on Therapeutic Targets. 2016;**20**(2):167-178. DOI: 10.1517/14728222.2016.1085971. Epub 2015 Sep 11. Review. PubMed PMID: 26357854

[104] Sriraman A, Li Y, Dobbelstein M. Fortifying p53 - beyond Mdm2 inhibitors. Aging (Albany NY). 2016 Sep 29;**8**(9):1836-1837. DOI: 10.18632/aging.101073. PubMed PMID: 27688014; PubMed Central PMCID: PMC5076439

[105] Burgess A, Chia KM, Haupt S, Thomas D, Haupt Y, Lim E. Clinical overview of MDM2/X-targeted therapies. Frontiers in Oncology. 2016 Jan 27;**6**:7. DOI: 10.3389/fonc.2016.00007. eCollection 2016. Review. PubMed PMID: 26858935; PubMed Central PMCID: PMC4728205

[106] Tosoni D, Pambianco S, Ekalle Soppo B, Zecchini S, Bertalot G, Pruneri G, Viale G, Di Fiore PP, Pece S. Pre-clinical validation of a selective anti-cancer stem cell therapy for Numb-deficient human breast cancers. EMBO Molecular Medicine. 2017 Mar 15. pii: e201606940. DOI: 10.15252/emmm.201606940. [Epub ahead of print] PubMed PMID: 28298340

[107] Zhu J, Zhao C, Zhuang T, Jonsson P, Sinha I, Williams C, Strömblad S, Dahlman-Wright K. RING finger protein 31 promotes p53 degradation in breast cancer cells. Oncogene. 2016 Apr 14;**35**(15):1955-1964. DOI: 10.1038/onc.2015.260 Epub 2015 Jul 6. PubMed PMID: 26148235; PubMed Central PMCID: PMC4833873

[108] Zhu J, Zhuang T, Yang H, Li X, Liu H, Wang H. Atypical ubiquitin ligase RNF31: The nuclear factor modulator in breast cancer progression. BMC Cancer. 2016 Jul 26;**16**:538. DOI: 10.1186/s12885-016-2575-8. PubMed PMID: 27460922; PubMed Central PMCID: PMC4962416

[109] Finn RS, Aleshin A, Slamon DJ. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. Breast Cancer Research. 2016 Feb 9;**18**(1):17. DOI: 10.1186/s13058-015-0661-5. Review. PubMed PMID: 26857361; PubMed Central PMCID: PMC4746893

[110] Vidula N, Rugo HS. Cyclin-dependent kinase 4/6 inhibitors for the treatment of breast cancer: A review of preclinical and clinical data. Clinical Breast Cancer. 2016 Feb;**16**(1):8-17. DOI: 10.1016/j.clbc.2015.07.005. Epub 2015 Jul 26. Review. PubMed PMID: 26303211

[111] FDA Approved drugs in Oncology/Centerwatch

[112] Wang Z, Fukushima H, Inuzuka H, Wan L, Liu P, Gao D, Sarkar FH, Wei W. Skp2 is a promising therapeutic target in breast cancer. Frontiers in Oncology. 2012 Jan 4;**1**(57). pii: 18702. PubMed PMID: 22279619; PubMed Central PMCID: PMC3263529

[113] Sun YJ, Wang XK, Li BJ. S-phase kinase-associated protein 2 expression interference inhibits breast cancer cell proliferation. Genetics and Molecular Research. 2015 Aug 10;**14**(3):9244-9252. DOI: 10.4238/2015.August.10.4. PubMed PMID: 26345857

[114] Cole AJ, Clifton-Bligh R, Marsh DJ. Histone H2B monoubiquitination: Roles to play in human malignancy. Endocrine-Related Cancer. 2015 Feb;**22**(1):T19-T33. DOI: 10.1530/ERC-14-0185. Epub 2014 Jun 2. Review. PubMed PMID: 24891457

[115] Lan X, Atanassov BS, Li W, Zhang Y, Florens L, Mohan RD, Galardy PJ, Washburn MP, Workman JL, Dent SY. USP44 is an integral component of N-CoR that contributes to gene repression by deubiquitinating histone H2B. Cell Reports. 2016 Nov22;**17**(9):2382-2393. DOI: 10.1016/j.celrep.2016.10.076. PubMed PMID: 27880911; PubMed Central PMCID: PMC5131803

[116] Wilson Xu C, Urasaki Y. Modulation of histone H2B monoubiquitination and treatment of. Cancer. Oct 2013

[117] Gajulapalli VN, Malisetty VL, Chitta SK, Manavathi B. Oestrogen receptor negativity in breast cancer: a cause or consequence? Bioscience Reports. 2016 Dec 23;**36**(6):e00432. DOI: 10.1042/BSR20160228. Print 2016 Dec. Review. PubMed PMID: 27884978; PubMed Central PMCID: PMC5180249

[118] Zhou Z, Qiao JX, Shetty A, Wu G, Huang Y, Davidson NE, Wan Y. Regulation of estrogen receptor signaling in breast carcinogenesis and breast cancer therapy. Cellular and Molecular Life Sciences. 2014 Apr;**71**(8):1549. DOI: 10.1007/s00018-013-1376-3. Retraction in: Cellular and Molecular Life Sciences. 2014 Apr;**71**(8):1549. PubMed PMID: 25031550; PubMed Central PMCID: PMC3962223

[119] McCubrey JA, Steelman LS, Bertrand FE, Davis NM, Sokolosky M, Abrams SL, Montalto G, D'Assoro AB, Libra M, Nicoletti F, Maestro R, Basecke J, Rakus D, Gizak A, Demidenko ZN, Cocco L, Martelli AM, Cervello M. GSK-3 as potential target for therapeutic intervention in cancer. Oncotarget. 2014 May 30;**5**(10):2881-2911 Review. PubMed PMID: 24931005; PubMed Central PMCID: PMC4102778

[120] Ugolkov et al. GSK3 inhibition overcomes chemoresistance in human breast cancer. Cancer Letters. 2016 Oct 1;**380**(2):384-392. DOI: 10.1016/j.canlet.2016.07.006.Epub 2016 Jul 14

[121] Wu W, Koike A, Takeshita T, Ohta T. The ubiquitin E3 ligase activity of BRCA1 and its biological functions. Cell Division. 2008 Jan 7;**3**:1. DOI: 10.1186/1747-1028-3-1

[122] Livraghi L, Garber JE. PARP inhibitors in the management of breast cancer: Current data and future prospects. BMC Medicine. 2015 Aug 13;**13**:188. DOI: 10.1186/s12916-015-0425-1. Review. PubMed PMID: 26268938; PubMed Central PMCID: PMC4535298

[123] Chustecka Z. Olaparib Positive Results in BRCA Metastatic Breast Cancer. Medscape; February 17 2017

[124] Kroese M, Zimmern RL, Pinder SE. HER2 status in breast cancer—an example of pharmacogenetic testing. Journal of the Royal Society of Medicine. 2007;**100**(7):326-329

[125] Vu T, Claret FX. Trastuzumab: Updated mechanisms of action and resistance in breast cancer. Frontiers in Oncology. 2012 Jun 18;**2**:62. DOI: 10.3389/fonc.2012.00062. eCollection 2012. PubMed PMID: 22720269; PubMed Central PMCID: PMC3376449

[126] Maher M. Current and emerging treatment regimens for HER2-positive breast cancer. Pharmacy and Therapeutics. 2014;**39**(3):206-212

[127] Jeong JH, An JY, Kwon YT, Li LY, Lee YJ. Quercetin-induced ubiquitination and downregulation of her-2/neu. Journal of Cellular Biochemistry. 2008 Oct 1;**105**(2):585-595. DOI: 10.1002/jcb.21859. PubMed PMID: 18655187; PubMed Central PMCID: PMC2575035

[128] Zhang X, Munster PN. New protein kinase inhibitors in breast cancer: Afatinib and neratinib. Expert Opinion on Pharmacotherapy. 2014 Jun;**15**(9):1277-1288. DOI: 10.1517/14656566.2014.913570. Epub 2014 Apr 30

[129] Rafaat A, Abd-Rabou A, Reda A. TRAIL combinations: The new 'trail' for cancer therapy (review). Oncology Letters. 2014 May;**7**(5):1327-1332. Epub 2014 Feb 27

[130] Manouchehri JM, Kalafatis M, Lindner D. Evaluation of the efficacy of TRAIL plus quercetin as a potential breast carcinoma therapeutic. Cancer Research. DOI: 10.1158/1538-7445.AM2016-1295. Published July 2016

[131] Nourashrafeddin S, Aarabi M, Modarressi MH, Rahmati M, Nouri M. The evaluation of WBP2NL-related genes expression in breast cancer. Pathology Oncology Research. 2015Apr;**21**(2):293-300. DOI: 10.1007/s12253-014-9820-8. Epub 2014 Nov 25. PubMed PMID:25417742

[132] Chen Y, van de Vijver MJ, Hibshoosh H, Parsons R, Saal LH. PTEN and NEDD4 in human breast carcinoma. Pathology Oncology Research. 2016 Jan;**22**(1):41-47. DOI: 10.1007/s12253-015-9971-2. Epub 2015 Aug 15. PubMed PMID: 26276352; PubMed Central PMCID: PMC4681749

[133] Ye X, Wang L, Shang B, Wang Z, Wei W. NEDD4: A promising target for cancer therapy. Current Cancer Drug Targets. 2014;**14**(6):549-556 Review. PubMed PMID:25088038; PubMed Central PMCID: PMC4302323

[134] Pal A, Donato NJ. Ubiquitin-specific proteases as therapeutic targets for the treatment of breast cancer. Breast Cancer Research. 2014;**16**(5):461 Review. PubMed PMID: 25606592; PubMed Central PMCID: PMC4384352

[135] Wu Y, Wang Y, Lin Y, Liu Y, Wang Y, Jia J, Singh P, Chi YI, Wang C, Dong C, Li W, Tao M, Napier D, Shi Q, Deng J, Mark Evers B, Zhou BP. Dub3 inhibition suppresses breast cancer invasion and metastasis by promoting Snail1 degradation. Nature Communications. 2017 Feb 15;**8**:14228. DOI: 10.1038/ncomms14228. PubMed PMID: 28198361; PubMed Central PMCID: PMC5316870

[136] Huang X, Dixit VM. Drugging the undruggables: Exploring the ubiquitin system for drug development. Cell Research. 2016 Apr;**26**(4):484-498. DOI: 10.1038/cr.2016.31. Epub 2016 Mar 22. Review. PubMed PMID: 27002218; PubMed Central PMCID: PMC4822129

[137] Traboulsi T, Ezzy M, Gleason J. Antiestrogens: structure-activity relationships and use in breast cancer treatment. Journal of Molecular Endocrinology. 2016 Dec;**58**(1):R15-R31. DOI: 10.1530/JME-16-0024. Epub 2016 Dec 3

[138] Yan SC, Liu YP, Zhang LY, Qu JL, Xu L, Liu J, Zhang Y, Hou KZ, Teng YE, Qu XJ. Ubiquitin ligase c-Cbl is involved in tamoxifen-induced apoptosis of MCF-7 cells by downregulating the survival signals. Acta Oncology. 2011 Jun;**50**(5):693-699. DOI: 10.3109/0284186X.2010.543144. Epub 2010 Dec 22

[139] Rawat A, Gopal G, Selvaluxmy G, Rajkumar T. Inhibition of ubiquitin conjugating enzyme UBE2C reduces proliferation and sensitizes breast cancer cells to radiation, doxorubicin, tamoxifen and letrozole. Celluar Oncology. 2013 Dec;**36**(6):459-467. DOI: 10.1007/s13402-013-0150-8. Epud 2013 Sep 27

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DNA damage response (DDR) and lesion repair are vital processes ensuring genome integrity through various pathways depending mainly on the nature of DNA injury and cell cycle stage. DDR is finely regulated at many levels in co-ordination with other ongoing processes as is genome replication and cell cycle progression.

Posttranslational modifications (PTMs), affecting both protein-protein and protein-DNA interactions, play a crucial role in finely tuning all processes involved in the restoration of genome lesions. Regarding damaged chromatin, PTMs serve in many cases as recruitment platforms for DNA repair mechanisms by facilitating binding sites or regulating interactions between involved proteins. Ubiquitination, the addition of ubiquitin moieties on a target protein, apart from controlling protein availability through degradation, is also involved, together with partner small ubiquitin-like modifier (SUMO), in controlling many pathways involved in DDR by modifying the structure-function relationship and thus interacting with partner molecules. The aim of this book is to cover a broad spectrum of current topics in ubiquitination and to a lesser extent SUMOylation involvement in regulation of DDR and repair in health and disease. This book is intended for pre- and postgraduate students and young scientists in this field. Members of both academic and research institutions, actively involved in the field, have described their current understanding of major mechanisms involved, highlighted key events, described ongoing applications in both developmental diseases and cancer and provided hints for future potential applications.

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