IntechOpen

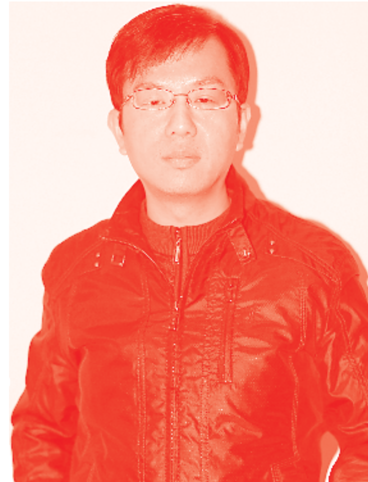
Oncogenes and Carcinogenesis

Edited by Pinar Erkekoglu

Oncogenes and Carcinogenesis

Edited by Pinar Erkekoglu

Published in London, United Kingdom



IntechOpen

Supporting open minds since 2005

Oncogenes and Carcinogenesis
http://dx.doi.org/10.5772/intechopen.74727
Edited by Pinar Erkekoglu

Contributors
Jun Hirayama, Yasemin Baskin, Burcin Baran, Nazlı Mert Ozupek, Gizem Calibasi Kocal, Max Kushner, Anna Riegel, Ghada Sharif, Wan Lam, Victor Martinez, Adam Sage, Erin Marshall, Miwa Suzuki, Aaron Goodarzi, Graham Dellaire, Pinar Erkekoglu

© The Editor(s) and the Author(s) 2019
The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com).
Violations are liable to prosecution under the governing Copyright Law.

Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 3.0 Unported License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be foundat http://www.intechopen.com/copyright-policy.html.

Notice
Statements and opinions expressed in the chapters are these of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2019 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Oncogenes and Carcinogenesis
Edited by Pinar Erkekoglu
p. cm.
Print ISBN 978-1-78985-171-7
Online ISBN 978-1-78985-172-4
eBook (PDF) ISBN 978-1-83962-062-1

We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,000+
Open access books available

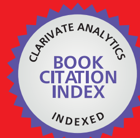
116,000+
International authors and editors

120M+
Downloads

151
Countries delivered to

Our authors are among the
Top 1%
most cited scientists

12.2%
Contributors from top 500 universities



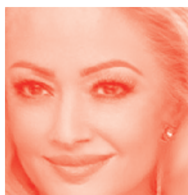
WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Meet the editor



Pınar Erkekoglu was born in Ankara, Turkey. She graduated from Hacettepe University Faculty of Pharmacy (BS). Later, she received her MSci and PhD degrees in Toxicology. She completed part of her PhD studies in Grenoble, France, at the Université Joseph Fourier and at CEA/INAC/LAN after receiving full scholarship from both the Erasmus Scholarship Program and CEA. She worked as a post-doc and a visiting associate at the MIT Biological Engineering Department. She has been working as an associate professor in Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, since 2013. Her main interests are carcinogenesis, endocrine disrupting chemicals, oxidative stress, and aromatic amines. She has published more than 140 papers in national and international journals. She has been a European registered toxicologist since 2014.

Contents

Preface **XIII**

Section 1
Introduction to Oncogene Concept **1**

Chapter 1 **3**
Introductory Chapter: Interactions between Environmental Chemicals and KRAS Oncogene in Different Cancers - Special Focus on Colorectal, Pancreatic, and Lung Cancers
by Pinar Erkekoglu

Section 2
Oncogenes and Circadian Clock **11**

Chapter 2 **13**
A Molecular Link between the Circadian Clock, DNA Damage Responses, and Oncogene Activation
by Yoshimi Okamoto-Uchida, Junko Izawa and Jun Hirayama

Section 3
Oncogenic Activation in Different Cancers **29**

Chapter 3 **31**
MicroRNAs (miRNAs) in Colorectal Cancer
by Burcin Baran, Nazli-Mert Ozupek, Gizem Calibasi-Kocal and Yasemin Basbinar

Chapter 4 **49**
The AIB1/NCOA3/SRC-3 Oncogene
by Max H. Kushner, Anna T. Riegel and Ghada M. Sharif

Chapter 5 **71**
Oncogenetics of Lung Cancer Induced by Environmental Carcinogens
by Victor D. Martinez, Adam P. Sage, Erin A. Marshall, Miwa Suzuki, Aaron A. Goodarzi, Graham Dellaire and Wan L. Lam

Preface

Most oncogenes are expressed as proto-oncogenes. They are involved in cell growth and proliferation or inhibition of apoptosis. If there are chemical, physical, or biological factors that cause mutations in such genes, these genes are mostly upregulated and cellular proliferation increases. In this case, they are termed "oncogenes." The cascade of events leading to proliferation usually predispose the cell to cancer. These genes are mutated and/or overexpressed at high levels in tumor cells. Tumors of the lung, breast, pancreas, and colon may display specific oncogenetic features. These tumors have been largely associated with exposure to environmental carcinogens and a variety of biological agents, including viruses. These carcinogens can induce specific genetic and epigenetic alterations (including modulation of DNA methylation, histone acetylation, and RNA expression) in these tissues, leading to aberrant functioning of oncogenes and tumor suppressor genes. On the other hand, microRNAs (miRNAs) are significant modifiers of transcription and translation of both oncogenes and tumor suppressor proteins, particularly in carcinogenesis. In the last 50 years, several oncogenes and miRNAs related to oncogenes have been identified in different types of human cancers. It is now clear that high expression of oncogenes, DNA damage response, and regulation of cell cycle are related to the circadian clock. There are several studies on cancer drugs that target the proteins encoded by oncogenes. In addition, the reversible nature of epigenetic modifications has led to the new field of "epigenetic therapy." Therefore, understanding the link between genetic alterations, epigenetic modifications, and expression of oncogenes can unravel the molecular targets for treating cancer. This book will mainly focus on the expressions of different oncogenes in breast, colon, and lung cancers. Moreover, the alterations in miRNAs in different types of cancers and the effects of the circadian clock on the expression of oncogenes in carcinogenesis will also be mentioned. Readers will mainly understand how the modulations and mutations in the expressions of oncogenes and related miRNAs lead to the promotion of carcinogenesis and how these alterations affect the carcinogenesis process in certain types of cancers. Moreover, readers will also gain knowledge of the relationship between the circadian clock and oncogenes.

Assoc. Prof. Dr. Pinar Erkekoglu
Editor,
Hacettepe University,
Faculty of Pharmacy,
Department of Toxicology,
Ankara, Turkey

Section 1

Introduction to Oncogene Concept

Chapter 1

Introductory Chapter: Interactions between Environmental Chemicals and KRAS Oncogene in Different Cancers - Special Focus on Colorectal, Pancreatic, and Lung Cancers

Pinar Erkekoglu

1. Introduction

v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is an oncogene. The KRAS gene is located on the twelfth chromosome and belongs to the Ras family of oncogenes. These proteins play important roles in cell division, cell differentiation, and apoptotic cell death. Induction of KRAS with different environmental chemicals leads to high expression of K-Ras protein, which in turn causes high cellular proliferation. These cascade of events finally initiate certain types of cancers, particularly colorectal (CRC), pancreatic, and lung cancers. High calorie intake, diets rich in meat and fat, smoking, and alcohol consumption are the major risk factors of CRCs, and it was estimated that in CRC, mutated KRAS has an incidence of ~50%. Exposure to certain environmental chemicals [organochlorine insecticides such as DDT and its metabolite dichlorodiphenyltrichloroethylene (DDE); herbicides such as EPTC and pendimethalin; N-nitrosamines; polychlorinated biphenyls (PCBs); benzene] and drugs (anti-diabetics drugs) can also contribute to the increased incidence of PC throughout the world. It was stated that in adenocarcinomas of the pancreas, mutated KRAS has an incidence of ~70–90%. Lung cancer is the leading cause of deaths worldwide. KRAS gene mutations are much more common in long-term tobacco smokers with lung cancer when compared to nonsmokers. KRAS gene mutations are observed in 15–25% of all lung cancer cases, being more frequent in whites vs. Asian populations. Lung cancers with KRAS gene mutations typically indicate a poor prognosis and are associated with resistance to several cancer treatments. This chapter mainly focuses on KRAS, interactions between environmental chemicals, and KRAS oncogene in different cancers, particularly in colorectal, pancreatic, and lung cancers.

Most oncogenes are expressed as proto-oncogenes, involved in cell growth and proliferation or inhibition of apoptosis. If there are chemical, physical, or biological factors that cause mutations in such genes promoting cellular growth, these genes are mostly upregulated and cellular proliferation increases [1]. The cascade of events leading to proliferation usually predisposes the cell to cancer. In this case,

IntechOpen

they are termed as "oncogenes" [1, 2]. These genes are mutated and/or overexpressed at high levels in tumor cells. Normally, cells repair themselves or undergo apoptosis if there is an interruption on the cell cycle. However, the high expression of multiple oncogenes, along with mutated apoptotic and/or tumor suppressor genes and exposure to environmental chemicals that trigger such mutations can all act in concert and finally cause tumorigenesis [1–3]. In the past 50 years, several oncogenes have been identified in different types of human cancers. There are many cancer drugs that target the proteins encoded by oncogenes [1–3].

Genetic and environmental interactions usually determine the profiles of cancers. v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is a very important oncogene for the initiation of cancer [1]. It is usually found to be mutated in different types of cancer, particularly in colorectal cancers (CRCs), pancreatic cancer (PC), and lung cancer [4–6]. Concerning KRAS, different chemicals such as polychlorinated biphenyls (PCBs), certain antidiabetic drugs, and pesticides may be leading causes of KRAS mutations, and such mutations increase the expression of K-Ras protein in different tissues, leading to high cellular proliferation and finally carcinogenesis [7–9]. This chapter mainly focuses on CRCs, PC, and lung cancer and KRAS. Moreover, the interactions between KRAS mutations and environmental factors in these particular cancers will also be mentioned.

2. KRAS gene

The most important oncogene for several types of cancer is KRAS. Cytogenetic location of this gene is 12p12.1 [the short (p) arm of chromosome 12 at position 12.1] [10]. The KRAS gene belongs to the Ras family of oncogenes. RAS family oncogenes also include two other genes: H-RAS and N-RAS. These proteins play important roles in cell division, cell differentiation, and apoptotic cell death. KRAS causes the initiation of cancer through deregulation of the G1 cell cycle [10].

The KRAS gene expresses a protein called "K-Ras," which is part of a signaling pathway known as "the RAS/microtubule-associated protein (MAP) kinase signaling (MAPK) pathway." The protein carries the mitogenic signals from the "epidermal growth factor receptor (EGFR)" on the cell surface to the cell nucleus. These signals provide instructions for growth, proliferation, maturation, or differentiation to the cell. The K-Ras protein converts a molecule called guanosine-5′-triphosphate (GTP) into another molecule called guanosine-5′-diphosphate (GDP), and therefore, it is a "GTPase." By such conversion, K-Ras protein almost acts like a "switch," which is turned on and off by the GTP and GDP molecules. In order to transmit signals, K-Ras must bind to GTP, and this turns on the protein [10]. However, K-Ras protein is inactivated when it converts the GTP to GDP. This means that when this particular protein is bound to GDP, it does not send signals to the nucleus. In several pathological conditions [cardiofaciocutaneous syndrome, Noonan syndrome, Costello syndrome, autoimmune lymphoproliferative syndrome (ALPS), and epidermal nevus] and different cancers [colorectal (CRC), pancreatic (PC), and lung cancer; cholangiocarcinoma; and core binding factor acute myeloid leukemia (CBF-AML)], KRAS mutations are observed in patients [10].

3. Cancers associated with KRAS

3.1 Colorectal cancers

Colorectal cancers (adenomas or carcinomas) occur as a combination of unbalanced diet, environmental exposures, accumulation of genetic and epigenetic

instability, and oncogenic gene activations [11, 12]. It is certainly clear that unbalanced diet is a major risk factor for the development of CRCs. A constant, high, or prolonged exposure of colon to carcinogens is the primary cause for malignant transformation of colonocytes [11, 12]. If hereditary disposition (in terms of mutations in key genes controlling cell cycle and replication) is already present, genome instability will accelerate tumorigenesis process [13]. It was estimated that in CRC, mutated K-Ras has an incidence of ~50% [14].

The major genetic pathways of colorectal cancers (CRCs) are usually divided into two pathways [15, 16]:

1. "The Chromosome Instability Pathway" representing the pathway of sporadic CRC through the KRAS, adenomatous polyposis coli (APC), and tumor suppressor protein 53 (P53) mutations.

2. The "Microsatellite Instability Pathway" representing the pathway of hereditary non-2 primary KRAS mutation generally leads to a self-limiting hyperplastic or borderline lesion and may be implicated in the serrated pathway through which serrated adenomas and carcinomas may also develop.

The KRAS mutation alone is not sufficient or necessary to drive the malignant transformation. Therefore, additional "drivers" should be present in the development of CRC. These additional factors include but are not limited to high calorie intake, diets rich in meat and fat, smoking, and alcohol consumption [17]. KRAS mutations are frequently found in <95% of early dysplasia, including aberrant crypt foci (ACF), and also in hyperplastic polyps [18–20]. The sequence in which the KRAS mutation occurs in relation to the APC mutation is important. The dysplastic lesion often progresses to carcinogenesis if a mutation in KRAS gene occurs right after an APC mutation [21, 22]. Because of the key role in EGFR signaling, the presence of a KRAS mutation predicts a very poor response to specific antibody (monoclonal antibodies) treatment with EGFR inhibitors such as panitumumab and cetuximab [23, 24].

3.2 Pancreatic cancer

Pancreatic cancer is a multifactorial and extremely aggressive type of cancer. Pancreatic tumors are usually highly chemoresistant, and many types of PC have very bad prognoses. Little information regarding the possible association of different risk factors with the known genetic alterations (such as activation of KRAS oncogene and inactivation of the p53 gene) is present in the literature [8, 25]. However, it was stated that in adenocarcinomas of the pancreas, mutated KRAS has an incidence of ~70–90% [14].

Increasing data on the molecular pathogenesis of PC have shown that genetic alterations, such as mutations of KRAS and particularly epigenetic dysregulation (DNA methylation, histone acetylation, or microRNA expressions) of tumor-associated genes [i.e., silencing of the tumor suppressor p16 (ink4a)], are suggested to be hallmarks of PC. Serine/threonine-protein kinase (Raf), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and Ral guanine nucleotide dissociation stimulator (RaLGDS) are the major effectors of KRAS in adenomas of pancreas [26, 27].

Repeated acute pancreatic injury and inflammation are important contributing factors in the development of PC. Alcohol consumption, cigarette smoking, diet (high coffee consumption), environmental chemicals [organochlorine insecticides such as DDT and its metabolite dichlorodiphenyltrichloroethylene (DDE); herbicides such as s-ethyl dipropylthiocarbamate (EPTC) and pendimethalin; N-nitrosamines; polychlorinated biphenyls (PCBs); benzene], and drugs [diabetes

drugs like glucagon-like peptide-1 (GLP-1) agonists, such as exenatide; dipeptidyl-peptidase-4 inhibitors (DPP-4), such as sitagliptin; calcium channel blockers such as nifedipine, nicardipine, and diltiazem] can also contribute to the highly increasing incidence of PC throughout the world. On the other hand, gall stones, diabetes, and obesity are the major pathological factors associated with PC [27–29]. In a study by Slebos et al., mutations in KRAS codon 12 were found in 75% of the PC patients. However, there were no differences in blood PCB levels between the KRAS wild-type and mutant groups [8].

3.3 Lung cancer

Lung cancer is the primary cause of cancer-related deaths worldwide. Active and passive smoking are the two of primary causes of lung cancer. Lung cancers are classified as small cell (non-epithelial) or non-small cell carcinomas (epithelial-derived). Small cell carcinomas are highly malignant; has the ability to metastasize easily and chemotherapy is the choice of treatment. However, treatment of non-small cell cancer primarily involves surgical excision, supplemented by radiation or chemotherapy. Although this treatment method may provide partial or full recovery, it also increases the risk for concurrent diseases. Using anti-cancer drugs with "high efficacy and low-toxicity" is the priority goal in this field [30, 31].

KRAS gene mutations are observed in 15–25% of all lung cancer cases. These mutations are more frequent in white populations than in Asian populations. About 25–50% of whites with lung cancer have KRAS gene mutations, whereas 5–15% of Asians with lung cancer have KRAS gene mutations [14].

In lung adenocarcinomas, both KRAS-activating mutations and in and EGFR mutations can be observed. KRAS appear to be mutually exclusive. Three different mutations in the KRAS gene have been associated with lung cancer [32]. Nearly all of the KRAS gene mutations associated with lung cancer change the amino acid glycine at position 12 or 13 (Gly12 or Gly13) or change the amino acid glutamine at position 61 (Gln61) in the K-Ras protein. These mutations cause a constantly activated KRAS, which directs the cells to proliferate in an uncontrolled way, and the high cellular proliferation leads to tumor formation [33].

Even though KRAS mutations were identified in non-small cell lung tumors more than 20 years ago, the clinical value of determining KRAS tumor status is recently gaining importance. Recent studies indicate that patients with mutant KRAS tumors fail to benefit from adjuvant chemotherapy and do not respond to EGFR inhibitors. There is a clear need for therapies specifically developed for patients with KRAS-mutant non-small cell lung cancers [34, 35]. KRAS gene mutations are much more common in long-term tobacco smokers with lung cancer when compared to nonsmokers. Lung cancers with KRAS gene mutations typically indicate a poor prognosis and are associated with resistance to several cancer treatments [33–35].

4. Conclusion

KRAS is a very important oncogene. K-Ras protein is upregulated in different cancers and can cause bad prognosis of the disease. However, KRAS mutations are not sometimes enough to initiate cancer. Therefore, along with KRAS mutations, several environmental chemicals and drugs may contribute to the cascade of events leading to cancer.

It can be stated that in CRCs, PC, and lung cancer, KRAS mutations should be evaluated in clinics. On the other hand, the exposures of different environmental

chemicals and drugs (pesticides, PCBs, tobacco smoke, alcohol, N-nitrosamines, benzene, antidiabetics, calcium channel blockers, etc.) should be evaluated along with KRAS mutations, and the patients with preneoplastic lesions should be warned about such exposures. As KRAS gene mutations generally indicate a poor prognosis and are associated with resistance to several cancer treatments, new drugs targeting different molecules in KRAS triggering pathways should be developed in order to overcome this resistance, particularly in CRCs, PC, and lung cancer.

Author details

Pinar Erkekoglu
Department of Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

*Address all correspondence to: erkekp@yahoo.com

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell. 2017;**170**(1):17-33

[2] Denis JA, Lacorte JM. Detection of RAS mutations in circulating tumor cells: Applications in colorectal cancer and prospects. Annales de Biologie Clinique. 2017;**75**(6):607-618

[3] Ryan MB, Corcoran RB. Therapeutic strategies to target RAS-mutant cancers. Nature Reviews. Clinical Oncology. 2018;**15**(11):709-720

[4] Ciliberto D, Staropoli N, Caglioti F, Chiellino S, Ierardi A, Ingargiola R, et al. The best strategy for RAS wild-type metastatic colorectal cancer patients in first-line treatment: A classic and Bayesian meta-analysis. Critical Reviews in Oncology/Hematology. 2018;**125**:69-77

[5] Li D, Jiao L. Molecular epidemiology of pancreatic cancer. International Journal of Gastrointestinal Cancer. 2003;**33**(1):3-14

[6] Zhang J, Park D, Shin DM, Deng X. Targeting KRAS-mutant non-small cell lung cancer: Challenges and opportunities. Acta Biochimica et Biophysica Sinica Shanghai. 2016;**48**(1):11-16

[7] Nakanishi Y, Bai F, Inoue K, Takayama K, Pei XH, Harada T, et al. Polychlorinated biphenyls promote 1-nitropyrene-induced lung tumorigenesis without the induction of K-ras gene mutation in a/J mice. Teratogenesis, Carcinogenesis, and Mutagenesis. 2001;**21**(6):395-403

[8] Slebos RJ, Hoppin JA, Tolbert PE, Holly EA, Brock JW, Zhang RH, et al. K-ras and p53 in pancreatic cancer: Association with medical history, histopathology, and environmental exposures in a population-based study. Cancer Epidemiology, Biomarkers & Prevention. 2000;**9**(11):1223-1232

[9] Szablewski L. Diabetes mellitus: Influences on cancer risk. Diabetes/Metabolism Research and Reviews. 2014;**30**(7):543-553

[10] Genetics Home Reference. US National Library of Medicine. KRAS gene KRAS proto-oncogene. GTPase. Available from: https://ghr.nlm.nih.gov/gene/KRAS

[11] World Cancer Research Fund, American Institute for Cancer Research. Continous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. 2011. Available from: http://www.aicr.org/reduce-your-cancer-risk/recommendations-for-cancer-prevention/

[12] Lynch HT, de la Chapelle A. Hereditary colorectal cancer. The New England Journal of Medicine. 2003;**348**:919-932

[13] Weitz J, Koch M, Debus J, Höhler T, Galle PR, Büchler MW. Colorectal cancer. Lancet. 2005;**365**:153-165

[14] Johnson L, Mercer K, Greenbaum D, Bronson RT, Crowley D, Tuveson DA, et al. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. Nature. 2001;**410**(6832):1111-1116

[15] Kukor Z, Mayerle J, Krüger B, Tóth M, Steed PM, Halangk W, et al. Presence of cathepsin B in the human pancreatic secretory pathway and its role in trypsinogen activation during hereditary pancreatitis. The Journal of Biological Chemistry. 2002;**277**(24):21389-21396

[16] Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, et al. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell. 2009;**15**(4):283-293

[17] Lee MS, Gu D, Feng L, Curriden S, Arnush M, Krahl T, et al. Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor-beta 1. The American Journal of Pathology. 1995;**147**(1):42-52

[18] Alrawi SJ, Schiff M, Carroll RE, Dayton M, Gibbs JF, Kulavlat M, et al. Aberrant crypt foci. Anticancer Research. 2006;**26**(1A):107-119

[19] Feng Y, Bommer GT, Zhao J, Green M, Sands E, Zhai Y, et al. Mutant KRAS promotes hyperplasia and alters differentiation in the colon epithelium but does not expand the presumptive stem cell pool. Gastroenterology. 2011;**141**:1003-1013. e1-10

[20] Otori K, Oda Y, Sugiyama K, Hasebe T, Mukai K, Fujii T, et al. High frequency of K-ras mutations in human colorectal hyperplastic polyps. Gut. 1997;**40**:660-663

[21] Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008;**135**:1079-1099

[22] Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nature Medicine. 2004;**10**:789-799

[23] Markman B, Javier Ramos F, Capdevila J, Tabernero J. EGFR and KRAS in colorectal cancer. Advances in Clinical Chemistry. 2010;**51**:71-119

[24] Kolodecik T, Shugrue C, Ashat M, Thrower EC. Risk factors for pancreatic cancer: Underlying mechanisms and potential targets. Frontiers in Physiology. 2014;**4**:415

[25] Nussinov R, Tsai CJ, Muratcioglu S, Jang H, Gursoy A, Keskin O. Principles of K-Ras effector organization and the role of oncogenic K-Ras in cancer initiation through G1 cell cycle deregulation. Expert Review of Proteomics. 2015;**12**(6):669-682

[26] Neureiter D, Jäger T, Ocker M, Kiesslich T. Epigenetics and pancreatic cancer: Pathophysiology and novel treatment aspects. World Journal of Gastroenterology. 2014;**20**(24):7830-7848

[27] Edderkaoui M, Eibl G. Risk factors for pancreatic cancer: Underlying mechanisms and potential targets. Frontiers in Physiology. 2014;**5**:490

[28] Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, et al. Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort. International Journal of Cancer. 2009;**124**(10):2495-2500

[29] Fritschi L, Benke G, Risch HA, Schulte A, Webb PM, Whiteman DC, et al. Occupational exposure to N-nitrosamines and pesticides and risk of pancreatic cancer. Occupational and Environmental Medicine. 2015;**72**(9):678-683

[30] Kutkowska J, Porębska I, Rapak A. Non-small cell lung cancer—Mutations, targeted and combination therapy. Postepy Higieny i Medycyny Doświadczalnej (Online). May 17 2017;**71**(0):431-445

[31] Lin PY, Chang YJ, Chen YC, Lin CH, Erkekoglu P, Chao MW, et al. Anti-cancer effects of 3,5-dimethylaminophenol in A549 lung cancer cells. PLoS One. 2018;**13**(10):e0205249

[32] Karachaliou N, Mayo C, Costa C, Magrí I, Gimenez-Capitan A, Molina-Vila MA, et al. KRAS mutations in lung cancer. Clinical Lung Cancer. 2013;**14**(3):205-214

[33] Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Research. 2012;**72**(10):2457-2467

[34] Chapman AM, Sun KY, Ruestow P, Cowan DM, Madl AK. Lung cancer mutation profile of EGFR, ALK, and KRAS: Meta-analysis and comparison of never and ever smokers. Lung Cancer. 2016;**102**:122-134

[35] Subramanian J, Govindan R. Molecular profile of lung cancer in never smokers. EJC Supplements. 2013;**11**(2):248-253

Section 2

Oncogenes and Circadian Clock

Chapter 2

A Molecular Link between the Circadian Clock, DNA Damage Responses, and Oncogene Activation

Yoshimi Okamoto-Uchida, Junko Izawa and Jun Hirayama

Abstract

Circadian clocks enhance the efficiency and survival of living things by organizing their behavior and body functions. There has been a long history of research seeking a link between circadian clock and tumorigenesis. Studies of animal models and human tumor samples have revealed that the dysregulation of circadian clocks is an important endogenous factor causing mammalian cancer development. The core circadian clock regulators have been implicated in the control of both the cell cycle and DNA damage responses (DDR). Conversely, several intracellular signaling cascades that play important roles in regulation of the cell cycle and the DDR also contribute to circadian clock regulation. This review describes selected regulatory aspects of circadian clocks, providing evidence of a molecular link of the circadian clocks with cellular DDR.

Keywords: circadian clock, DNA damage response, DNA repair, oncogenes

1. Introduction

Circadian (derived from Latin "around the day") clocks constitute ubiquitous processes that regulate various biochemical and physiological events occurring with a 24 h periodicity, even in the absence of external cues [1, 2]. Under natural conditions, clocks are entrained to a 24 h day by environmental time cues, most commonly light. Circadian clocks are established in cell-autonomous oscillators, referred to as cellular clocks, which are controlled by a transcription/translation-based negative feedback loop [3, 4]. In humans, the circadian clock generates circadian rhythms in synthesis and release of hormones and cardiovascular activities such as heart rate, blood pressure, and vascular tone [5, 6]. Moreover, immune responses show temporal changes in antibody levels and total number of lymphocytes, which are related to circadian variations [7]. Therefore, dysfunction of the clock can cause a variety of diseases. In particular, it has been reported that the circadian clocks are associated with tumor suppression in vivo, indicative of the theoretical foundations for cancer chronotherapy [8, 9].

At the molecular level, the circadian clocks can be divided into three conceptual components [10, 11]. The first is the pacemaker, dedicated to generating and sustaining circadian rhythms by receiving and integrating signals from external

IntechOpen

time cues. The second component is the input which refers to the pathway through which these cues are perceived and act upon the central pacemaker. The third element applies to how the clock affects physiology, which is achieved through the output pathways. In vertebrates, the cellular clocks are comprised of the circadian locomotor output cycles kaput (CLOCK), neuronal PAS domain-containing protein 2 (NPAS2), brain and muscle arnt-like protein-1 (BMAL), period (PER), and cryptochrome (CRY) proteins (**Figure 1A**) [11]. CLOCK or NPAS2 heterodimerize with BMAL to form an active transcription complex that transactivates clock-controlled genes, including *Cry* and *Per*. Once the CRY and PER proteins have been translated, they are translocated to the nucleus, where they inhibit CLOCK(NPAS2):BMAL-mediated transcription through a direct protein-protein interaction. Importantly,

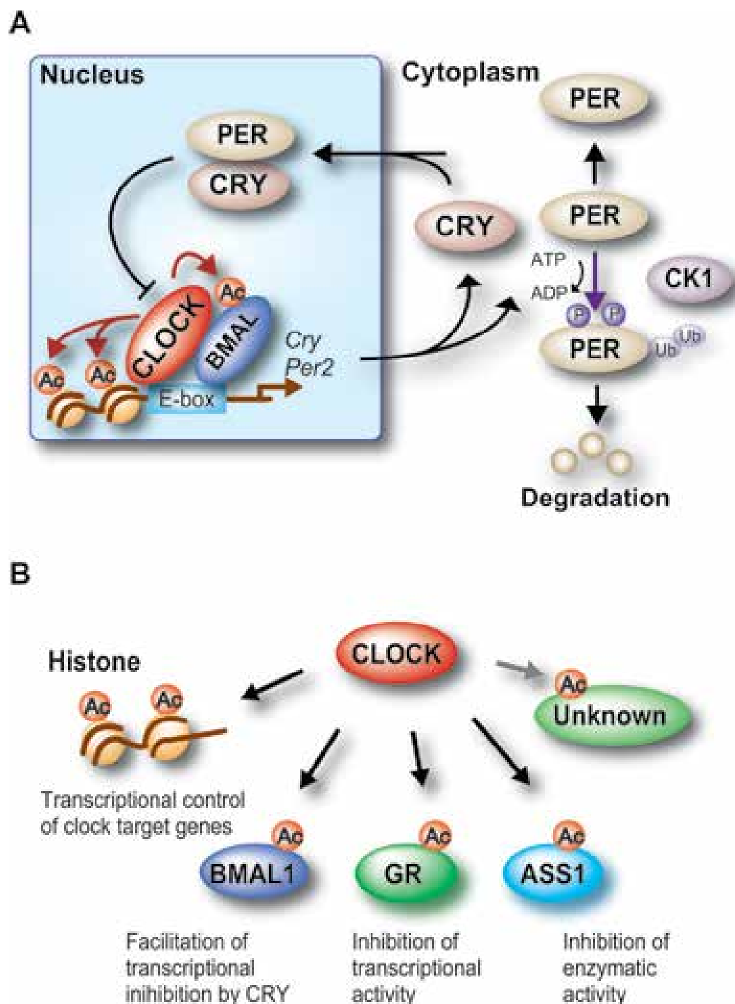


Figure 1.

Molecular mechanisms establishing circadian clocks in vertebrates. (A) Model of the vertebrate cellular clocks. Two basic helix-loop-helix PAS domain-containing transcription factors CLOCK and BMAL constitute the positive elements. When these transcription factors heterodimerize, they bind to E-boxes to drive the transcription of the negative components of the clock, Per and Cry genes. The products of these clock genes then negatively regulate their own expression, setting up the rhythmic oscillations of gene expression that drive the circadian clocks. CLOCK:BMAL complex also regulates clock-controlled genes, whose products mediate the "output" function of the clocks. CK1 phosphorylates PER protein, which is required for ubiquitination of PER and its subsequent degradation. An essential prerequisite for the circadian feedback loop is a short half-life of clock proteins. Thus, CK1-mediated degradation of PER is critical for maintenance of circadian rhythmicity of cellular clock. (B) Schematic representation of the proteins that are acetylated by CLOCK protein.

when active, the CLOCK (NPAS2):BMAL complex stimulates the transcription of many other clock-controlled genes. These genes in turn influence functions external to the oscillatory mechanism itself and mediate the "output" function of the clock. This accounts in part for the presence of circadian rhythms in a variety of physiological processes.

The phenotypes of mice with targeted disruptions of the genes encoding cellular clock's components have revealed direct links between the circadian clock and non-circadian aspects of animal physiology [6, 9]. In particular, these findings argue in favor of a major role played by the circadian machinery in cellular genotoxic stress responses and reveal intriguing links between the DNA damage responses (DDR) pathways and the circadian clocks. In this review, we summarize the evidence and explore the implications of such a link.

2. The relationship between transcriptional regulation of oncogenes and circadian clocks

The disruption of circadian clocks can have a profound effect on animal health and is linked to abnormal development and cancer [6, 9]. Expression of the circadian clock genes has been reported to be dysregulated in human cancers [12]. The circadian transcriptional machinery, cellular clock, has been reported to control expression of tumor suppressors. Thus, the abnormal control of clock genes' expression in cancer cells activates oncogenic signaling pathways by functional inhibition of tumor suppressors, such as ataxia telangiectasia mutated (ATM), p53, p21, and WEE1 [12].

The Wingless-related integration site (Wnt) signaling pathways collectively play important roles in developmental, proliferative, and cell death processes [13]. Mutations in genes encoding the various components of Wnt pathways have been identified that contribute to various types of cancer including hepatocellular carcinoma, pancreatic tumors, ovarian cancer, and breast cancer. Importantly, there are several lines of evidence that suggest the existence of an interaction between circadian clocks and Wnt signaling pathways. Previous study have performed microarray-based screening for circadian genes in several mouse tissues and have constructed a publicly accessible database, by which users can query for finding circadianly regulated genes or for the study of the temporal expression patterns of their genes of interest [14]. Interestingly, in this database, several Wnt signaling pathway genes, such as *Axin2*, *Frizzled3* (*Fzd3*), and *Disheveled* (*Dvl1*), show a circadian pattern of expression, suggesting the possibility that circadian clocks control transcription of Wnt signaling pathway genes. The future study of the connecting routes that link the circadian transcriptional machinery to Wnt signaling pathway will reveal a molecular link between circadian clock deficiency and tumorigenesis.

3. Possible roles of clock proteins in functional regulation of crucial components of DDR pathways

The activities associated with the physiological processes are organized in daily manner: during the daytime, the animal's physiology is given over to the catabolic processes, whereas at night, it concentrates on the anabolic functions of growth, repair, and consolidation [5, 6]. Disrupt, the time-dependent regulation of physiological functions in animals has profound effects on their health. In particular, many studies have provided evidence that disruption of the circadian clocks results

in tumorigenesis [8, 9]. Importantly, mice with mutations in the *Bmal1* gene show premature aging phenotype [15]. In addition, human CLOCK has been suggested to be involved in metastasis of colorectal cancer [16]. These findings implicate the core circadian machinery in the regulation of DDR and the cell cycle. Indeed, the circadian regulators have been demonstrated to interact with crucial components of cellular stress response pathways including the ATM, the checkpoint kinase 2 (Chk2) kinase [17], sirtuin1 (SIRT1) deacetylase [18], and nuclear receptors 19, 20], whereas it has been reported that DNA damage can act as a resetting cue for the mammalian circadian clock [21].

Histone acetyltransferases (HATs) such as CBP/p300 are known to acetylate nonhistone targets and have also been recognized as tumor suppressors [22, 23]. Translocation, amplification, overexpression, or mutation of HAT genes are known to occur in several forms of cancer, and several key cell cycle proteins (including p53 and c-MYC) are known targets of HATs. These observations suggest that HATs can also affect cell proliferation and differentiation in multiple ways, in addition to chromatin remodeling. It was previously reported that a core circadian regulator, CLOCK, has intrinsic HAT activity [24] and further that it acetylates a nonhistone target, the heterodimeric CLOCK-binding partner BMAL (**Figure 1B**) [25]. CLOCK also acetylates the glucocorticoid receptor and the argininosuccinate synthase, negatively regulating the transactivation capacity and the enzymatic activity, respectively [20, 26]. It is conceivable that CLOCK would directly interact with and regulate key DDR regulators, leading to the acetylation of these proteins and thereby modulating their activities (**Figure 1B**).

4. Roles of circadian clocks in regulation of cell cycle

Circadian clock proteins appear to play roles in cell cycle control, acting as tumor suppressors. They control the timing of cell proliferation by transcriptional control of key cell cycle genes such as *Wee1*, *c-Myc*, and cyclin-dependent kinase inhibitor 1d (20 kDa protein, *p20*) [27–29]. In mammals, PER proteins directly interact with ATM and Chk2 proteins, inducing cell growth inhibition, cell cycle arrest, and apoptosis [17]. In addition, it has been also reported that PER1 and PER2 interact with the androgen receptor (AR) or estrogen receptor (ER), respectively, in that PER1 inhibits AR-dependent transcription and PER2 induces ER degradation [19, 30]. These findings support the idea that clock proteins act as key players in the cell cycle by interacting directly with and regulating the functions of the cell cycle regulators.

In zebrafish, the cell cycle is directly regulated by light [31, 32]. Light determines the timing of mitosis (M phase) and DNA synthesis (S phase), establishing a circadian rhythm for cell cycle progression. At the molecular level, cellular clocks establish the circadian expression of the cell cycle genes, zebrafish *Wee1* and *p20* [29, 32]. The Wee-l kinase controls the timing of the G_2/M transition by directly phosphorylating and inhibiting cell division cycle2 (Cdc2)/cyclin B, leading to the suppression of mitotic cell division. In contrast, p20 regulates the G1/S transition of the cell cycle. Thus, the circadian control of these cell cycle regulators could be a mechanism establishing the circadian rhythm of cell cycle. Both cell cycle and circadian clock are endogenous pacemakers, and these mechanisms coexist in most eukaryotic cells and share several conceptual characteristics. The abovementioned findings point to functional links between the cell cycle and circadian clock in different organisms.

5. Posttranslational modifications contributing to both the circadian clock regulation and the cellular DDR

Posttranslational modifications of proteins regulate various biological processes at molecular levels, including gene expression, chromatin remodeling, and protein stabilization. These molecular events have essential roles in appropriately regulating biological phenomena, including development and circadian clock, by maintaining cellular functions, such as proliferation and molecular clocks, respectively. Posttranslational modifications, such as phosphorylation, sumoylation, and acetylation, control the transcriptional activity, subcellular localization, and stability of circadian clock regulators in multiple ways [4, 33]. In particular, the defects in phosphorylating the circadian clock regulators have been implicated in human sleep disorders [34, 35]. It is also well established that posttranslational modifications are vital for the regulation of the cell cycle and DDR. SIRT1 and casein kinase2 (CK2), already identified as responsible factors for posttranslational modifications of clock proteins [18, 36–39], have also been implicated in posttranslational modifications of proteins such as p53, forkhead box class O (FoxO), and E-cadherin that are involved in cellular metabolism, the cell cycle, and DDR [40, 41]. These findings support the hypothesis that the circadian clocks may be linked to other cellular processes, such as cell cycle control and DDR, through shared posttranslational modifications.

6. Studies on light-dependent regulation of zebrafish circadian clock have revealed links of circadian clock with DNA repair and cellular DDR

To guarantee that an organism's behavior remains tied to the rhythms of its environment, the circadian clocks must respond to environmental stimuli to be reset [2, 10]. The main cue for animals is light, which is provided by the day-night cycle. The mammalian route for circadian entrainment by light uses the retinohypothalamic tract, which connects directly to the central clock located in the suprachiasmatic nucleus of the brain [42]. This makes it difficult to analyze the light entrainment mechanisms of the circadian clocks, especially at cellular levels. Zebrafish peripheral cellular clocks display a striking feature as they are directly light responsive [43]. Notably, in the zebrafish-cultured cell lines, oscillations of clock gene expression can be entrained to new light-dark cycle, and expression of clock genes, such as zebrafish *Cryptochrome1a* (*zCry1a*) and *Period2* (*zPer2*), is transactivated by an acute light pulse [44–46]. These observations show that zebrafish cultured cells have the clock components required for a light-induced reset of circadian clock, therefore, providing a valuable tool for the study of general light-dependent regulation of cellular clocks.

Studies using zebrafish-cultured cells have contributed to identification of cellular signaling cascades involved in the light-dependent regulation of cellular clocks [47]. In several organisms, external stimuli are connected to a cell's nucleus via MAPK signaling pathways, such as p38 and extracellular signal-regulated kinase (ERK) [48]. Light has been reported to activate these signaling cascades in zebrafish cells (**Figure 2**) [49]. By a pharmacological approach, it has also been reported that the light-induced ERK activation triggers expression of *zPer2* and *zCry1a* genes, whereas the light-induced p38 activation suppresses it, highlighting a MAPK-mediated cross-regulatory mechanism of the expression of circadian clock genes [49, 50]. Importantly, an increased understanding of the light-dependent cellular clock regulation in zebrafish has suggested intriguing associations of the circadian clock with DNA repair and cellular DDR as described below.

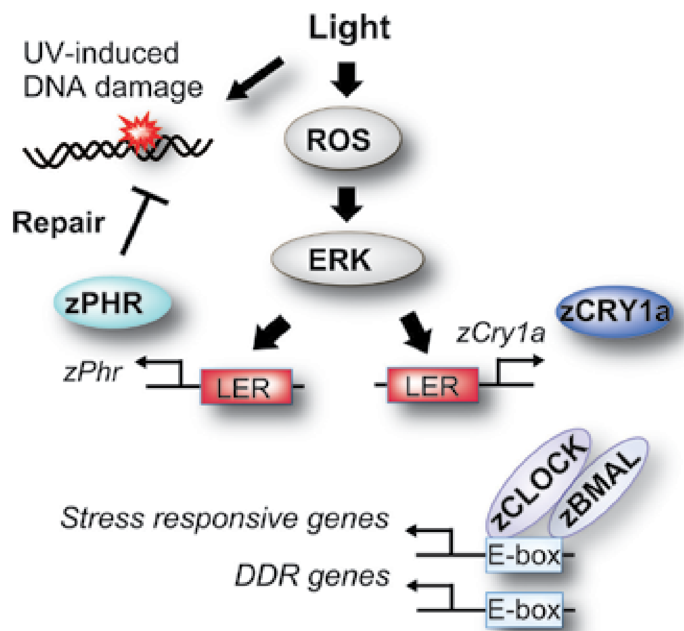


Figure 2.
A proposal model of light signaling pathways involved in shared control of the circadian clock and DNA repair in zebrafish. In a variety of organisms, light induces ROS production. In zebrafish cells, the light-induced ROS stimulate intracellular MAPK/ERK signaling pathway, which transduces photic signal to zCry1a expression. The light-induced zCRY1a interacts directly with the zCLOCK:zBMAL complex and modifies its transcriptional capacity. Notably, the zCLOCK:zBMAL complex regulates the transcription of a variety of genes involved in cellular stress responses and DDR. UV component of sunlight induces DNA damage. Light-induced ROS and activation of MAPK/ERK pathway also induce expression of a DNA repair enzyme, zPHR. The induced zPHR repairs UV-damaged DNA in a light-dependent manner.

7. Shared regulatory pathway for circadian clock and DNA repair in zebrafish

Although solar light has several beneficial uses, including the regulation of circadian clocks, the UV component of solar light is harmful to living cells because it produces cytotoxic and mutagenic lesions in DNA called cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts [(6-4) photoproducts] (**Figure 2**) [51, 52]. Natural selective pressure has forced the development of a self-defense system mediated by photoreactivation. Photoreactivation is the light-dependent DNA repair mechanism mediated by DNA photolyases (PHRs), which bind to and repair the UV-induced DNA damage using visible light as an energy source [53]. Two classes of PHRs have been identified, one specific for CPDs and the other specific for (6-4) photoproducts. Importantly, both the induction of PHRs in response to light and subsequent light-dependent repair of DNA by PHRs are essential for a successful photoreactivation in zebrafish cells [42, 54, 55]. Notably, the expression of the zebrafish *Phr* repairing (6-4) photoproduct (*z64Phr*) is regulated by the same light-induced MAPK cascades as those controlling the expression of the clock genes *zCry1a* and *zPer2* (**Figure 2**) [49]. The light-induced ERK activation triggers the expression of *z64Phr*, whereas the light-induced p38 activation inhibits it. Thus, the light-dependent DNA repair and regulation of the circadian clock are governed by shared regulatory pathways. Both CRYs and PHRs belong to the DNA photolyase/cryptochrome protein family and have highly similar amino acid sequences [42, 55, 56]. Evolutionary studies have shown that the animal CRY proteins functionally diverged first from the CPD photolyase and then further

to generate 64PHR [57]. These facts, together with the observation that *zCry1a* and *z64Phr* share regulatory pathways, strongly indicate an evolutionary link between the circadian clock and DNA repair. Importantly, evolutionary links functionally coupling the circadian clock and DNA repair also have been reported in other organisms. For example, *Neurospora* PRD-4, an orthologue of mammalian Chk2, transduces stress signals into the core circadian clock machinery, contributing the regulation of circadian clock [58]. Additionally, in the diatom *Phaeodactylum tricornutum*, *Phaeodactylum tricornutum* cryptochrome/photolyase family1 (PtCPF1), a novel cryptochrome/photolyase family member, not only repairs UV-induced DNA damage but also acts as a transcriptional repressor of the circadian clock [59].

8. Cellular responses to photooxidative stress are the candidate evolutionary origin of circadian clocks

Cellular reactive oxygen species (ROS) were originally thought to solely act as toxic metabolites because they react with components of DNA, proteins, and lipids and exert oxidative stress. However, ROS are also ideally suited as signaling molecules because they are small and can easily diffuse to short distances within a cell [60]. In addition, mechanisms for ROS production and the rapid removal (such as via catalase) are present in almost all cell types [61]. Much evidence has accumulated indicating significant roles of ROS in circadian clock controls that have resulted in the functional coupling of the circadian clock and DDR. For example, in *Drosophila*, a genome-wide screen identified several redox molecules as essential for the light entrainment of the circadian clock [62]. Similarly, a study in mammals showed that changes in reduced NADPH and NADH levels altered the affinity of the NPAS2:BMAL1 complex for its target DNA *in vitro* [63]. Thus, redox state may be an important determinant of circadian oscillations in mammalian cells. Nuclear factor erythroid-derived 2-like 2 (NRF2) is one of the components involved in the major cellular antioxidant defense pathways [64]. It induces a transcriptional program that maintains cellular redox balance and protects cells from oxidative insults. Importantly, it has been reported in mouse that cellular clock generates circadian rhythm in NRF2 level, which is essential in regulating the rhythmic expression of antioxidant genes involved in glutathione redox homeostasis in the lung [65].

In zebrafish, the transcriptional induction of *zCry1a* and *zPer2* genes has been proposed to be required for the light entrainment of cellular clocks [45, 66, 67]. The light-dependent transcription of *zCry1a* and *zPer2* is controlled through the production and removal of cellular ROS [66, 68]. The light-induced ROS stimulate the intracellular ERK signaling pathway and transduce photic signals to the transactivation of *zCry1a* and *zPer2* (**Figure 2**). Importantly, light increases the intracellular catalase activity by increasing the expression *of catalase*, an event that occurs after the maximum expression of the *zCry1a* and *zPer2* genes has been reached. This increased catalase activity diminishes the light-induced cellular ROS levels, resulting in decreased expression levels of *zCry1a* and *zPer2* genes. These findings provide evidence that ROS induced by light are the second messenger coupling photoreception to the entrainment of the circadian clock in zebrafish and further indicate that cellular responses to photooxidative stress would be the evolutionary origin of circadian clocks.

9. The light entrainment of the circadian clock in zebrafish would reflect a cellular response to photooxidative stress

It is conceivable that the development of circadian clocks is one way to segregate daytime from nighttime processes with light-dark cycles acting as selective forces

[69, 70]. In this scenario, increasing levels of oxygen-free radicals during the daytime may be a decisive factor in relegating the anabolic processes of mitosis, growth, and consolidation to the dark hours. Thus, it is reasonable that cellular signaling cascade mediated by ROS is utilized in the regulation of the circadian clocks and that common regulatory pathways mediate both cellular responses to photooxidative stress and the light-dependent regulation of the circadian clocks (**Figure 2**).

In addition to the photooxidative stress derived from sunlight, the UV component of it is major source of harm to organisms [51, 52]. In zebrafish, the light induces expression of PHRs which repair UV-damaged DNA in a light-dependent manner (**Figure 2**) [49, 71]. Importantly, this light induction of *DNA Phr* expression appears to be mediated by photooxidative stress [68, 72]. These observations are consistent with the idea that photooxidative stress may be utilized as a signal to activate DNA repair enzymes that can protect the organism's DNA from UV-induced damage. The fact that ROS, a well-known inducer of oxidative stress, can activate *zCry1a* transcription in zebrafish cells [66], together with the finding that *zCry1a* and *DNA Phr* are governed by shared light-induced signaling pathways [49], strongly suggests that, at least in zebrafish, the light entrainment of the circadian clock reflects a long-standing cellular response to photooxidative stress (**Figure 2**). The zCRY1a protein interacts directly with the CLOCK (NPAS2):BMAL complexes and regulates its transcriptional capacity [67, 73]. The complexes regulate a variety of key genes involved in cellular stress responses, DNA repair, and cell cycle regulation [14, 74]. Thus, the circadian clock protein zCRY1a may be the key integrator of oxidative stress that controls the core circadian machinery to regulate the transcription of genes responsible for DDR and cell cycle adjustments.

10. Conclusion

Many studies have identified a link between the circadian clock and tumorigenesis [8, 12]. The core of the circadian clock mechanism is the cell-autonomous and self-sustained transcriptional machinery called the cellular clock. Importantly, the cellular clocks have been reported to regulate transcription of tumor suppressors and cell cycle regulators [6, 12]. In addition, circadian proteins appear to play roles in cell cycle control, acting as tumor suppressors [9]. For example, it has been hypothesized that a core circadian regulator, CLOCK, directly interacts with key checkpoint proteins, leading to the acetylation of these proteins and thereby modulating their activities. In support of this idea, *Clock* mutant mice have been reported to be tumor-prone [9].

Cancer chronotherapy relies on the asynchrony that exists in cell proliferation and drug sensitivities between normal and malignant cells [8, 12]. The administration of cancer therapy based on circadian timing has had encouraging results, but still lacks a strong mechanistic foundation. Thus, identification of detailed molecular links between the circadian clocks and tumorigenesis will provide the functional basis of cancer chronotherapy.

Acknowledgements

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research [16K08521 and 18KT0068 (J.H.)]. This work was also supported by grant from Komatsu University (J.I. and J.H.).

Author details

Yoshimi Okamoto-Uchida[1], Junko Izawa[2] and Jun Hirayama[2]*

1 Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan

2 Department of Clinical Engineering, Faculty of Health Sciences, Komatsu University, Ishikawa, Japan

*Address all correspondence to: jun.hirayama@komatsu-u.ac.jp

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002;**418**(6901): 935-941. PubMed PMID: 12198538

[2] Okamura H. Clock genes in cell clocks: Roles, actions, and mysteries. Journal of Biological Rhythms. 2004;**19**(5):388-399. PubMed PMID: 15534319

[3] Dunlap JC. Molecular bases for circadian clocks. Cell. 1999;**96**(2):271-290. PubMed PMID: 9988221

[4] Hirayama J, Sassone-Corsi P. Structural and functional features of transcription factors controlling the circadian clock. Current Opinion in Genetics & Development. 2005;**15**(5):548-556. PubMed PMID: 16095901

[5] King DP, Takahashi JS. Molecular genetics of circadian rhythms in mammals. Annual Review of Neuroscience. 2000;**23**:713-742. PubMed PMID: 10845079

[6] Sahar S, Sassone-Corsi P. Metabolism and cancer: The circadian clock connection. Nature Reviews. Cancer. 2009;**9**(12):886-896. Epub 2009/11/26. DOI: 10.1038/nrc2747. PubMed PMID: 19935677

[7] Haus E, Smolensky MH. Biologic rhythms in the immune system. Chronobiology International. 1999;**16**(5):581-622. PubMed PMID: 10513884

[8] Fu L, Lee CC. The circadian clock: Pacemaker and tumour suppressor. Nature Reviews Cancer. 2003;**3**(5): 350-361. PubMed PMID: 12724733

[9] Uchida Y, Hirayama J, Nishina H. A common origin: Signaling similarities in the regulation of the circadian clock and DNA damage responses. Biological & Pharmaceutical Bulletin. 2010;**33**(4):535-544. PubMed PMID: 20410582

[10] Pando MP, Sassone-Corsi P. Signaling to the mammalian circadian clocks: In pursuit of the primary mammalian circadian photoreceptor. Science's STKE. 2001;**2001**(107):RE16. PubMed PMID: 11698692

[11] Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nature Reviews Genetics. 2017;**18**(3):164-179. DOI: 10.1038/nrg.2016.150. Epub 2016/12/19. PubMed PMID: 27990019. PubMed Central PMCID: PMCPMC5501165

[12] Fu L, Kettner NM. The circadian clock in cancer development and therapy. Progress in Molecular Biology and Translational Science. 2013;**119**:221-282. DOI: 10.1016/b978-0-12-396971-2.00009-9. Epub 2013/08/01. PubMed PMID: 23899600. PubMed Central PMCID: PMCPMC4103166

[13] Steinhart Z, Angers S. Wnt signaling in development and tissue homeostasis. Development. 2018;**145**(11). pii: dev146589. DOI: 10.1242/dev.146589. Epub 2018/06/10. PubMed PMID: 29884654

[14] Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, et al. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell. 2002;**109**(3):307-320. PubMed PMID: 12015981

[15] Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. Genes & Development. 2006;**20**(14):1868-1873. PubMed PMID: 16847346

[16] Wang Y, Sun N, Lu C, Bei Y, Qian R, Hua L. Upregulation of circadian gene 'hClock' contribution to metastasis of colorectal cancer. International Journal of Oncology. 2017;**50**(6):2191-2199. DOI: 10.3892/ijo.2017.3987. Epub 2017/05/13. PubMed PMID: 28498393

[17] Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. Molecular Cell. 2006;**22**(3):375-382. PubMed PMID: 16678109

[18] Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, et al. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell. 2008;**134**(2):329-340. PubMed PMID: 18662547

[19] Gery S, Virk RK, Chumakov K, Yu A, Koeffler HP. The clock gene Per2 links the circadian system to the estrogen receptor. Oncogene. 2007;**26**(57):7916-7920. PubMed PMID: 17599055

[20] Nader N, Chrousos GP, Kino T. Circadian rhythm transcription factor CLOCK regulates the transcriptional activity of the glucocorticoid receptor by acetylating its hinge region lysine cluster: Potential physiological implications. The FASEB Journal. 2009;**23**(5):1572-1583. PubMed PMID: 19141540

[21] Oklejewicz M, Destici E, Tamanini F, Hut RA, Janssens R, van der Horst GT. Phase resetting of the mammalian circadian clock by DNA damage. Current Biology. 2008;**18**(4):286-291. PubMed PMID: 18291650

[22] Ducasse M, Brown MA. Epigenetic aberrations and cancer. Molecular Cancer. 2006;**5**:60. DOI: 10.1186/1476-4598-5-60. Epub 2006/11/10. PubMed PMID: 17092350. PubMed Central PMCID: PMCPMC1636665

[23] Di Cerbo V, Schneider R. Cancers with wrong HATs: The impact of acetylation. Briefings in Functional Genomics. 2013;**12**(3):231-243. DOI: 10.1093/bfgp/els065. Epub 2013/01/18. PubMed PMID: 23325510

[24] Doi M, Hirayama J, Sassone-Corsi P. Circadian regulator CLOCK is a histone acetyltransferase. Cell. 2006;**125**(3):497-508. PubMed PMID: 16678094

[25] Hirayama J, Sahar S, Grimaldi B, Tamaru T, Takamatsu K, Nakahata Y, et al. CLOCK-mediated acetylation of BMAL1 controls circadian function. Nature. 2007;**450**(7172):1086-1090. PubMed PMID: 18075593

[26] Lin R, Mo Y, Zha H, Qu Z, Xie P, Zhu ZJ, et al. CLOCK acetylates ASS1 to drive circadian rhythm of ureagenesis. Molecular Cell. 2017;**68**(1):198-209.e6. DOI: 10.1016/j.molcel.2017.09.008. Epub 2017/10/07. PubMed PMID: 28985504

[27] Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. Science. 2003;**302**(5643):255-259. PubMed PMID: 12934012

[28] Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell. 2002;**111**(1):41-50. PubMed PMID: 12372299

[29] Laranjeiro R, Tamai TK, Peyric E, Krusche P, Ott S, Whitmore D. Cyclin-dependent kinase inhibitor p20 controls circadian cell-cycle timing. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(17):6835-6840. DOI: 10.1073/pnas.1217912110.

Epub 2013/04/10. PubMed PMID: 23569261. PubMed Central PMCID: PMCPMC3637774

[30] Cao Q, Gery S, Dashti A, Yin D, Zhou Y, Gu J, et al. A role for the clock gene per1 in prostate cancer. Cancer Research. 2009;**69**(19):7619-7625. PubMed PMID: 19752089

[31] Dekens MP, Santoriello C, Vallone D, Grassi G, Whitmore D, Foulkes NS. Light regulates the cell cycle in zebrafish. Current Biology. 2003;**13**(23):2051-2057. PubMed PMID: 14653994

[32] Idda ML, Kage E, Lopez-Olmeda JF, Mracek P, Foulkes NS, Vallone D. Circadian timing of injury-induced cell proliferation in zebrafish. PLoS One. 2012;**7**(3):e34203. DOI: 10.1371/journal.pone.0034203. Epub 2012/04/06. PubMed PMID: 22479565. PubMed Central PMCID: PMCPMC3315524

[33] Gallego M, Virshup DM. Post-translational modifications regulate the ticking of the circadian clock. Nature Reviews Molecular Cell Biology. 2007;**8**(2):139-148. PubMed PMID: 17245414

[34] Toh KL, Jones CR, He Y, Eide EJ, Hinz WA, Virshup DM, et al. An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. Science. 2001;**291**(5506):1040-1043. PubMed PMID: 11232563

[35] Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N, et al. Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature. 2005;**434**(7033):640-644. PubMed PMID: 15800623

[36] Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell. 2008;**134**(2):317-328. PubMed PMID: 18662546

[37] Maier B, Wendt S, Vanselow JT, Wallach T, Reischl S, Oehmke S, et al. A large-scale functional RNAi screen reveals a role for CK2 in the mammalian circadian clock. Genes & Development. 2009;**23**(6):708-718. PubMed PMID: 19299560

[38] Tamaru T, Hirayama J, Isojima Y, Nagai K, Norioka S, Takamatsu K, et al. CK2alpha phosphorylates BMAL1 to regulate the mammalian clock. Nature Structural & Molecular Biology. 2009;**16**(4):446-448. PubMed PMID: 19330005

[39] Tsuchiya Y, Akashi M, Matsuda M, Goto K, Miyata Y, Node K, et al. Involvement of the protein kinase CK2 in the regulation of mammalian circadian rhythms. Science Signaling. 2009;**2**(73):ra26. PubMed PMID: 19491384

[40] Gorospe M, de Cabo R. AsSIRTing the DNA damage response. Trends in Cell Biology. 2008;**18**(2):77-83. PubMed PMID: 18215521

[41] Meggio F, Pinna LA. One-thousand-and-one substrates of protein kinase CK2? The FASEB Journal. 2003;**17**(3):349-368. PubMed PMID: 12631575

[42] Sancar A. Cryptochrome: The second photoactive pigment in the eye and its role in circadian photoreception. Annual Review of Biochemistry. 2000;**69**:31-67. PubMed PMID: 10966452

[43] Whitmore D, Foulkes NS, Strahle U, Sassone-Corsi P. Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators. Nature Neuroscience. 1998;**1**(8):701-707. PubMed PMID: 10196586

[44] Whitmore D, Foulkes NS, Sassone-Corsi P. Light acts directly on organs and cells in culture to set the vertebrate circadian clock. Nature. 2000;**404**(6773):87-91. PubMed PMID: 10716448

[45] Pando MP, Pinchak AB, Cermakian N, Sassone-Corsi P. A cell-based system that recapitulates the dynamic light-dependent regulation of the vertebrate clock. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(18):10178-10183. PubMed PMID: 11517315

[46] Hirayama J, Cardone L, Doi M, Sassone-Corsi P. Common pathways in circadian and cell cycle clocks: Light-dependent activation of Fos/AP-1 in zebrafish controls CRY-1a and WEE-1. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(29):10194-10199. PubMed PMID: 16000406

[47] Okamoto-Uchida Y, Hirayama J. The zebrafish as an attractive animal model for the study of circadian clocks. In: Hayes N, editor. Circadian Clock: Regulations, Genetic and External Factors. New York, United States of America: Nova Science Publishers; 2018. pp. 103-127

[48] Huang C, Jacobson K, Schaller MD. MAP kinases and cell migration. Journal of Cell Science. 2004;**117**(Pt 20): 4619-4628. DOI: 10.1242/jcs.01481. Epub 2004/09/17. PubMed PMID: 15371522

[49] Hirayama J, Miyamura N, Uchida Y, Asaoka Y, Honda R, Sawanobori K, et al. Common light signaling pathways controlling DNA repair and circadian clock entrainment in zebrafish. Cell Cycle. 2009;**8**(17):2794-2801. PubMed PMID: 19652538

[50] Cermakian N, Pando MP, Thompson CL, Pinchak AB, Selby CP, Gutierrez L, et al. Light induction of a vertebrate clock gene involves signaling through blue-light receptors and MAP kinases. Current Biology. 2002;**12**(10):844-848. PubMed PMID: 12015122

[51] Brash DE. UV mutagenic photoproducts in *Escherichia coli* and human cells: A molecular genetics perspective on human skin cancer. Photochemistry and Photobiology. 1988;**48**(1):59-66. PubMed PMID: 3064116

[52] Mitchell DL, Nairn RS. The biology of the (6-4) photoproduct. Photochemistry and Photobiology. 1989;**49**(6):805-819. PubMed PMID: 2672059

[53] Kelner A. Photoreactivation of ultraviolet-irradiated *Escherichia coli*, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. Journal of Bacteriology. 1949;**58**(4):511-522. PubMed PMID: 18148831

[54] Todo T, Takemori H, Ryo H, Ihara M, Matsunaga T, Nikaido O, et al. A new photoreactivating enzyme that specifically repairs ultraviolet light-induced (6-4) photoproducts. Nature. 1993;**361**(6410):371-374. PubMed PMID: 8426655

[55] Todo T. Functional diversity of the DNA photolyase/blue light receptor family. Mutation Research. 1999;**434**(2):89-97. PubMed PMID: 10422537

[56] Todo T, Ryo H, Yamamoto K, Toh H, Inui T, Ayaki H, et al. Similarity among the Drosophila (6-4) photolyase, a human photolyase homolog, and the DNA photolyase-blue-light photoreceptor family. Science. 1996;**272**(5258):109-112. PubMed PMID: 8600518

[57] Cashmore AR, Jarillo JA, Wu YJ, Liu D. Cryptochromes: Blue light receptors

for plants and animals. Science. 1999;**284**(5415):760-765. PubMed PMID: 10221900

[58] Pregueiro AM, Liu Q, Baker CL, Dunlap JC, Loros JJ. The Neurospora checkpoint kinase 2: A regulatory link between the circadian and cell cycles. Science. 2006;**313**(5787):644-649. PubMed PMID: 16809488

[59] Coesel S, Mangogna M, Ishikawa T, Heijde M, Rogato A, Finazzi G, et al. Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Reports. 2009;**10**(6):655-661. PubMed PMID: 19424294

[60] Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany. 2002;**53**(372):1237-1247. PubMed PMID: 11997372

[61] Hancock JT, Desikan R, Neill SJ. Role of reactive oxygen species in cell signalling pathways. Biochemical Society Transactions. 2001;**29**(Pt 2):345-350. PubMed PMID: 11356180

[62] Sathyanarayanan S, Zheng X, Kumar S, Chen CH, Chen D, Hay B, et al. Identification of novel genes involved in light-dependent CRY degradation through a genome-wide RNAi screen. Genes & Development. 2008;**22**(11):1522-1533. PubMed PMID: 18519643

[63] Rutter J, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science. 2001;**293**(5529):510-514. PubMed PMID: 11441146

[64] Kang KW, Lee SJ, Kim SG. Molecular mechanism of nrf2 activation by oxidative stress. Antioxidants & Redox Signaling. 2005;**7**(11-12):1664-1673. DOI: 10.1089/ars.2005.7.1664. Epub 2005/12/17. PubMed PMID: 16356128

[65] Pekovic-Vaughan V, Gibbs J, Yoshitane H, Yang N, Pathiranage D, Guo B, et al. The circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant defense pathway to modulate pulmonary fibrosis. Genes & Development. 2014;**28**(6):548-560. DOI: 10.1101/gad.237081.113. Epub 2014/03/19. PubMed PMID: 24637114. PubMed Central PMCID: PMCPMC3967045

[66] Hirayama J, Cho S, Sassone-Corsi P. Circadian control by the reduction/oxidation pathway: Catalase represses light-dependent clock gene expression in the zebrafish. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(40):15747-15752. PubMed PMID: 17898172

[67] Tamai TK, Young LC, Whitmore D. Light signaling to the zebrafish circadian clock by Cryptochrome 1a. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(37):14712-14717. PubMed PMID: 17785416

[68] Osaki T, Uchida Y, Hirayama J, Nishina H. Diphenyleneiodonium chloride, an inhibitor of reduced nicotinamide adenine dinucleotide phosphate oxidase, suppresses light-dependent induction of clock and DNA repair genes in zebrafish. Biological & Pharmaceutical Bulletin. 2011;**34**(8):1343-1347. PubMed PMID: 21804230

[69] Tauber E, Last KS, Olive PJ, Kyriacou CP. Clock gene evolution and functional divergence. Journal of Biological Rhythms. 2004;**19**(5):445-458. PubMed PMID: 15534324

[70] Chen Z, McKnight SL. A conserved DNA damage response pathway

responsible for coupling the cell division cycle to the circadian and metabolic cycles. Cell Cycle. 2007;**6**(23):2906-2912. PubMed PMID: 18000400

[71] Tamai TK, Vardhanabhuti V, Foulkes NS, Whitmore D. Early embryonic light detection improves survival. Current Biology. 2004;**14**(3):R104-R105. PubMed PMID: 14986634

[72] Mitani H, Shima A. Induction of cyclobutane pyrimidine dimer photolyase in cultured fish cells by fluorescent light and oxygen stress. Photochemistry and Photobiology. 1995;**61**(4):373-377. PubMed PMID: 7740081

[73] Hirayama J, Nakamura H, Ishikawa T, Kobayashi Y, Todo T. Functional and structural analyses of cryptochrome. Vertebrate CRY regions responsible for interaction with the CLOCK:BMAL1 heterodimer and its nuclear localization. The Journal of Biological Chemistry. 2003;**278**(37):35620-35628. PubMed PMID: 12832412

[74] Gavriouchkina D, Fischer S, Ivacevic T, Stolte J, Benes V, Dekens MP. Thyrotroph embryonic factor regulates light-induced transcription of repair genes in zebrafish embryonic cells. PLoS One. 2010;**5**(9):e12542. PubMed PMID: 20830285

Section 3

Oncogenic Activation in Different Cancers

Chapter 3

MicroRNAs (miRNAs) in Colorectal Cancer

Burcin Baran, Nazli-Mert Ozupek, Gizem Calibasi-Kocal and Yasemin Basbinar

Abstract

Colorectal cancer (CRC) is the third most common cancer in the world and third leading cause of cancer-related deaths in men and women as well. While early screening procedures and removal of small polyps improve the survival rates among the patients, there is still need for new diagnostic and therapeutic approaches for developing more effective treatments. MicroRNAs (miRNAs) are short noncoding RNA fragments, which involve in posttranscriptional regulation of gene expression, and they are shown to involve in tumorigenesis either targeting oncogenes or tumor suppressor genes. Based on the current studies, miRNAs are now suggested as potential biomarkers for CRC diagnosis, prognosis, and therapeutic responses. In this chapter, the latest findings on the role of miRNA in CRC in many aspects are reviewed: diagnosis (role of circular miRNAs in blood and miRNAs from tissue biopsies and their potential role in pathophysiology and diagnosis of CRC), prognosis (miRNAs related with metastasis, recurrence, and survival rates in CRC), and therapeutic responses (role of miRNAs both in chemotherapies and/or in targeted therapies in CRC). In conclusion, miRNAs are promising molecules for diagnosis, prognosis, and therapeutic responses of CRC.

Keywords: colorectal cancer, diagnosis, miRNA, prognosis, therapeutic response

1. Introduction

MicroRNAs are a subgroup of small noncoding RNAs containing 18–25 nucleotides, and they do not carry any genetic information for protein expression. They regulate the posttranslational gene expression by binding 3′ untranslated region (UTR) of the target messenger RNA (mRNA). Approximately 30% of protein coding genes are regulated by miRNAs, and they have important roles in cellular functions including proliferation, differentiation, apoptosis, signaling, metabolism, and tumorigenesis. Due to their effect on crucial processes, miRNAs are significant modifiers of transcription and translation of both oncogenes and tumor suppressor proteins. Hence, some of them are classified as oncomiR and tumor suppressor miRNA in the cellular processes of tumor [1].

First miRNA, lin-4, was discovered in *Caenorhabditis elegans* in 1993, and it had role on the regulation of larval development by the repression of a nuclear protein encoded by *lin-14*. The second discovered miRNA, let-7, is expressed in late development and complementary to the 3′ UTR of the several genes including lin-14,

lin-28, lin-41, lin-42, and daf-12. After the discovery of lin-4 and let-7, miRNAs were shown in other organisms including plants and animals [2, 3], and over 10,000 miRNAs have been identified in various organisms. In humans, over 2500 types of encoded miRNAs have been determined [4].

2. Biogenesis of miRNA

The biogenesis of miRNA is a complicated process, starting in the nucleus, following with posttranslational modifications, and finalized in the cytoplasm. Similar to gene encoding, biogenesis of primary miRNAs (pri-miRNAs) is starting with the transcription by RNA polymerase II or RNA polymerase III enzyme. In the nucleus, pri-miRNA is recognized and cleaved by Drosha enzyme to form precursor miRNA (pre-miRNA). The pre-miRNA is exported to cytoplasm by exportin-5. In the cytoplasm, pre-miRNA is bound to cytoplasmic RNase Dicer and RNA-induced silencing complex (RISC), which is composed of argonaute 2 (AGO2) and transactivation response (TAR) RNA-binding protein (TRBP). Firstly, AGO2 cleaves the pre-miRNA from its 3′ end, and the cleaved pre-miRNA is further cleaved by Dicer into mature miRNA duplex. Mature miRNA duplex is then unwounded; while one strand of the miRNA remains on AGO2 protein, and the other strand (passenger strand) is degraded. Mostly, miRNAs are recognizing the complementary sequence of 3′ UTR of mRNAs, hence directing RISC to cleave mRNAs and translational repression of mRNAs [5, 6] (**Figure 1**).

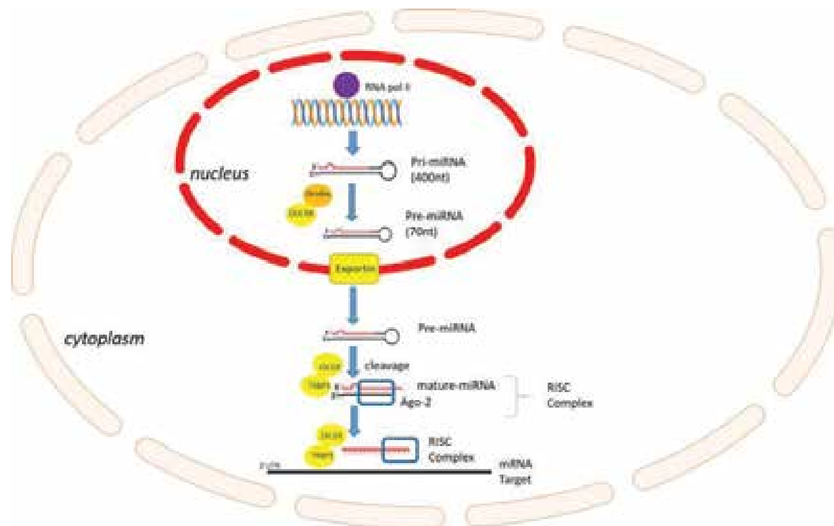


Figure 1.
miRNA biogenesis. The pathway starts miRNA transcription by RNA polymerase II or III to generate the primary transcripts (pri-miRNAs). Pri-miRNA is processed by the Drosha-DiGeorge syndrome critical region gene 8 (DGCR8, Pasha Pasha in Drosophila melanogaster and Caenorhabditis elegans) complex (also known as the microprocessor complex) that generates ~70 nucleotide (nt) pre-miRNAs. Pre-miRNA, which is recognized by the nuclear export factor exportin-5, is transferred to the cytoplasm. In the cytoplasm, the cytoplasmic RNase Dicer cleaves the pre-miRNA hairpin to its mature length. Dicer in complex with the transactivation response (TAR) RNA-binding protein (also known as TRBP and TARBP2) and argonaute (AGO) 1–4 mediate the processing of pre-miRNA and the assembly of the RISC (RNA-induced silencing complex). With the formation of this complex structure, one strand of the miRNA duplex is removed and single-stranded miRNA is generated. Interaction between microRNA complex and target mRNA induces post-transcriptional silencing by destabilization of mRNA and suppression of translation [7, 8].

3. Involvement of microRNAs in cancer

microRNA studies were began in *C. elegans*, as lin-4 and let-7 were identified as noncoding RNAs functioning in larval development. Soon after, the research groups focused on the function of these noncoding RNAs and discovered their homologs in vertebrates as well. The role of miRNAs in tumorigenesis was first reported in chronic lymphocytic leukemia (CLL) by two different groups in 2002. Hemizygous or homozygous loss of 13q14 chromosome was frequently observed among CLL patients [9]. Two different miR-15 and miR-16 expression levels were shown to be downregulated with the deletion of this locus [10]. Both miR-15/16 levels are inversely correlated with antiapoptotic B cell lymphoma-2 (Bcl-2) protein level in the cells. Introduction of miR-15/16 to the leukemic cell lines repressed Bcl-2 expression and induced apoptosis in these cells [11]. It is now very well established that aberrant miRNA expression contributes to cancer [12]. miRNAs are targeting the genes, which involve in cell proliferation, migration, invasion, and metastasis; hence dysregulation of these miRNAs leads to transformation and malignancy of cells [13, 14]. miRNA dysregulation in cancer cells can be result of genomic deletion, mutations, amplification, or epigenetic silencing [14]. A single miRNA can target a variety of mRNAs involved in different cell signaling pathways; interestingly, a single mRNA can be targeted by several miRNAs also [15], such as Let-7, which is one of the initially discovered miRNAs, targets human rat sarcoma (RAS), high-mobility group AT-hook 2 (HMGA2), and MYC mRNAs and downregulates their expression [16]. Phosphotensin homolog (PTEN), which is an important regulator of cell cycle, can be targeted by several different miRNAs including miR-21, miR-22, miR-106b-25, miR-17-92 [17].

In tumorigenesis, miRNAs either act as tumor suppressor or as an oncogene; interestingly, their expression is repressed or induced by transcription factors such as p53 or MYC via their promoter regions. miR-145 is one of the initial examples of tumor suppressor miRNAs. miR-145 was found to be downregulated in a variety of tumors including colon, breast carcinomas [18, 19]. It is interesting that tumor suppressor protein p53 induces miR-145 expression via p53 response element in its promoter. Later, miR-145 targets c-Myc or insulin receptor substrate I (IGF-R1) protooncogenes and silences their expressions, hence preventing tumor cell proliferation [18, 20] . Furthermore miR-145 inhibits invasion and metastasis by targeting Fli-1 or Mucin-1 [20, 21]. miR-145 also targets estrogen receptor-α (ER-α) via its two complementary sites and downregulates ER-α expression [22]. miR-34 family is another target of p53 tumor suppressor protein [23]. Another important tumor suppressor miRNA is miR-34 family. miR-34 family comprises three members: miR-34a, miR-34b, and miR-34c. While miR-34a is ubiquitously expressed in every tissue, expression of miR-34b and miR-34c is restricted to fallopian tubes, lungs, and brain [24, 25]. miR-34a is a very potential tumor suppressor since it is targeting many mRNAs related with proliferation [such as cyclin-dependent kinase-4 (CDK4) and cyclin-dependent kinase-6 (CDK6)], cellular growth [such as Notch2, platelet-derived growth factor receptor A (PDGFRA)], antiapoptosis [Bcl-2, sirtuin 1 (SIRT1), survivin], invasion, and migration [MET, SNAIL, cluster of differentiation (CD44)] [26–28]. Downregulation of miR-34 is observed among many malignancies and associated with poor prognosis [29, 30]. As a result of its role as a tumor suppressor, miR-34 has been applied either alone or in combination with conventional therapies on several tumor cell lines and mouse tumor models and showed promising results [31–34]. miR-34 was first miRNA tested in human Phase I trial (NCT01829971). MRX34, liposomal miR-34 mimic, was tested among patients with

solid advanced tumors. While MRX34 treatment showed evidence of antitumor activity in a subset of patients, it exerts some toxicities in patients. Hence, there is need for further studies for improving tolerability among the patients [35, 36].

In addition to tumor suppressor miRNAs, miRNAs behave like oncogenes, called as "oncomiRs." mir-21 is the first miRNA identified as oncogenic; it is significantly upregulated in many tumors including colon cancer, breast cancer, hepatocellular carcinoma, and glioblastoma [37]. miR-21 overexpression contributes to cell proliferation and antiapoptotic responses by targeting important downstream proteins such as phosphotensin homolog (PTEN), programmed cell death protein 4 (PDC4), and tropomyosin I [38–40]. Besides this, miR-21 was shown to be bona fide oncogene by causing pre-B-cell lymphoma in mouse models by overexpression. When mir-21 expression was inactivated, tumors regressed completely in few days [41].

As the importance of miRNAs became evident, miRNA expression profiles for each tumor type have been studied with several methodologies including microarray, QRT-PCR, and next-generation sequencing [15, 42]. miRNA expression profiles can reflect embryonic or development origin of the tissue and able to classify the origin of tissue with high accuracy (>90%), even separate cell subtypes (stem cells vs. progenitor cells) in the same tissue [43–45]. These miRNA profiling studies open the way for biomarker studies. In the biomarker studies, it is aimed to find diagnostic, prognostic, and predictive markers for better characterization of the disease and therapy response as an outcome [46].

4. miRNA and colorectal cancer

Colorectal cancer (CRC) is the second most common cancer among the women and third most common cancer among men. In 2016, more than 1.4 million men and women in the USA have been diagnosed with CRC [47]. Despite the availability of successful treatment options such as surgery, chemotherapy, and radiotherapy, the prognosis of CRC is not promising. Relapse or metastatic spread occurs after surgery in many CRC patients. Colorectal cancer is divided into two phenotypes according to mutational status. In chromosomal instability phenotype (CIN), high rate of inactivating mutations in adenomatous polyposis coli (*APC*) and tumor protein P53 (*TP53*) genes are found as well as activating mutations in Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene. However mutations in DNA repair genes, transforming growth factor-beta receptor II *(TGFBRII) gene, Bcl2- associated C protein (BAX) and BRAF* genes are commonly existed in microsatellite instability-high tumors (MSI-H) [48]. Certainly, genomic background affects the miRNA expression in CRC, such as *TP53* mutations affect miR-145 expression levels, which is downregulated among many CRC patients [49, 50]. Furthermore, miR-193a-3p expression was found as specifically downregulated in *BRAF*-mutated CRC cases [51]. The distinction between these phenotypes became more prominent in disease progression and therapy response, which will be discussed in the following sections. In CRC, to date, totally, 1870 original studies were retrieved in PubMed (as of May 2018), in which 38 of them were clinical trials investigating miRNA expression patterns in both CRC tissue specimen and plasma samples and compared them with normal samples. Bunch of miRNAs were found to be dysregulated in CRC samples in these studies [52–54]. While some of these miRNAs are related with early stages of tumorigenesis and can be used as diagnostic markers, the others are associated with therapeutic response, resistance to chemotherapy, and survival prognosis, hence aiding the physician in making therapeutic decisions as prognostic and predictive biomarkers [55].

4.1 miRNAs in colorectal cancer diagnosis

Early diagnosis is essential for CRC patients since they have more favorable prognosis. Fecal blood test and colonoscopy techniques are being currently used for early screening. However, fecal blood tests are not very efficient for detecting early carcinoma formation. Colonoscopy is a gold standard technique, it reduces cancer risk about 30–75%, yet it is invasive and expensive technique and highly uncomfortable for a patient [56]. Therefore, noninvasive and inexpensive screening and diagnostic methods or biomarkers are needed. miRNAs are promising candidates for noninvasive biomarker diagnosis. Diagnostic miRNAs can be isolated from blood or stool samples as well as tumor tissues [57] (**Table 1**).

There are different miRNA profiling studies comparing CRC samples with normal healthy tissue samples; however, each study emphasized on different set of miRNAs in CRC diagnosis and progression. According to miRNA profile study, miR-18a, -20a, -21, -29a, -92a, -106b, -133a, -143, and -145 expression levels were found to be significantly changed in CRC patients when compared with normal patients, and these markers can be used for CRC diagnosis [59]. In a systematic review, miR-106a, -30a-3p, -139, -145, -125a, and -133a were proposed as diagnostic biomarkers [60]. In another study, miR-143, -145, -21, -320, -126, -484-5p, -143, -145, -16, -125b, -21, and -106 were found to be candidate for diagnostic biomarkers [57]. While studies share some common miRNAs (such as miR143, miR145, miR106, miR21), they are differing in their list of miRNAs. In fact, the type of miRNAs can be differed due to the type of sample (blood or stool), experimental procedures, and used microRNA platforms. Another handicap of these studies is that they have been conducted with a small number of samples. Larger sample studies and additional meta-analyses are need for better determination of CRC-related diagnostic markers. Still, it can be said that miRNAs are very promising noninvasive markers for tumor diagnosis.

4.2 miRNAs in colorectal cancer prognosis

Taking part in CRC diagnosis, miRNAs are also affecting prognosis and therapeutic response. As mentioned before, the expression and deregulation of miRNAs in CRC patients are affected by chromosomal abnormalities and microsatellite instability [61, 62]. In CRC, miRNA expression dysregulation is shown especially in microsatellite instability (MSI-high) tumors. MSI-high groups are distinct population among CRC patients, which accounts for 15% of all cases, observed in hereditary cases such as Lynch syndrome or in sporadic cases mostly as a result of hypermethylation or inactivation of mismatch repair (MMR) genes [63]. These MSI tumors characterized by distinct behavior are associated with proximal tumor localization and high infiltration of lymphocytes. These phenotypes showed less distant organ metastasis than MSI stable tumors and have better prognosis [64]. Several miRNAs have been shown in participating in inactivation of several DNA mismatch repair genes, such as miR-155 downregulates mutL protein homolog 1 (MLH1), mutS homolog 2 (MSH2), and mutS homolog 6 (MSH6) mRNAs expression, whereas miR-21 targets MSH2 and MSH6 mRNA and inactivates them [65, 66]. Overall 94 miRNAs are differently expressed in microsatellite stable and in microsatellite instable tumors [67]. Upregulation (miR-17, miR-20, miR-25, miR-31, miR92, miR-93, miR-133b, miR-135a, miR-183, miR-203, and miR-223) and downregulation (miR-16, miR-26b, miR-143, miR-145, miR-191, miR-192, miR-215, and let-7a) are generally observed in MSI-high tumors [68]. miRNA expression is also differed among *TP53* and *KRAS* mutated tumors as well. miR-125p targets 3′ UTR region of p53 and represses p53 expression and accelerates the tumor growth;

miRNAs	Expression	Target genes
miR-15a	Upregulate	*Bcl-2*
miR-17-3p	Upregulate	*E2F*, *CDKN1A*
miR-18a	Upregulate	*SMAD4*, *KRAS*
miR-19a/miR-19b	Upregulate	*PTEN*
miR-20a	Upregulate	*BECN1*, *ATG16L1*, *SQSTM1*
miR-21	Upregulate	*PDCD4*, *PTEN*, *SPRY2*, *TPMI*
miR-24	Downregulate	*Topoisomerase-1*
miR-29a	Upregulate	*DNMT3*
miR-31	Downregulate	*WNT*, *β-catenin*
miR-34a	Downregulate	*SMAD4*, *FRAT1*, *Bcl-2*, *c-Met*
miR-92a	Upregulate	*PHLPP2*, *VHL*, *Bim*
Let-7g	Upregulate	*KRAS*, *Cdk6*, *Cdc25*, *HMGA2*
miR-106b	Upregulate	*P21*, *E2F1*
miR-133a	Downregulate	*MCL1*, *BCL2L2*
miR-143	Downregulate	*Erk5*, *DNMT3*, *KRAS*
miR-145	Downregulate	*EGFR*, *IRS-1*
miR-181b	Downregulate	*ATM*
miR-203	Downregulate	*ABL1*, *TP63*
miR-223	Upregulate	*STMN1*
miR-302	Upregulate	*GAB2*, *AKT2*
miR-320a	Downregulate	*VDAC*, *STAT3*, *SOX4*
miR-335	Upregulate	*RASA-1*
miR-375	Downregulate	*SLC7A11*, *IGFR1*, *SEC23A*
miR-422a	Downregulate	*TGF-β*, *CD73*
miR-423-5p	Downregulate	*RFVT3*
miR-601	Downregulate	*PTP4A1*
miR-760	Downregulate	*PHLPP2*

Abbreviations: Bcl-2, B cell lymphoma-2; E2F, E2F transcription factor 1; CDKN1A, cyclin-dependent kinase inhibitor 1A; KRAS, Kirsten rat sarcoma viral oncogene homolog; PTEN, phosphotensin homolog; BECN1, Beclin 1; ATG16L1, autophagy-related 16 like 1; SQSTM1, sequestosome 1; PDCD4, programmed cell death 4; SPRY2, sprouty RTK signaling antagonist 2; DNMT3, DNA methyl transferase 3; FRAT1, WNT signaling pathway regulator; PHLPP2, PH domain leucine-rich repeat protein phosphatase 2; VHL, von Hippel-Lindau tumor suppressor; Cdk6, cyclin-dependent kinase 6; Cdc25, cell division cycle 25A; HMGA2, high-mobility group gene; P21, CDKN1A, cyclin-dependent kinase inhibitor 1A; E2F1, E2F transcription factor 1; MCL1, BCL2 family apoptosis regulator; BCL2L2, BCL2 like 2; EGFR, epidermal growth factor receptor; IRS-1, insulin receptor substrate 1; ATM, ataxia telangiectasia mutated; ABL1, v-abl Abelson murine leukemia viral oncogene homolog 1; TP63, tumor protein p63; STMN1, stathmin 1; GAB2, GRB2-associated binding protein 2; AKT2, v-akt murine thymoma viral oncogene homolog 2; VDAC, voltage-dependent anion channel; SOX4, SRY (sex-determining region Y)-box 4; SLC7A11, solute carrier family 7 member 11; IGFR1, insulin-like growth factor 1 receptor; TGF-β, transforming growth factor-beta; CD73, cluster of differentiation 73; RFVT3, known as SLC52A3 (solute carrier family 52 member 3); PTP4A1, protein tyrosine phosphatase 4a1.

Table 1.
Simplified list of diagnostic miRNA markers for colorectal cancer (modified from Refs. [58, 59]).

hence, expression levels of miR-125p are associated with poor survival among CRC patients [69]. However, miR34 expression is a good prognostic marker. miR-34 is one of the targets of p53 protein and it increases miRNA expression. miR-34 then suppresses the expression of WNT pathway and epithelial mesenchymal transition (EMT)-related genes. Increase of miR-34b and miR-34c levels in stromal tissue is

leading to poor prognosis in colon cancer [70–72]. miR-122, miR-214, miR-372, miR-15b, let-7e, and miR-17 are other dysregulated miRNAs found in *TP53* mutated tumors [73]. miR-148-b and miR-221 are also important diagnostic markers associated with *p53* mutational status, and their overexpression is associated with worse prognosis [74, 75]. miR-143 and miR-145 are frequently downregulated in CRC and their one of the targets is KRAS mRNA; hence, they are important prognostic and predictive biomarkers in CRC [76, 77]. Let-7 role is one of the well-studied tumor suppressor miRNAs, which targets RAS. Let-7a expression is higher in *KRAS* mutated metastatic samples than normal mucosa or nonmetastatic disease [78]. Decrease Let-7b expression is worse prognostic marker, which is associated with recurrence and low overall survival of patients [79]. Furthermore, decrease in miR-487b levels is associated with liver metastasis in CRC patients [80]. Not only KRAS-associated miRNAs act as tumor suppressor, some of them are acting oncogenic in prognosis. miR-200 and miR-221 are downstream miRNAs of RAS pathway, and high expression of these miRNAs is related with worse prognosis [81].

Furthermore, exosome-containing miRNAs (miR-17/92 cluster and miR-19a cluster) are evaluated as biomarkers for early diagnosis and high recurrence in patients with CRC [82]. miR-21-5p, miR-29-3p, and miR-148-3p levels were studied in CRC samples and show that dysregulation in these miRNAs is associated with high mortality risk [83].

4.3 miRNAs in treatment response prediction of colorectal cancer

A variety of therapeutic advances are existed for CRC treatment such as conventional chemotherapy (5-fluorouracil, capecitabine, irinotecan, oxaliplatin), immunotherapy, radiotherapy, and chemoradiotherapy. miRNAs play an important role in the regulation of effectiveness and resistance to these therapies and prediction of personalized therapy response [84, 85]. Resistance to therapy is still the biggest challenge for defeating cancer. It may be caused by a variety of reasons such as reduction in transportation and intracellular accumulation of drugs by modulating the activity of drug transporters such ATP-binding cassette subfamily B (ABCB)/multidrug resistance (MDR) transporters (which is reviewed in reference [86]), dysregulation in DNA damage repair mechanisms, insufficient or oncogenic immune response, blockage of apoptosis, emergence of inflammation, and altered expression of oncogenes and tumor suppressor genes related with therapy response. miRNAs are actively participating in all of these resistance mechanisms [87, 88].

4.3.1 Chemotherapy

Although there are advances in cytotoxic and targeted therapy in CRC, drug resistance is one of the most important obstacles in front of successful chemotherapy [89]. Fluoropyrimidine-based chemotherapy (5-FU or capecitabine), vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR)-targeted, and epidermal growth factor receptor (EGFR)-targeted therapies are the main therapeutic methods for CRC [87]. miRNAs have role in chemotherapy resistance in terms of deregulation of drug metabolism-related enzymes, increased efflux of chemotherapeutics, impairment of chemotherapeutic-induced apoptosis, modulation of DNA damage repair, and autophagy [87].

miR-92b-3p, miR-3156-5p, miR-10a-5p, and miR-125a-5 were found to be related with progression-free survival in meta;static CRC patients treated with 5-FU/oxaliplatin/bevacizumab regime [90]. A negative relationship was found between miR-27b, miR-148a, and miR-326 expression levels and progression-free

survival in metastatic colorectal cancer patients receiving first-line oxaliplatin-based treatment [91]. The expression of miR-326 was related with decreased overall survival. These results proposed that plasma miRNAs can be used as noninvasive biomarkers for evaluating drug response in metastatic CRC patients who are treated with 5-FU and oxaliplatin-based chemotherapy [91] (**Table 2**).

4.3.2 Immunotherapy

Since chemo/radio therapies and surgery have limitations, immunotherapy is a good alternative to treat CRC patients. Immunotherapy aimed to evoke immune system to eliminate tumors either using immune stimulatory cytokines (vaccines, etc.) or checkpoint inhibitors [such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) receptor, and its ligands (PD-L1/2)] [92]. Interestingly, immune cell filtrates more in MSI-high CRC, and these subtypes are responding better to immunotherapies [93].

miRNAs	Therapy	Expression	Target genes
miR-7	EGFR-targeted	Downregulate	*EGFR*, *RAF-1*
miR-10b	5-FU	Upregulate	*BIM*
miR-20a	Oxaliplatin	Upregulate	*BNIP2*
miR-21	5-FU	Upregulate	*MSH2*
miR-22	5-FU	Downregulate	*BTG-1*
miR-23a	5-FU	Upregulate	*APAF-1*, *ABCF-1*
miR-27a, miR-27b	5-FU	Downregulate	*DPYD*
miR-133b	EGFR-targeted	Downregulate	*EGFR*
miR-139-5p	5-FU	Downregulate	*Bcl-2*
miR-143	Oxaliplatin	Downregulate	*IGF-1R*
miR-153	Oxaliplatin	Upregulate	*FOXO3a*
miR-199-5p, miR-375	EGFR	Upregulate	*PHLPP1*
miR-203	5-FU	Downregulate	*TYMS*
miR-203	Oxaliplatin	Upregulate	*ATM*
miR-204	5-FU	Downregulate	*HMGA2*
miR-218	5-FU	Downregulate	*TYMS*, *BIRC5*
miR-302, miR-369, miR-200c	5-FU	Upregulate	*MRP8*
miR-409-3p	Oxaliplatin	Downregulate	*Beclin-1*
miR-425-5p	5-FU	Upregulate	*PDCDIO*
miR-494	5-FU	Downregulate	*DPYD*
miR-519c	5-FU	Downregulate	*ABCG2*, *HuR*
miR-520g	Oxaliplatin	Upregulate	*P21*

Abbreviations: 5-FU, 5-fluorouracil; EGFR, epidermal growth factor; RAF-1, Raf protooncogene; BNIP2, BCL2-interacting protein 2; MSH2, human mutS homolog 2; BTG-1, BTG antiproliferation factor 1; APAF-1, apoptotic peptidase-activating factor 1; ABCF-1, ATP-binding cassette subfamily D member 1; DPYD, dihydropyrimidine dehydrogenase; Bcl-2, B cell lymphoma-2; IGF-1R, insulin-like growth factor 1 receptor; FOXO3a, forkhead box class O3; PHLPP1, Phlpp1 PH domain and leucine-rich repeat protein; TYMS, thymidylate synthase; ATM, ataxia telangiectasia mutated; HMGA2, high mobility group AT-hook 2; BIRC5, baculoviral IAP repeat containing 5; MRP8, myeloid-related protein 8; ABCG2, ATP-binding cassette subfamily G member 2; P21, cyclin-dependent kinase inhibitor 1A.

Table 2.
The expression profile of miRNAs that have role on chemotherapy response in colorectal cancer (modified from Ref. [85]).

miRNAs are essential in regulation of the immune response as well. The role of miR-34 has been mentioned earlier. Upregulation of miR-34a elicits the activation of tumor-infiltrating $CD8^+$ T cells by targeting PD-L1 [94]. miRNAs are also involved in innate immunity by macrophages and NK cells, and adaptive immunity by B cells, T cells, and dendritic cells. miR-124 modulates signal transducer and activator of transcription 3 (STAT3) pathway and enhances the T cell-mediated immune clearance [95]. miR-491 regulates the proliferation and apoptosis of $CD8^+$ T cells [96]. miR-491 inhibits the activation of $CD8^+$ T cells and promotes its apoptosis via targeting B-cell lymphoma-extra-large (Bcl-xL), cyclin-dependent kinase-4 (CDK4), and T cell factor 1 (TCF1), hence aiding tumor cells escaping from immune system. Tumor-derived TGF-β also induces the miR-491 expression. Thus, miR-491 can be evaluated as a new immunotarget for CRC treatment [96].

miR-196b, miR-378a, and miR-486-5p are evaluated as predictive biomarkers for the efficacy of the vaccine treatment in CRC [97]. miRNAs were enrolled in Phase II studies. In 16 patients, high expression of miR-196b-5p and low expression of miR-378a-3p and miR-486-5p are associated with better prognosis after vaccine treatment. Hence, these miRNAs can be determined as novel biomarkers for prediction of outcome responses of patients [97].

4.3.3 Potential candidates

miRNAs are also involving in radiotherapy responses. The expression of miRNA-processing enzymes Drosha and Dicer was found to be upregulated in radioresistant cell lines when compared with radiosensitive cell lines [98]. The role of miRNAs in radiotherapy response was evaluated further in the study cited as reference [87]. In the study, biomarkers for the prediction of chemoradiotherapy response in CRC were identified by using integrative and systematic bioinformatics analysis. The unique target genes of miR-198 and miR-765 were altered significantly upon transfection of specific miRNA mimics in the radiosensitive cell line. Thus, it could be said that miR-198, miR-202, miR-371-5p, miR-513a-5p, miR-575, miR-630, and miR-765 could be used for predicting the response of CRC to preoperative chemo-radiotherapy [87]. Still, further studies are needed to understand the miRNA role in radiotherapy/radiochemotherapy prediction.

5. Concluding remarks and limitations

By the discovery of miRNAs, a significant number of studies have been conducted to indicate the utility of miRNAs. According to the highlighted studies, miRNAs in body fluids have potential to be predictive, diagnostic or prognostic biomarkers; and also they can be therapeutic targets due to their inducer ability on tumorigenesis. Basically, miRNAs offer promising practice for screening, diagnosis, prognosis, and treatment of cancer. Therefore, these noncoding RNA fragments may be used alone or combined with other protocols to screen, diagnose, prognose, and treat cancer. However, their clinical importance is still not conclusive, and validation studies are needed for routine-based clinical application.

Evidences showed that inhibition of oncomiRs or replacement of tumor suppressive miRNAs could be used to develop innovative treatment approaches. Further studies are needed to reveal the molecular mechanisms on the regulation of miRNA biogenesis. Determination of miRNA target genes, molecular interactions between target mRNA and miRNAs, and signaling pathways will help to interpret molecular mechanisms of cancer. Besides investigations on miRNA expression patterns and

their molecular mechanisms, studies on technological developments for reliable and cost-effective miRNA applications are also extremely important to enhance minimally invasive routine miRNA applications. Methodological variability among different clinical centers is the biggest limitation for the successful combination of miRNAs in cancer management. Standardization and normalization of essential steps of miRNA applications, such as miRNA extraction, processing, biobanking, and quantitation, eliminate the clinical facility-based variations. Using internal controls and enrollment of the laboratory accreditation/validation programs may present benefits for standardization. miRNAs have potential to be therapeutic targets and treatment options. But determination of mRNAs and miRNAs interactions and obtaining the large population-based multicenter cohorts are essential to use miRNAs in therapy. Especially before the implementation of miRNAs in clinics, evaluation of miRNA panels on large patient cohorts must be achieved.

Author details

Burcin Baran[1,2†], Nazli-Mert Ozupek[1,2†], Gizem Calibasi-Kocal[1] and Yasemin Basbinar[1,3*]

1 Department of Basic Oncology, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey

2 Institute of Health Sciences, Dokuz Eylul University, Izmir, Turkey

3 Personalized Medicine and Pharmacogenomics Research Center, Dokuz Eylul University, Izmir, Turkey

† These authors contributed equally.

*Address all correspondence to: yasemin.baskin@deu.edu.tr

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Vannini I, Fanini F, Fabbri M. Emerging roles of microRNAs in cancer. Current Opinion in Genetics & Development. 2018;**48**:128-133. DOI: 10.1016/j.gde.2018.01.001

[2] Lee RC, Feinabum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;**75**:843-854. DOI: 10.1016/0092-8674(93)90529-Y

[3] Harfe BD. MicroRNAs in vertebrate development. Current Opinion in Genetics & Development. 2005;**15**:410-415. DOI: 10.1016/j.gde.2005.06.012

[4] Huang Y, Shen XJ, Zou Q, Wang SP, Tang SM, Zhang GZ. Biological functions of microRNAs: A review. Journal of Physiology and Biochemistry. 2011;**67**(1):129-139. DOI: 10.1007/s13105-010-0050-6

[5] Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: Synthesis, mechanism, function and recent clinical trials. Biochimica et Biophysica Acta. 2010;**1803**:1231-1243. DOI: 10.1016/j.bbamcr.2010.06.013

[6] Garzon R, Fabbri M, Cimmino A, et al. MicroRNA expression and function in cancer. Trends in Molecular Medicine. 2006;**12**:580-587. DOI: 10.1016/j.molmed.2006.10.006

[7] Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nature Cell Biology. 2009;**11**(3):228-234. DOI: 10.1038/ncb0309-228

[8] He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nature Reviews. Genetics. 2004;**5**(7):522-531. Review. Erratum in: Nat Rev Genet. 2004 Aug;5(8):631. DOI: 10.1038/nrg1379

[9] Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. The New England Journal of Medicine. 2000;**343**:1910-1916. DOI: 10.1056/NEJM200012283432602

[10] Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. PNAS. 2002;**99**:15524-15529. DOI: 10.1073/pnas.242606799

[11] Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. PNAS. 2005;**102**(39):13944-13949. DOI: 10.1073/pnas.0506654102

[12] Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors. Oncogene. 2006;**25**:6188-6196. DOI: 10.1038/sj.onc.1209913

[13] Price C, Chen J. MicroRNAs in cancer biology and therapy: Current status and perspectives. Genes & Diseases. 2014;**1**(1):53-63. DOI: 10.1016/j.gendis.2014.06.004

[14] Garzon R, Calin G, Croce CA. MicroRNAs in cancer. Annual Review of Medicine. 2009;**60**: 167-179. DOI: 10.1146/annurev.med.59.053006.104707

[15] Hayes J, Peruzzi P, Lawler S. MicroRNAs in cancer: Biomarkers, functions and therapy. Trends in Molecular Medicine. 2014;**20**(8): 460-469. DOI: 10.1016/j.molmed.2014.06.005

[16] Lee H, Han S, Kwon CS, Lee D. Biogenesis and regulation of the

let-7 miRNAs and their functional implications. Protein & Cell. 2016;**7**(2):100-113. DOI: 10.1007/s13238-015-0212-y

[17] Bermúdez Brito M, Goulielmaki E, Papakonstanti EA. Focus on PTEN regulation. Frontiers in Oncology. 2015;**5**:166. DOI: 10.3389/fonc.2015.00166

[18] Shi B, Sepp-Lorenzino L, Prisco M, Linsley P, de Angelis T, Baserga R. Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. The Journal of Biological Chemistry. 2007;**282**: 32582-32590. DOI: 10.1074/jbc.M702806200

[19] Wang S, Bian C, Yang Z, Bo Y, Li J, Zeng L, et al. miR-145 inhibits breast cancer cell growth through RTKN. International Journal of Oncology. 2009;**34**:1461-1466. DOI: 10.3892/ijo_00000275

[20] Sachdeva M, Yuan MY. MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. Cancer Research. 2010;**70**(1):378-387. DOI: 10.1158/0008-5472.CAN-09-2021

[21] Larsson E, Fuchs PF, Heldin J, et al. Discovery of microvascular miRNAs using public gene expression data: miR-145 is expressed in pericytes and is a regulator of Fli1. Genome Medicine. 2009;**1**(11):108. DOI: 10.1186/gm108

[22] Spizzo R, Nicoloso MS, Lupini L, et al. miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-α in human breast cancer cells. Cell Death and Differentiation. 2010;**17**(2):246-254. DOI: 10.1038/cdd.2009.117

[23] Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Molecular Cell. 2007;**26**(5):745-752. DOI: 10.1016/j.molcel.2007.05.010

[24] Slabáková E, Culig Z, Remšík J, Souček K. Alternative mechanisms of miR-34a regulation in cancer. Cell Death & Disease. 2017;**8**:e3100. DOI: 10.1038/cddis.2017.495

[25] Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. 2007;**8**:166. DOI: 10.1186/1471-2164-8-166

[26] Agostini M, Knight RA. miR-34: From bench to bedside. Oncotarget. 2014;**5**(4):872-881. DOI: 10.18632/oncotarget.1825

[27] Bommer GT, Gerin I, Feng Y, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Current Biology. 2007;**17**:1298-1307. DOI: 10.1016/j.cub.2007.06.068

[28] Rokavec M, Li H, Jiang L, Hermeking H. The p53/miR-34 axis in development and disease. Journal of Molecular Cell Biology. 2014;**6**(3): 214-230. DOI: 10.1093/jmcb/mju003

[29] Vogt M, Munding J, Grüner M, et al. Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. Virchows Archiv. 2011;**458**(3):313-322. DOI: 10.1007/s00428-010-1030-5

[30] Lodygin D, Tarasov V, Epanchintsev A, et al. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. Cell Cycle. 2008;**7**(16):2591-2600. DOI: 10.416/cc.7.16.6533

[31] Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. Oncogene.

2007;**26**(34):5017-5022. DOI: 10.1038/sj.onc.1210293

[32] Ji Q, Hao X, Zhang M, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PLoS One. 2009;**4**:e6816. DOI: 10.1371/journal.pone.000681

[33] Wiggins JF, Ruffino L, Kelnar K, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Research. 2010;**70**(14):5923-5930. DOI: 10.1158/0008-5472.CAN-10-0655

[34] Zarone MR, Misso G, Grimaldi A, et al. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Scientific Reports. 2017;7:17949. DOI: 10.1038/s41598-017-18186-0

[35] Bader AG. miR-34—A microRNA replacement therapy is headed to the clinic. Frontiers in Genetics. 2012;**3**:120. DOI: 10.3389/fgene.2012.00120

[36] Beg MS, Brenner AJ, Sachdev J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. Investigational New Drugs. 2017;**35**(2):180-188. DOI: 10.1007/s10637-016-0407-y

[37] Li C, Sun J, Xiang Q, et al. Prognostic role of miRNA21 in expression in gliomas: A meta analysis. Journal of Neuro-Oncology. 2016;**130**(1):11-17. DOI: 10.1007/s11060-016-2233-7

[38] Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007;**133**(2):647-658. DOI: 10.1053/j.gastro.2007.05.022

[39] Asangani IA, Rasheed SAK, Nikolova DA. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene. 2008;**27**(15):2128-2136. DOI: 10.1038/sj.onc.1210856

[40] Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1. The Journal of Biological Chemistry. 2007;**282**:14328-14336. DOI: 10.1074/jbc.M611393200

[41] Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010;**467**(7311):86-90. DOI: 10.1038/nature09284

[42] Berindan-Neagoe I, Monroig Pdel C, Pasculli B, Calin GA. MicroRNAome genome: A treasure for cancer diagnosis and therapy. CA: A Cancer Journal for Clinicians. 2014;**64**(5):311-336. DOI: 10.3322/caac.21244

[43] Lu J, Getz G, Miska EA. MicroRNA expression profiles classify human cancers. Nature. 2005;**435**:834-838. DOI: 10.1038/nature03702

[44] Rosenfeld N, Aharonov R, Meiri E. MicroRNAs accurately identify cancer tissue origin. Nature Biotechnology. 2008;**26**(4):462-469. DOI: 10.1038/nbt1392

[45] Liu C, Kelnar K, Vlassov AV. Distinct microRNA expression profiles in prostate cancer stem/progenitor cells and tumor-suppressive functions of let-7. Cancer Research. 2012;**72**(13):3393-3404. DOI: 10.1158/0008-5472.CAN-11-3864

[46] Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: Approaches and considerations. Nature Reviews. Genetics. 2012;**13**(5):358-369. DOI: 10.1038/nrg3198

[47] Miller KD, Siegel RS, Lin CC, et al. Cancer treatment and survivorship

statistics, 2016. CA: A Cancer Journal for Clinicians. 2016;**66**:271-289. DOI: 10.3322/caac.21349

[48] Müller MF, Ibrahim AE, Arends MJ. Molecular pathological classification of colorectal cancer. Virchows Archiv. 2016;**469**(2):125-134. DOI: 10.1007/s00428-016-1956-3

[49] Yang L, Belaguli N, Berger DH. MicroRNA and colorectal cancer. World Journal of Surgery. 2009;**33**:638-646. DOI: 10.1007/s00268-008-9865-5

[50] Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Molecular Cancer Research. 2003;**1**:882-891. Online ISSN 1557-3125

[51] Takahashi H, Takahashi M, Ohnuma S, et al. microRNA-193a-3p is specifically down-regulated and acts as a tumor suppressor in BRAF-mutated colorectal cancer. BMC Cancer. 2017;**17**:723. DOI: 10.1186/s12885-017-3739-x

[52] Cekaite L, Eide PW, Lind GE, et al. MicroRNAs as growth regulators, their function and biomarker status in colorectal cancer. Oncotarget. 2016;**7**:6476-6505. DOI: 10.18632/oncotarget.6390

[53] Danese E, Minicozzi AM, Benati M, et al. Reference miRNAs for colorectal cancer: Analysis and verification of current data. Scientific Reports. 2017;**7**:8413. DOI: 10.1038/s41598-017-08784-3

[54] Luo X, Burwinkel B, Tao S, Brenner H. MicroRNA signatures: Novel biomarker for colorectal cancer? Cancer Epidemiology, Biomarkers & Prevention. 2011;**20**(7):1272-1286. DOI: 10.1158/1055-9965.EPI-11-0035

[55] Schetter AJ, Okayama H, Harris CC. The role of microRNAs in colorectal cancer. Cancer Journal. 2012;**18**(3):244-252. DOI: 10.1097/PPO.0b013e318258b78f

[56] Sovich JL, Sartor Z, Misra S. Developments in Screening Tests and Strategies for Colorectal Cancer. Biomedical Research International. 2015;**2015**:326728. DOI: 10.1155/2015/326728

[57] Masuda T, Hayashi N, Kuroda Y, et al. MicroRNAs as biomarkers in colorectal cancer. Cancers (Basel). 2017;**9**(9):124. DOI: 10.3390/cancers9090124

[58] Luo X, Stock C, Burwinkel B, Brenner H. Identification and evaluation of plasma microRNAs for early detection of colorectal cancer. PLoS One. 2013;**8**(5):e62880. DOI: 10.1371/journal.pone.0062880

[59] Giráldez MD, Lozano JJ, Ramírez G, et al. Circulating microRNAs as biomarkers of colorectal cancer: Results from a genome-wide profiling and validation study. Clinical Gastroenterology and Hepatology. 2013;**11**(6):681-8.e3. DOI: 10.1016/j.cgh.2012.12.009

[60] Ma Y, Zhang P, Yang J, et al. Candidate microRNA biomarkers in human colorectal cancer: Systematic review profiling studies and experimental validation. International Journal of Cancer. 2012;**130**:2077-2087. DOI: 10.1002/ijc.26232

[61] Lindblom A. Different mechanisms in the tumorigenesis of proximal and distal colon cancers. Current Opinion in Oncology. 2001;**13**(1):63-69. PMID: 11148689

[62] Earle JS, Luthra R, Romans A, Abraham R, Ensor J, Yao H, et al. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. The Journal of Molecular Diagnostics. 2010;**12**(4):433-440. DOI: 10.2353/jmoldx.2010.090154

[63] Gatalica Z, Vranic S, Xiu J, Swensen J, Reddy S. High microsatellite instability (MSI-H) colorectal carcinoma: A brief review of predictive biomarkers in the era of personalized medicine. Familial Cancer. 2016;**15**(3):405-412. DOI: 10.1007/s10689-016-9884-6

[64] Kloor M, Staffa L, Ahadova A, von Knebel Doeberitz M. Clinical significance of microsatellite instability in colorectal cancer. Langenbeck's Archives of Surgery. 2014;**399**(1):23-31. DOI: 0.1007/s00423-013-1112-3

[65] Valeri N, Gasparini P, Fabbri M, et al. Modulation of mismatch repair and genomic stability by miR-155. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(15):6982-6987. DOI: 10.1073/pnas.1002472107

[66] Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(7):2257-2261. DOI: 10.1073/pnas.0510565103

[67] Slattery ML, Herrick JS, Mullany LE, et al. Colorectal tumor molecular phenotype and miRNA: Expression profiles and prognosis. Mod Pathol. 2016;**29**(8):915-927. DOI: 10.1038/modpathol.2016.73

[68] Moridikia A, Mirzaei H, Sahebkar A, Salimian J. MicroRNAs: Potential candidates for diagnosis and treatment of colorectal cancer. Journal of Cellular Physiology. 2018;**233**(2):901-913. DOI: 10.1002/jcp.25801

[69] Nishida N, Yokobori T, Mimori K, Sudo T, Tanaka F, Shibata K, et al. MicroRNA miR-125b is a prognostic marker in human colorectal cancer. International Journal of Oncology. 2011;**38**:1437-1443. DOI: 10.3892/ijo.2011.969

[70] Kim NH, Kim HS, Kim NG, Lee I, Choi HS, Li XY, et al. p53 and microRNA-34 are suppressors of canonical Wnt signaling. Science Signaling. 2011;**4**:ra71. DOI: 10.1126/scisignal.2001744

[71] Siemens H, Jackstadt R, Hünten S, Kaller M, Menssen A, Götz U, et al. miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. Cell Cycle. 2011;**10**:4256-4271. DOI: 10.4161/cc.10.24.18552

[72] Hiyoshi Y, Schetter AJ, Okayama H, Inamura K, Anami K, Nguyen GH, et al. Increased microRNA-34b and-34c predominantly expressed in stromal tissues is associated with poor prognosis in human colon cancer. PLoS One. 2015;**10**(4):e0124899. DOI: 10.1371/journal.pone.0124899

[73] Kanaan Z, Rai SN, Eichenberger MR, Barnes C, Dworkin AM, Weller C, et al. Differential microRNA expression tracks neoplastic progression in inflammatory bowel disease-associated colorectal cancer. Human Mutation. 2012;**33**:551-560. DOI: 10.1002/humu.22021

[74] Pu XX, Huang GL, Guo HQ, et al. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. Journal of Gastroenterology and Hepatology. 2010;**25**(10):1674-1680. DOI: 10.1111/j.1440-1746.2010.06417.x

[75] Wang G, Cao X, Lai S, Luo X, Feng Y, Wu J, et al. Altered p53 regulation of miR-148b and p55PIK contributes to tumor progression in colorectal cancer. Oncogene. 2015;**34**:912-921. DOI: 10.1038/onc.2014.30

[76] Meng WJ, Yang L, Ma Q, Zhang H, Adell G, Arbman G, et al. MicroRNA Expression Profile

Reveals miR-17-92 and miR-143-145 Cluster in Synchronous Colorectal Cancer. Medicine (Baltimore). 2015;**94**(32):e1297. DOI: 10.1097/MD.0000000000001297

[77] Pichler M, Winter E, Stotz M, et al. Down-regulation of KRAS-interacting miRNA-143 predicts poor prognosis but not response to EGFR-targeted agents in colorectal cancer. British Journal of Cancer. 2012;**106**:1826-1832. DOI: 10.1038/bjc.2012.175

[78] Vickers MM, Bar J, Gorn-Hondermann I, Yarom N, Daneshmand M, Hanson JE, et al. Stage-dependent differential expression of microRNAs in colorectal cancer: Potential role as markers of metastatic disease. Clinical & Experimental Metastasis. 2012;**29**:123-132. DOI: 10.1007/s10585-011-9435-3

[79] Kahlert C, Klupp F, Brand K, Lasitschka F, Diederichs S, Kirchberg J, et al. Invasion front-specific expression and prognostic significance of microRNA in colorectal liver metastases. Cancer Science. 2011;**102**:1799-1807. DOI: 10.1111/j.1349-7006.2011.02023.x

[80] Hata T, Mokutani Y, Takahashi H, Inoue A, Munakata K, Nagata K, et al. Identification of microRNA-487b as a negative regulator of liver metastasis by regulation of KRAS in colorectal cancer. International Journal of Oncology. 2017;**50**(2):487-496. DOI: 10.3892/ijo.2016.3813

[81] Cheng H, Zhang L, Cogdell DE, Zheng H, Schetter AJ, Nykter M, et al. Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One. 2011;**6**(3):e17745. DOI: 10.1371/journal.pone.0017745

[82] Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, et al. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. British Journal of Cancer. 2015;**113**(2):275-281. DOI: 10.1038/bjc.2015.201

[83] Dong Y, Yu J, Ng SS. MicroRNA dysregulation as a prognostic biomarker in colorectal cancer. Cancer Management and Research. 2014;**6**: 405-422. DOI: 10.2147/CMAR.S35164

[84] Xie T, Huang M, Wang Y, Wang L, Chen C, Chu X. MicroRNAs as regulators, biomarkers and therapeutic targets in the drug resistance of colorectal cancer. Cellular Physiology and Biochemistry. 2016;**40**(1-2):62-76. DOI: 10.1159/000452525

[85] Heneghan HM, Miller N, Kerin MJ. MiRNAs as biomarkers and therapeutic targets in cancer. Current Opinion in Pharmacology. 2010;**10**(5):543-550. DOI: 10.1016/j.coph.2010.05.010

[86] An X, Sarmiento C, Tan T, Zhu H. Regulation of multidrug resistance by microRNAs in anti-cancer therapy. Acta Pharmaceutica Sinica B. 2017;**7**(1):38-51. DOI: 10.3389/fgene.2012.00180

[87] Zhu Y, Peng Q, Lin Y, Zou L, Shen P, Chen F, et al. Identification of biomarker microRNAs for predicting the response of colorectal cancer to neoadjuvant chemoradiotherapy based on microRNA regulatory network. Oncotarget. 2017;**8**(2):2233. DOI: 10.18632/oncotarget.13659

[88] Ramos P, Bentires-Alj M. Mechanism-based cancer therapy: Resistance to therapy, therapy for resistance. Oncogene. 2015;**34**(8):3617-3626. DOI: 10.1038/onc.2014.314

[89] Hammond WA, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: A review. Therapeutic Advances in Medical Oncology. 2016;**8**(1):57-84. DOI: 10.1177/1758834015614530

[90] Kiss I, Mlčochová J, Součková K, Fabian P, Poprach A, Halamkova J, et al. MicroRNAs as outcome predictors in patients with metastatic colorectal cancer treated with bevacizumab in combination with FOLFOX. Oncology Letters. 2017;**14**(1):743-750. DOI: 10.3892/ol.2017.6255

[91] Kjersem JB, Ikdahl T, Lingjaerde OC, Guren T, Tveit KM, Kure EH. Plasma microRNAs predicting clinical outcome in metastatic colorectal cancer patients receiving first-line oxaliplatin-based treatment. Molecular Oncology. 2014;**8**(1):59-67. DOI: 10.1016/j.molonc.2013.09.001

[92] Markman JL, Shiao SL. Impact of the immune system and immunotherapy in colorectal cancer. Journal of Gastrointestinal Oncology. 2015;**6**(2):208-223. DOI: 10.3978/j.issn.2078-6891.2014.077

[93] Giannakis M, Mu XJ, Shukla SA, et al. Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Reports. 2016;**15**(4):857-865. DOI: 10.1016/j.celrep.2016.03.075

[94] Cortez MA, Ivan C, Valdecanas D, Wang X, Peltier HJ, Ye Y, et al. PDL1 regulation by p53 via miR-34. Journal of National Cancer Institute. 2016;**108**:djv303. DOI: 10.1093/jnci/djv303

[95] Wei J, Wang F, Kong LY, Xu S, Doucette T, Ferguson SD, et al. miR-124 inhibits STAT3 signaling to enhance T cell-mediated immune clearance of glioma. Cancer Research. 2013;**73**: 3913-3926. DOI: 10.1158/0008-5472.CAN-12-4318

[96] Yu T, Zuo QF, Gong L, Wang LN, Zou QM, Xiao B. MicroRNA-491 regulates the proliferation and apoptosis of CD8+ T cells. Scientific Reports. 2016;**6**:30923. DOI: 10.1038/srep30923

[97] Ozasa H, Hazama S, Shimizu R, Etoh R, Inoue Y, Kanekiyo S, et al. miR-196b and miR-486 as predictive biomarkers for the efficacy of the vaccine treatment: From the results of phase I and II studies for metastatic colorectal cancer. Oncology Letters. 2017;**14**(2):1355-1362. DOI: 10.3892/ol.2017.6303

[98] Kraemer A, Barjaktarovic Z, Sarioglu H, et al. Cell survival following radiation exposure requires miR-525-3p mediated suppression of ARRB1 and TXN1. PLoS One. 2013;**8**(10):e77484. DOI: 10.1371/journal.pone.0077484

Chapter 4

The AIB1/NCOA3/SRC-3 Oncogene

Max H. Kushner, Anna T. Riegel and Ghada M. Sharif

Abstract

A member of the NCOA/SRC/p160 co-activator family, AIB1 is amplified and overexpressed in multiple cancer types, notably breast, ovarian, and pancreatic cancer. Common to all members of the NCOA/SRC/p160 family are bHLH-PAS, receptor interaction, and CBP/p300 interacting activation domains. The protein acts as a scaffold to support the transcriptional activity of many DNA binding transcription factors, such as the ER, AP-1, E2F, NFκB, and TEADs. In doing so, the multi-domain protein facilitates chromatin remodeling and oncogenic gene transcription. Further, the AIB1Δ4 isoform promotes tumorigenesis and metastasis through interaction with chromatin in the nucleus or at the periphery of the cell. Pathologically, AIB1 promotes the transformation of normal tissue to cancerous lesions in multiple diseases, and loss delays progression. AIB1 has also been implicated in cancer recurrence and pharmacological resistance. We will discuss the structure and isoforms of AIB1, the physiological consequences of its interaction with transcription factors and hormone receptors, and clinical significance of the protein.

Keywords: AIB1, NCOA3, SRC-3, nuclear coactivator, steroid receptor co-activator, oncogene, breast cancer, transcriptional co-activation, chromatin modification

1. Introduction

Amplified in breast cancer 1 (AIB1) is a transcriptional co-activator and a member of the nuclear co-activator (NCOA) family; the protein was discovered concurrently by many groups and given a variety of names, including AIB1 [1], ACTR [2], TRAM-1 [3], RAC-3 [4], CIP1 [5], and SRC-3 [6]. AIB1 is an oncogene that is amplified and overexpressed in cancer, and acts by recruiting and stabilizing chromatin remodeling complexes [1, 2, 7]. In its well-known capacity, AIB1 interacts with nuclear receptors such as the estrogen, progesterone, and androgen receptor, to promote hormone dependent transcription and repression. Less well-studied, AIB1 promotes disease progression and de-differentiation by potentiating oncogenic signaling through interaction with a diversity of transcription factor interactions in hormone-independent disease contexts [8–11]. Thus, AIB1 acts as an oncogene by stabilizing transcription complexes, recruiting chromatin modifying enzymes, and thereby amplifying oncogenic signals. Unsurprisingly, high AIB1 levels are a poor prognostic marker across many cancer types, and also the protein can facilitate resistance to therapeutics in patients. Herein, we describe the form and function of AIB1, and its role in cancer and the clinical setting.

IntechOpen

2. Structure of the oncogene

2.1 The nuclear coactivator (NCOA) family

The NCOA/SRC/p160 co-activator family consists of NCOA1 (also called SRC-1), NCOA2 (also called SRC-2, GRIP1, or TIF2), and AIB1 (also called SRC-3, NCOA3, ACTR, TRAM-1, and RAC-3). The three family members share ~55% sequence homology, and common to all members is a bHLH-PAS domain, nuclear receptor interaction domains, and C-terminal activation domains (reviewed in [12]). As a result, the members of the NCOA family interact with many different transcription factors, nuclear receptors, and chromatin modifying enzymes. Apart from mediating transcription factor interactions (detailed in Section 6 of this review), the homologous domains and residues are critical to protein stability and turnover. The bHLH-PAS domain contains residues essential for proteasomal mediated protein turnover (K17 and R18) as well as a nuclear localization signal; site-directed mutagenesis of these residues promote protein hyperstability [13]. Though the bHLH-PAS domain shares homology to DNA recognizing motifs found in both Drosophila and Humans, the NCOA family members do not directly bind the DNA, but rather facilitate transcriptional activity of binding partners [14]. The bHLH-PAS domain mediates the NCOA members' interaction with transcription factors, repressors, and coactivators, but is lost in the clinically relevant Δ4 isoform (see Section 2.2). The middle region of the NCOA family proteins contains three a-helical LXXLL motifs (NR Boxes) that mediate nuclear receptor (NR) recognition and specificity [15, 16]. The affinity of the NCOA is both dependent on the individual nuclear receptors, as well as the specific bound ligands [17]. Interaction with nuclear receptors, including the well understood interaction with the Estrogen Receptor, is detailed in Section 4. The C-terminus contains two activation domains, as well as a glutamine-rich region. These activation domains are critical to the recruitment of chromatin remodeling enzymes CBP/p300, P/CAF, CARM1, and PRMT1 [2, 18]. Similar to the bHLH-PAS domain, the C-terminal activation domains are critical for a variety of transcription factor interactions. Furthermore, SRC-1 and AIB1 both have minimal acyltransferase activity, associated with their C-terminal activation domains, which function as histone acetyltransferases [2, 19]. **Figure 1** illustrates the structure of AIB1 and some of its known binding partners.

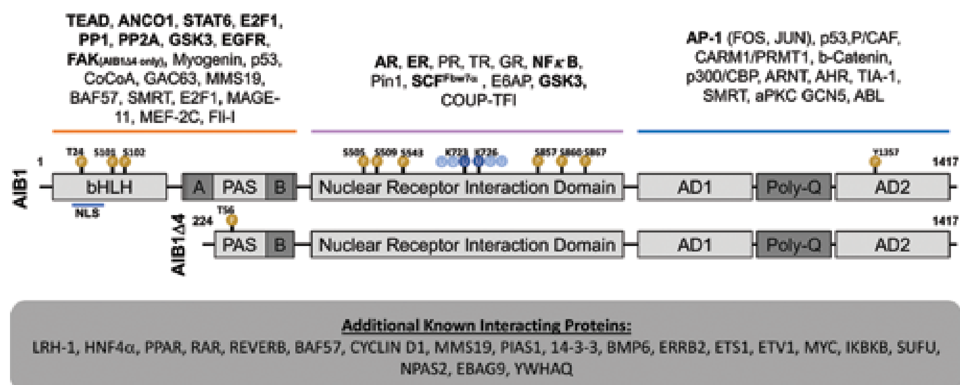


Figure 1.

AIB1 interacts with many proteins through its independent domains. Line diagram showing some of the proteins that AIB1 and AIB1Δ4 interact with in a cell. Bolded proteins are described in the text of this chapter. Critical residues for phosphorylation (yellow) and ubiquitination (blue) are shown. bHLH = basic helix-loop-helix domain; PAS = PER-ARNT-SIM domain. AD = activation domain.

It is the independent functions of the multiple domains of AIB1 that allow it to perform a variety of different cellular activity. Of note, the capacity of AIB1 to act as a co-activator is dependent not on mutually exclusive interactions, but rather on many simultaneous binding events in which it acts as a scaffold to support multi-protein complex formation.

2.2 The AIB1Δ4 isoform

We have identified an isoform of the AIB1 oncogene that lacks the N-terminal bHLH-PAS A domain, originally designated Δ3, now called AIB1Δ4. Alternative splicing results in the loss of exon 4, thereby shifting the start codon to exon 7 and forming a N-terminal truncated, yet functional protein, missing 224 amino acids [20, 21]. As the isoform retains its nuclear receptor interacting domain, poly-Q region, and its C-terminal activation domains, the isoform can localize to estrogen response elements in the nucleus and recruits CBP/p300 to activate hormone dependent transcription [22]. Moreover, Δ4 isoform can also utilize its C-terminal activation domains, meaning that it can still co-activate many of hormone receptors and transcription factors on which it usually acts (see Sections 4 and 6). While retaining the ability to perform a significant portion of its normal activity, AIB1Δ4 cannot bind an endogenous repressor, ANCO1, and this may contribute to a significant portion of its hyperactivity [23, 24].

AIB1Δ4 has been shown to be highly responsive to growth factor and estrogen signaling. While much of this chapter will be devoted to the role of AIB1 on promoting transcription, it can also act as a key player in repression complexes. The ANCO1 repressor binds and represses the AIB1-ER complex on an estrogen response element in the Her2 gene body. This mechanism allows for estrogen mediated repression of the Her2 gene, as AIB1 in turn recruits histone deacetylases to the site to suppress transcription. The AIB1Δ4 isoform is not repressed by this mechanism, as it lacks the bHLH-PAS domain that ANCO1 requires as a binding interface, and thus maintains transcription of the Her2 growth factor receptor. As a result, the AIB1Δ4 isoform enhances growth factor signaling input via increasing the mRNA levels of the receptor [24].

Interestingly, by lacking the N-terminal bHLH-PAS domain containing the nuclear localization signal, the Δ4 isoform localizes both to the nucleus and cytoplasm. In the cytoplasm, the isoform interacts with both EGFR and FAK to promote cell migration through promotion of Src phosphorylation. The bHLH-PAS domain generally acts as an inhibitor of this interaction [25]. Knockdown of AIB1 in ovarian cells also results in a mislocalization of FAK, which may be due to loss of the isoform [26].

The role of AIB1Δ4 has been validated in vivo. AIB1Δ4 has been shown to increase mammary cell proliferation in tissue specific genetically engineered mouse models, potentially by increasing levels of IGF-1R levels on the cell surface [27]. Overexpression of the isoform resulted in similar hyperplasia as compared to the overexpression of the full-length protein, however it further presented with an increase in progesterone receptor signaling and cell cycle promoting cyclin levels. Compared to the full-length protein, AIB1Δ4 acted synergistically with ERα to promote mammary gland stromal and epithelial hyperplasia [28]. The isoform is not only seen in estrogen dependent breast cancer, as AIB1Δ4 expression levels are increased in metastatic triple negative (hormone-independent) and pancreatic cancer cell lines, compared to their isogenic parental lines [22]. More data is needed to explain the complete function of AIB1Δ4, which clearly plays a role in normal physiology, and may contribute to disease progression and invasion.

3. Modifications and PTMs regulate AIB1 activity

Post-translational modifications (PTM) are critical to modulating the stability and activity of AIB1. Within the 165 kDa protein, there are multiple phosphorylation and ubiquitination sites, some of which have been well described by recent experimentation.

3.1 Transcriptional regulation of AIB1

AIB1 protein level is regulated by multiple processes, with levels primarily peaking during the cell cycle. AIB1 autoregulates its own expression and is recruited to its own promoter in complex with E2F1 (see Section 6.3). AIB1 transcription is responsive to cell cycle cues mediated by Rb hypophosphorylation and resulting activation of E2F; thus, AIB1 levels increase during G1, and attenuate during S phase (when comparing relative levels during the cell cycle) [29]. Downregulation of AIB1 protein levels is, in part, mediated by the FoxG1 tumor suppressor, which acts by interacting with AIB1 and disrupting the interaction with E2F1 on its own promoter (FoxG1 additionally interrupts AIB1's activity with other transcription factors, such as NFκB, AP-1, and the Estrogen Receptor) [30]. Tight control of the AIB1 activity is regulated by its own positive feedback and tempered by inhibitory protein interactions.

AIB1 is also targeted by microRNA that regulates its expression. miR-17-5p targets at least two sites on the AIB1 mRNA, and miR-20b also binds to AIB1 mRNA. The two miRs are negatively correlated with AIB1 expression and loss of miR expression is associated with taxol resistance in breast cancer [31, 32]. These miRs, in addition to down regulating AIB1, interact with multiple other proteins to differentially regulate their gene expression. As a result, they are implicated in the progression or suppression of several cancers, in a context dependent manner.

3.2 The AIB1 life cycle

The stability and half-life of the AIB1 protein, once translated, is regulated by a series of phosphorylation and ubiquitination events (see **Figure 1**). There is a balance achieved between multi-mono-ubiquitination and long chain poly-ubiquitination that creates a phosphorylation dependent 'time clock' for the stability of AIB1. GSK3 phosphorylates AIB1 at S505 and S509 in the absence of AKT signaling to promote its multi-mono- or poly-ubiquitination by $SCF^{Fbw7\alpha}$ at K723 and K726. Amplified AKT signaling inactivates GSK3. Multi-(mono)ubiquitination of AIB1 then promotes hormone dependent activity through interaction with the estrogen receptor, whereas poly-ubiquitination promotes degradation of AIB1 [33].

An alternative variety of phosphatases regulate proteasome mediated turnover by binding and dephosphorylating AIB1. PDXP, PP1, and PP2A dephosphorylate AIB1 at S101 and S102, and negatively regulate Estrogen Receptor binding and cooperation. PP1 specifically stabilizes AIB1 by preventing proteasomal turnover by dephosphorylating the N-terminal C-region degron at the S101 and S102 residues. Though more stable, decreased affinity for the estrogen receptor significantly reduced AIB1 mediated cell growth in estrogen dependent cell lines [34]. Regulation of AIB1 protein levels is thus regulated by a complex series of phosphorylation and ubiquitination events in response to cell signaling and stimulation. The phosphorylation and ubiquitination sites may be unique to the pathways promoting turnover, and thus further study is warranted to better understand the signaling cascades that control AIB1 levels.

3.3 AIB1 post-translational modifications modulate activity

AIB1 is phosphorylated at different serine and threonine residues throughout the different domain structures by a variety of kinases. As a result, AIB1 is responsive to many different upstream signaling cascades, contributing to its oncogenic nature. JNK, p38, ERK, IKK, and PKA can all phosphorylate AIB1 at different residues to promote interaction with CBP and subsequently activate transcription (see **Figure 1**). Phosphorylation sites within AIB1 have been well profiled as mediators of certain protein-protein interactions. Six phosphorylation sites were originally described as essential for interaction with the estrogen and androgen receptors (T24, S505, S543, S857, S860, and S867). Conversely, phosphorylation at only T24 and S867 was required for TNF mediated NFκB interaction and activity [35]. These phosphorylation events in response to estradiol, TNFα, and upstream IGF signaling thus activate AIB1 to interact with partners and potentiate transcription [35–37]. Additionally, we have found that Abl kinase phosphorylates AIB1 at Y1357 in response to IGF, EGF and estradiol stimulation, which results in AIB1 interaction with essential chromatin modifying enzymes. This phosphorylation event is critical for AIB1's coactivator function [38]. Phosphorylation of AIB1 is thus a critical step in activation of the protein and is mediated by a variety of upstream signals that converge on the oncogenic coactivator. Some phosphorylation sites are required for all described activity, yet much work needs to be done to better understand what regulates the selectivity of AIB1 to bind with specific transcription factors and nuclear receptors.

4. AIB1 potentiates hormone receptor signaling

Estrogen, progesterone and androgen receptors (ER, PR, AR) are nuclear steroid receptors, which play a major role in sexual development and reproduction. Upon binding to their ligands, these receptors dimerize and translocate to the nucleus. They act as transcription factors by binding directly to unique DNA sequences termed response elements. Subsequently, histone modifying enzymes and transcriptional co-regulators are recruited to activate target gene transcription. AIB1 has been shown to directly interact with ER, PR and AR via its LXXLL motifs [39, 40]. There are two classes of estrogen receptors; ERα and ERβ. AIB1 binds and enhances ERα receptor-stimulated gene transcription in a ligand-dependent manner [6]. Upon binding to ERα, AIB1 recruits chromatin-remodeling histone acetyl-transferases enzymes and thus increases ERα transcriptional activity. On the other hand, AIB1 can regulate ERα protein levels when bound to estradiol (E2). AIB1 recruits ubiquitin-proteasome complex to the ligand bound ERα leading to its degradation. Studies have shown that when AIB1 levels are reduced, ERα levels are stabilized [41]. In AIB1−/− mice, delays in puberty and mammary gland development as well as aberrant reproductive functions have been reported [5]. Similar observations are seen in both PR−/− and ER−/− mice emphasizing the essential role of AIB1 in ER and PR dependent functions. In breast cancer, AIB1 potentiates the development of hormone-dependent tumors and contributes to antiestrogen resistance [42, 43]. Lacking the inhibitory domain, AIB1Δ4 isoform has been shown to potentiate ER and PR transcription activity to a much greater extent than AIB1 [20]. In breast tumor samples, the association between AIB1 level and both ER and PR levels has not been clearly determined. One study showed that amplification of AIB1 in breast cancer correlates with high expression of ER and PR [44]. Yet another study showed that overexpression of AIB1 in breast cancer samples was associated

with loss of both ER and PR expression [45]. Moreover, AIB1 strongly binds AR and co-activates its target gene transcription. In prostate cancer, AIB1 is shown to be overexpressed and its levels correlate with higher tumor grade and increased disease recurrence but did not correlate with serum PSA levels [46]. Several studies have established AIB1 as a preferred coactivator for hormone-activated AR. Mutations in AR that alter its binding potency to AIB1 has been found in prostate cancer patients, suggesting an oncogenic role of AIB1 in prostate cancer [40].

5. AIB1 potentiates membrane receptor signaling

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase (RTK). It is activated by EGF which mediates several functions including cell proliferation, cell survival, and development. AIB1 knockdown abrogated EGF growth response in lung, breast, and pancreatic cancer cell lines. This was a result of reduced tyrosine phosphorylation of EGFR at multiple residues both at autophosphorylation and Src kinase phosphorylation sites via less recruitment of Src homology 2 domain-containing proteins to the EGFR. EGF-dependent phosphorylation of HER2 was also decreased yet no effect was seen on phosphorylation of platelet-derived growth factor receptor (PDGFR), HER3 or other RTKs. This suggests that the oncogenic effect of AIB1 may be mediated by EGFR and HER2 signaling pathways [47]. In a MMTV-Neu mouse model, homozygous deletion of AIB1 completely inhibits Neu-induced mammary tumor formation. The role of AIB1 in HER2/Neu oncogenic activity was elucidated in the Neu/AIB1+/− tumors showing decreased phosphorylated Neu, cyclin D1, and cyclin E [48]. In addition to its role as a transcription coactivator, AIB1 isoform, AIB1Δ4, can act in the periphery of the cell mediating EGFR and FAK direct interaction. Overexpression of AIB1Δ4 increased cell migration and MDA-MB-231-induced breast tumor metastasis [25].

The insulin-like growth factor (IGF)-I regulates protein turnover and has a role in cell proliferation and differentiation. IGF-I binds to its receptor activating a cascade of intracellular tyrosine kinases which phosphorylate downstream substrates including IRS and Shc [49]. AIB1 is rate-limiting for IGF-I signaling and functions in human breast cancer cells. Knockdown of AIB1 in MCF7 cells reduced IGF-1-stimulated anchorage-independent proliferation and IGF-I-dependent anti-anoikis [37]. In AIB1−/− mice, impaired insulin-like growth factor I pathway reduced mammary tumorigenesis and metastasis with no change in ER or PR regulated genes [50]. In addition, AIB1 regulates the expression of proteins involved in the IGF-1 signaling pathway. For example, inhibition of mTOR prevented mammary hyperplasia and hypertrophy that was caused by AIB1 overexpression in the mouse mammary gland. In mice, mTOR inhibition prevented the growth of xenografts from AIB1-induced mammary tumors [51].

6. AIB1 interacts with transcription factors

Though AIB1 has been primarily studied for its interaction with the Estrogen receptor (see Section 4), AIB1 interacts with a diverse set of transcription factors which may explain its oncogenic role in cancer. We will focus on the interaction of AIB1 with known oncogenic transcription factors to highlight the importance of AIB1 as a transcriptional co-activator across a variety of signaling pathways. As a general pattern, AIB1 acts to potentiate transcription of signaling pathways; when it binds to a transcription factor, it tends to increase the expression of target genes synergistically. Additionally, many pathways simultaneously activate their effectors and act on AIB1 in

the cytosol to increase nuclear translocation. Thus, many growth factors signaling cascades converge on transcription factors and AIB1. We have selected a few of the most well studied oncogenic transcription factors whose activity is increased by AIB1 in cancer. **Figure 2** illustrates these interactions and their phenotypic consequences.

6.1 AP-1

The activator protein transcription factor is a heteromeric complex consisting of Fos, Jun, ATF, and MAF family members, and can act as an oncogene that drives proliferative signaling. The complexes regulate a large swath of human gene expression and can contribute to both pro- and anti-tumorigenic gene expression (reviewed in [52]). AIB1 uses its C-terminal activation domains to interact with the Fos and Jun family members to activate and potentiate signaling, as measured by synthetic luciferase reporter assays and target gene expression [10]. Clinically, this interaction has been identified to be relevant in driving many pro-proliferative cancer genes. In hormone independent prostate cancer, responsiveness to IGF-Akt signaling by AIB1-AP-1 cooperation synergizes their effects on the transcription of target genes, thereby promoting cell growth and division [53]. In both hormone-dependent and -independent breast cancer (in which AIB1 is amplified), AIB1 interaction with and co-activation of AP-1 specifically promotes the transcription of matrix metalloproteinases, contributing to invasive progression [54]. Such invasive behavior has also been linked to turnover of Focal Adhesions by AIB1 through an AP-1 dependent interaction [55]. Of note, the AIB1Δ4 isoform contains the domains necessary to interact with AP-1 and FAK, which may explain some of its endogenous role, as well as a dual functionality of this oncogenic isoform [25, 55].

6.2 The TEADs

Many recent publications have implicated YAP and TAZ, effectors negatively regulated by Hippo Signaling pathway, as potent oncogenes critical to the

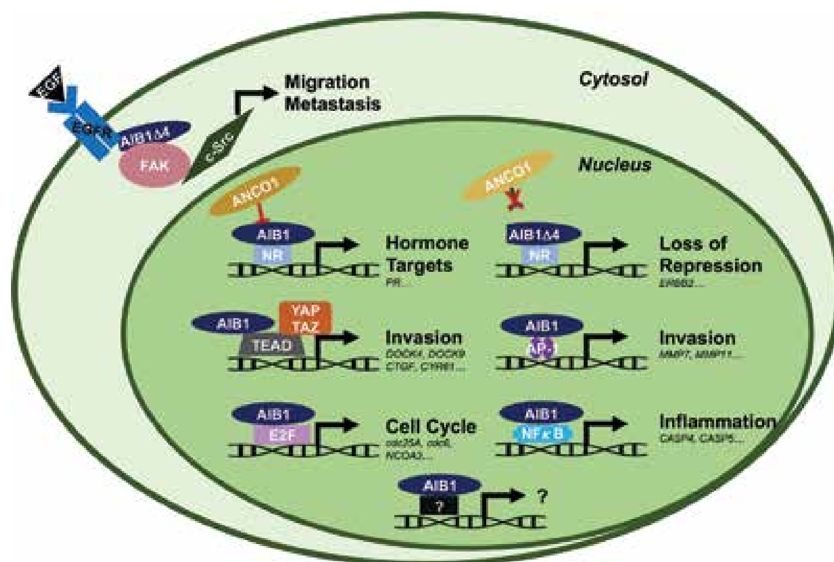


Figure 2.
Oncogenic effects and gene expression changes involving direct AIB1 interaction with transcription factors and membrane proteins. Graphical depiction of AIB1 interaction with binding partners, resulting phenotypic behavior, and select transcriptional targets (italicized).

transformation of normal tissue and the progression of disease. The effectors bind with the TEAD family of proteins to exert their oncogenic potential as a transcription factor complex [56–58]. Before paralogs YAP and TAZ, were implicated as transactivators of the TEAD family of transcription factors, the TEAD family was shown to interact with all the members of Nuclear Coactivator Family [8]. Specifically, the bHLH-PAS domain of AIB1 interacts with TEADs, likely in a larger complex with YAP or TAZ, and may then recruit histone modifying proteins to propagate transcription as measured by target genes [59]. Similarly, knockdown of AIB1 in cell lines significantly reduces TEAD target genes CTGF and CYR61 [60]. Currently, it is unclear to what extent the oncogenic YAP/TAZ-TEAD complex requires AIB1 or other members of the NCOA family to act as oncogenes. Knockdown of AIB1 in multiple studies has resulted in a modest, but significant reduction of TEAD transcriptional targets [60]. Interestingly, the Drosophila homolog of AIB1, called Taiman, contains PPxY motifs that are known to interact with the YAP homolog at its conserved WW domain. These PPxY motifs, however, are neither conserved in human AIB1, nor any member of the NCOA/SRC/p160 family [61].

Importantly, AIB1 may be the critical mediator of TEAD cooperation with AP-1. Early reports show an oncogenic signature associated with AP-1 and TEAD co-occupancy in triple negative breast cancer that promotes more aggressive disease [62]; this interaction was recently show to be mediated by AIB1 acting as a bridge between AP-1 and TEAD [59]. However, a clear panel of genes co-regulated by AIB1-TEAD interaction has yet to be elucidated. Further, while reports have shown AIB1 to be part of the TEAD-SRF (Serum Response Factor) complex, the data is unclear as to whether AIB1 is required for complex formation, or whether YAP can recruit SRF in the absence of AIB1 [60, 63]. It is apparent that TEAD, AP-1, and SRF all have coordinated responses to external growth stimuli [64], but the extent to which AIB1 and other co-activators are required to propagate and potentiate oncogenic signaling remains an exciting and unanswered question.

6.3 E2F family

The E2F family of transcription factors are direct targets of the hypo-phosphorylated Rb cell cycle regulation machinery, so many E2F members promote the transcription of pro-proliferative genes and controls the entry into S phase (reviewed in [65, 66]). AIB1 interacts with E2F family members through its N-terminal bHLH-PAS domain to promote the transcription of cdc25A, cdc6, MCMs, cyclins and Cdk. Depletion of AIB1 prevents cells from entering S-phase and undergoing mitosis. Furthermore, AIB1 controls its own expression through binding to E2F1 on its own promoter. As a result, AIB1 levels increase during G1 [9, 29]. Not only does E2F interact with AIB1 at its own promoter, but it also acts on other transcription factors, such as SP1, to further augment AIB1 expression [67]. This shows that direct and indirect binding of cell cycle effectors promote transcription of AIB1. Recently, the importance of an AIB1-E2F1 axis was highlighted while studying the efficacy of CDK4/6 inhibitor Palbociclib across all subtypes of breast cancer; AIB1 loss partially phenocopied Her2 inhibition and correlated with the CDK4/6 inhibitor treatment [68]. Thus, AIB1 contributes to cell cycle progression through E2F interaction, which is commonly dysregulated in cancer. This directly links AIB1 to regulation of cell cycle progression, implicating AIB1 further in pro-proliferative activities separate from external stimuli and nuclear receptor interaction.

6.4 ETS factors

The Ets1, Ets2, and Pea3 members of the Ets family of transcription factors bind to DNA in response to upstream Her2 activation and resulting kinase cascades, mediated by ERK and JNK [69]. The Ets family members have been shown to be co-expressed with AIB1 and both independently serve as a negative prognostic marker in breast and lung cancer [70, 71]. AIB1 was later shown to interact directly with the Ets family members to potentiate transcription of matrix metalloproteinases such as MMP2 to promote cell invasion and metastasis in vitro and in patients [71–73]. Once phosphorylated by ERK3 at S857, AIB1 specifically localized to the promoters of MMP2 and MMP9 in complex with Pea3 to promote invasive behavior [74]. Interestingly, these MMP targets seem highly dependent on AIB1 levels, as knock-down of AIB1 abrogated most of their expression [71].

6.5 NFκB pathway

Though many binding partners are shared between members of the NCOA family, mostly between SRC-1 and AIB1, cooperation with IKK is unique to AIB1. IKK mediates the degradation of IkB, the inhibitor of NFκB, in response to TNFα stimulation. Interestingly, in parallel to this, AIB1 is phosphorylated by IKK, increasing its nuclear localization and then can act on NFκB bound to DNA through its C-terminal activation domain [11, 75]. This emphasizes the ability of AIB1 to be to play a multi-faceted role within a signaling pathway, and the importance of its regulation to control its potency.

6.6 STAT6

Opposed to its role in the NFκB pathway, where AIB1 is the unique family member interacting with a kinase, STAT6 solely interacts with SRC-1. However, this does not mean AIB1 does not play a critical role. While SRC-1 directly interacts with STAT6 on the chromatin via its bHLH domain, AIB1 cannot. Recruited p300 bound to STAT6 can then recruit AIB1, which potentiates STAT6 signaling. This represents a unique cooperation between SRC-1 and AIB1, as SRC-1 is required for the co-activation of STAT6, and AIB1 then potentiates the transcription complex's activity. This was found to be an IL-4 dependent interaction, which acts in a dose-dependent manner [76–79]. Such interactions are controlled by PP2A, as described in Section 3.2 [80].

We attempted to highlight some of the most categorized interactions between AIB1 and transcription factors that are well studied oncogenes. See **Figure 1** for a more complete list of proteins that interact with AIB1.

7. AIB1 as an oncogene

7.1 Genetically engineered mouse models

Genetic models have also clearly established AIB1 as an oncogene in multiple cancer types. Genetically engineered mouse models that overexpress AIB1 have been shown to increase incidence and growth of tumors, as well as significantly increase hyperplasia in the breast [7]. This is not solely due to its interaction with the estrogen receptor, the same mice presented an increase in tumors in hormone

independent tissues, such as lung, skin, and bone, suggesting the oncogenic role of AIB1 may be mediated by a variety of different tissue specific transcription factor interactions [46, 81]. Removal of the NCOA3 gene that encodes AIB1 in v-Ha-Ras driven mouse model of breast cancer also delayed tumor formation by negatively impacting growth factor signaling [50]. In breast cancer studies, it is clear that AIB1 exerts its oncogenic potential through hormone receptor signaling and by positively affecting many pro-proliferative pathways.

There have also been genetic models implicating AIB1 as a critical mediator of the development and maintenance of hormone responsive and castration resistant prostate cancer. In hormone sensitive prostate cancer, AIB1 mediates its effects through androgen receptor activity, eventual castration resistant/hormone insensitive disease was marked by AIB1 potentiation of Akt-mTOR signaling (similar to studies in the breast) [81–83]. Taken together, these data suggest that AIB1 is critical for the formation and progression of many cancer types, in both hormone dependent and independent settings. Especially in the hormone-independent diseases, it is critical to study the binding partners of AIB1 in order to better understand how AIB1 is acting as an oncogene.

7.2 AIB1 levels and clinical outcome

Patient data has shown that the levels of AIB1 correlate with the severity and stage of disease. In each tissue type of origin, it is likely that AIB1 is acting as an oncogene in a different capacity or selectively amplifying a variety of oncogenic signals. We have described a few specific cancer types below that detail the predictive capacity of AIB1 in disease progression.

- Breast cancer: our group and others have shown that AIB1 is overexpressed and amplified in breast cancer compared to normal breast tissue [1, 20]. It is estimated that the mRNA expression in tumors is up between 10 and 60% in primary tumors, and increased 30% in metastatic sites [21, 84]. When stratified by grade of lesion, there is a clear positive correlation of AIB1 mRNA levels with worsening stage, with nearly a 65% increase in expression compared to normal tissue in grade 3 tumors [45]. We have also shown that increasing mRNA levels are associated with worse patient outcome [43]. These patients have dysregulated signaling pathways as previously described: augmented estrogen receptor signaling (in ER+ disease) and increased IGF/growth factor levels and enhanced in PI3K-Akt-mTOR pathway activity.
    - Early stage breast cancer: only 20–30% of women with stage 0 Ductal Carcinoma In Situ (DCIS) will progress to invasive disease. It is still unclear what factors promote the invasion, as genetic expression signatures of DCIS patients resemble those of invasive disease [85, 86]. We have shown that AIB1 is required for the formation of DCIS lesions in mice, and loss of AIB1 increases tumor necrosis, and decreases proliferation and tumor burden. Further, genetic ablation of AIB1 significantly reduces CD44+/CD24– breast cancer initiating cells, thus more closely resembling differentiated luminal epithelium. This is in part due to disruption and downregulation of the Notch and Her2 signaling pathways, where AIB1 was shown to regulate mRNA expression of Notch, Jag, and DLL family members [87]. Thus, AIB1 may be promoting a breast cancer initiating cell subpopulation that is required to promote the invasive transition.

- Ovarian cancer: AIB1 was also found to be overexpressed and amplified in ovarian cancer [1]. In high grade ovarian cancer samples, 64% of patients

stained positive for nuclear AIB1, whereas less than 10% of patients with cystomas or borderline cancer cases stained positive. [26] AIB1 polymorphs at the sequence level may also be predictive of ovarian cancer. CAG sequence polymorphisms within the glutamate track (poly-Q region) of AIB1 may also be predictive of ovarian cancer aggressiveness—codon lengths can vary between 24 and 30 repeats. The shorter the track, the shorter the time to disease recurrence compared to patients with longer sequences [88].

- Pancreatic cancer: AIB1 is rarely expressed in normal pancreas ducts (<6% of patients) yet is increased in pancreatitis and high-grade neoplasia between 15 and 20% of samples. Finally, upon progressing to pancreatic ductal adenocarcinoma (PDAC), nearly 65% of patients are positive for AIB1 mRNA and protein. Some patients also present with increased copy number, which may explain some, but not all of the overexpression of AIB1. Of note, the AIB1Δ4 isoform is present in pancreatic cancer cell lines, suggesting it acts not only through dysregulated hormone receptor signaling, but may also be playing unexplored roles [89]. AIB1 has also been shown to increase inflammatory conditions by upregulating CXCL1, CXCL2, and CXCL5 during disease development in a mouse model [90]. This may partially explain why the increase of mRNA and protein in pancreatitis and early stage disease is selected for as PDAC progresses in patients.

- Prostate cancer: levels of AIB1 are associated with severity/grade of prostate cancer. Higher levels across all stages are a negative prognostic marker in recurrence free survival. The Kaplan Meyer curves are similar for PSA and nuclear AIB1, suggesting it could serve as a biomarker for disease prognosis and progression [46, 81]. Of note, the same polymorphic CAG sequence that may be prognostically relevant to ovarian cancer may be relevant to prostate cancer—a case study of Chinese men suggested an optimal length of mid-quantity CAG repeats [91]. However, these data need to be repeated in a population with a higher incidence and risk of the disease.

- Colorectal cancer: overexpression of AIB1 was detected in 35% of samples, and amplification of the NCOA3 gene was detected in 10% of patients with colorectal cancer [92]. Levels not only varied significantly when compared to normal tissue, but also significantly increased by tumor grade [82]. One potential role of AIB1 in colorectal cancer is interaction with Estrogen Receptor Beta, which is expressed in CRC. In 110 patients, increasing grade of lesion showed significant upregulation in the levels of expression of AIB1, ERb, and SRC-2. Paradoxically, the same study noted both an increase in invasion associated with higher AIB1 levels, but an increase in survival outcome [93]. AIB1−/− mice were also unsusceptible to colorectal cancer induction by azoxymethane/dextran sodium sulfate treatment [94]. Clearly, more data is needed to explain the role of AIB1 in colorectal cancer, and whether AIB1 may modulate pro- and anti-tumorigenic behavior.

7.3 AIB1 promotes metastasis

As AIB1 acts to potentiate a variety of signaling cascades, it contributes not only to the growth of the primary cancerous lesion, but also promotes metastasis to distant sites. AIB1 loss suppressed lung metastasis in MMTV-PyMT breast cancer models, significantly reducing the ability of the cells to metastasis. Following transplantation of the AIB1−/− tumors from knockout mice to wild-type PyMT mice, metastasis was completely lost [74]. Pancreatic and breast tumors formed in AIB1−/− also exhibit a more epithelial, E-cad high tumor phenotype, suggesting

a repression of epithelial-mesenchymal transition [74]. This is reflected in patient data, where more PDAC patients samples with lymph node metastasis (68%) stain positive for nuclear AIB1, as opposed to patients without metastasis, who also stain negative for AIB1 (42%) [95]. Similarly, in papillary thyroid cancer, levels of AIB1 positive increase significantly from high grade lymph node positive disease (73.2%), compared to non-metastatic disease (41.2%) [96]. Patient samples highlight the role of AIB1 in metastasis, as presence of nuclear staining not only correlates with increased disease grade, but metastasis rate.

In molecular studies, specific transcription factor interactions with AIB1 have been identified as integral to promoting metastasis. The ERK3 mediated interaction of AIB1 with the Ets family member PEA3 results in the oncogenic transcription of matrix metalloproteinases MMP2 and MMP9, which promote an EMT phenotype and destruction of the surrounding extracellular matrix, leading to invasion [74]. Reduction of ERK3 or AIB1 by shRNA attenuates metastasis in lung cancer models; unsurprisingly ERK3 is also upregulated in lung cancer clinical samples [71]. Similarly, AIB1 interaction with AP-1 upregulates MMP7 and MMP10, leading to increased metastasis in breast cancer, regardless of hormone receptor status. Alternatively, AIB1 binds to the promoters of Notch intracellular domain targets to enhance Notch proliferative signaling and effecting cell cycle progression in colorectal cancer. AIB1 reduction by genetic knockout reduced the Notch targets HES1 as well as Cyclins (likely controlled by E2F1-AIB1, however). Reduction of AIB1 levels yields significantly reduced tumor burden and lung metastasis [94].

Finally, an underexplored area of AIB1 activity may be its role in promoting invasion and migration by facilitating oncogenic transcription factor cooperation. Recently, AIB1 was shown to be recruited to larger TEAD and AP-1 transcription factor complexes, and promote cooperative transcription of DOCK4 and DOCK9, thereby increasing mobility [59]. Endogenous TEAD and AP-1 share a significant degree of genomic co-enriched ChIP-seq peaks, and it remains to be seen exactly how much of this is mediated by AIB1, as the two transcription factors are potent oncogenes [62]. Further, transcription factor cooperation has been suggested with TEAD, AP-1, SRF, and other stimuli responsive transcription factors—determining to what extent AIB1 or other NCOA family members are relevant to such signaling networks is an unanswered question [60, 63, 64].

7.4 The role of AIB1Δ4 in metastasis

The AIB1Δ4 isoform is an N-terminally truncated splice variant that lacks the bHLH-PAS domain. In vitro it localizes significantly less in the nucleus, and shuttles more often between the nucleus and cytoplasm. It may be more readily retained in the cytoplasm because of its interaction with FAK and its role in focal adhesions (see Section 2.2) [25]. Interestingly we have found that metastatic MDA-MB-231 triple negative cell lines and metastatic Colo357 pancreatic ductal adenocarcinoma lines have significantly higher AIB1Δ4/AIB1 ratio than their parental counterparts [22]. This aggressive phenotype may be due to loss of transcriptional repression by ANCO1, as we have shown previously [24].

In the cell periphery, AIB1Δ4 interacts with FAK in lamellipodia and filopodia in response to EGF stimulation in breast cancer cells. The isoform in unable to bind to the promoters of the MMPs previously described. Instead, it is phosphorylated by PAK1 which facilitates interaction with EGFR and FAK to promote transwell migration in vitro. These findings correlated with in vivo studies showing overexpression AIB1Δ4 significantly increased metastasis to the lymph node and lung [25]. These data match our earlier findings in isotypic cell lines and highlight the many ways this understudied isoform may be contributing to normal and disease biology.

7.5 Therapeutics and AIB1

Briefly, AIB1 can contribute to chemo- or hormonal therapeutic resistance, promoting disease relapse. Early indications of this 'escape route' came about when stratifying AIB1 levels and outcomes of ER+ patients treated with tamoxifen, a selective estrogen receptor modulator that is a first line treatment. Patients with high AIB1 levels after receiving treatment had significantly worse outcomes than those with low AIB1 (oddly, patients who never received tamoxifen and also had AIB1 did significantly better; this has yet to be explained). The same study showed conclusively that high levels of Her2/Neu and AIB1 significantly increase the chance of relapse, likely due to crosstalk between growth factor and hormone signaling pathways that thereby circumvent estrogen dependence [42, 97, 98]. Later, it was shown that direct competition between AIB1 and PAX2 occurs on the ERBB2 gene body encoding Her2. Loss of PAX2 reverses repression of Her2 transcription mediated by tamoxifen, and ectopic expression reverts AIB1 mediated tamoxifen resistance [99]. Finally, upregulation of AIB1 has also been shown to be a mechanism of taxol resistance in breast cancer [31].

8. Discussion and conclusion

AIB1 in an oncogene that contributes to disease progression in multiple cancers. It primarily acts to augment transcriptional activity, thereby amplifying pro-proliferative and pro-tumorigenic signaling cascades through binding to its many partners. Though primarily studied for its role in interacting with nuclear hormone receptors, AIB1 has been clearly implicated to play an oncogenic role in hormone independent cancers. Genetic manipulation or removal of the NCOA3 gene has almost universally slowed cancer progression wherever studied, likely by dampening all of the pathways it usually effects. From this role, its clinical importance is obvious, as its elevated levels is usually a negative prognostic marker.

Most clinical studies have underscored the importance of AIB1 in the progression of human disease. Overwhelmingly, expression of AIB1 is correlated with poor prognosis in breast, ovarian, pancreatic, prostate, and colon cancer, as well as increased metastasis [25, 100]. Heightened AIB1 levels have successfully been used in the clinic as a negative prognostic marker in post-menopausal breast cancer [101] and may mark tamoxifen resistance [102]. Further, preclinical investigation of compounds that promote the degradation of AIB1 have shown promising results in attenuating the effects of the oncogene [103, 104]. Interestingly, pharmacological hyperstimulation of AIB1 has also been shown to induce cell death by increasing cell stress [105].

Clarifying the extent to which AIB1 is critical in bridging cooperating transcription factors will further explain intracellular signaling biology and may also provide new targets for therapeutic development. Also, there remains a gap in knowledge surrounding the role of the AIB1Δ4 in the nucleus, especially in regard to global binding and transcriptional patterns of the isoform. Finally, elucidating the importance of AIB1 as an effector of growth factor and cytokine signaling may explain its potent oncogenic nature. It is clear that AIB1 may be both a clinically relevant prognostic marker and a promising therapeutic target, as evidenced by the promising preclinical data.

Conflict of interest

The authors declare no conflicts of interest.

Author details

Max H. Kushner, Anna T. Riegel and Ghada M. Sharif*
Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA

*Address all correspondence to: gms58@georgetown.edu

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, et al. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science. 1997;**277**:965-968

[2] Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, et al. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell. 1997;**90**:569-580. DOI: 10.1016/S0092-8674(00)80516-4

[3] Takeshita A, Cardona GR, Koibuchi N, Suen CS, Chin WW. TRAM-1, a novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. Journal of Biological Chemistry. 1997;**272**:27629-27634

[4] Li H, Gomes PJ, Chen JD. RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:8479-8484

[5] Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW. The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**: 6379-6384. DOI: 10.1073/pnas.120166297

[6] Suen CS, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR, Frail DE. A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. Journal of Biological Chemistry. 1998;**273**:27645-27653

[7] Torres-Arzayus MI, de Mora JF, Yuan J, Vazquez F, Bronson R, Rue M, et al. High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. Cancer Cell. 2004;**6**:263-274. DOI: 10.1016/j.ccr.2004.06.027

[8] Belandia B, Parker MG. Functional interaction between the p160 coactivator proteins and the transcriptional enhancer factor family of transcription factors. Journal of Biological Chemistry. 2000;**275**:30801-30805. DOI: 10.1074/jbc.C000484200

[9] Louie MC, Zou JX, Rabinovich A, Chen HW. ACTR/AIB1 functions as an E2F1 coactivator to promote breast cancer cell proliferation and antiestrogen resistance. Molecular and Cellular Biology. 2004;**24**:5157-5171. DOI: 10.1128/MCB.24.12.5157-5171.2004

[10] Lee SK, Kim HJ, Na SY, Kim TS, Choi HS, Im SY, et al. Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. Journal of Biological Chemistry. 1998;**273**:16651-16654

[11] Werbajh S, Nojek I, Lanz R, Costas MA. RAC-3 is a NF-kappa B coactivator. FEBS Letters. 2000;**485**:195-199

[12] Johnson AB, O'Malley BW. Steroid receptor coactivators 1, 2, and 3: Critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy. Molecular and Cellular Endocrinology. 2012;**348**:430-439. DOI: 10.1016/j.mce.2011.04.021

[13] Li C, Wu R-C, Amazit L, Tsai SY, Tsai M-J, O'Malley BW. Specific amino acid residues in the basic helix-loop-helix domain of SRC-3 are essential for its nuclear localization and proteasome-dependent turnover. Molecular and

Cellular Biology. 2007;**27**:1296-1308. DOI: 10.1128/MCB.00336-06

[14] Huang ZJ, Edery I, Rosbash M. PAS is a dimerization domain common to Drosophila period and several transcription factors. Nature. 1993;**364**:259-262. DOI: 10.1038/364259a0

[15] Chang CY, Norris JD, Grøn H, Paige LA, Hamilton PT, Kenan DJ, et al. Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: Discovery of peptide antagonists of estrogen receptors alpha and beta. Molecular and Cellular Biology. 1999;**19**:8226-8239

[16] Coulthard VH, Matsuda S, Heery DM. An extended LXXLL motif sequence determines the nuclear receptor binding specificity of TRAP220. Journal of Biological Chemistry. 2003;**278**:10942-10951. DOI: 10.1074/jbc.M212950200

[17] Bramlett KS, Wu Y, Burris TP. Ligands specify coactivator nuclear receptor (NR) box affinity for estrogen receptor subtypes. Molecular Endocrinology. 2001;**15**:909-922. DOI: 10.1210/mend.15.6.0649

[18] Koh SS, Chen D, Lee YH, Stallcup MR. Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. Journal of Biological Chemistry. 2001;**276**:1089-1098. DOI: 10.1074/jbc.M004228200

[19] Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, et al. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature. 1997;**389**:194-198. DOI: 10.1038/38304

[20] Reiter R, Wellstein A, Riegel AT. An isoform of the coactivator AIB1 that increases hormone and growth factor sensitivity is overexpressed in breast cancer. Journal of Biological Chemistry. 2001;**276**:39736-39741. DOI: 10.1074/jbc.M104744200

[21] List HJ, Reiter R, Singh B, Wellstein A, Riegel AT. Expression of the nuclear coactivator AIB1 in normal and malignant breast tissue. Breast Cancer Research and Treatment. 2001;**68**:21-28

[22] Chien CD, Kirilyuk A, Li JV, Zhang W, Lahusen T, Schmidt MO, et al. Role of the nuclear receptor coactivator AIB1-Delta4 splice variant in the control of gene transcription. The Journal of Biological Chemistry. 2011;**286**:26813-26827. DOI: 10.1074/jbc.M110.216200

[23] Zhang A, Yeung PL, Li C-W, Tsai S-C, Dinh GK, Wu X, et al. Identification of a novel family of ankyrin repeats containing cofactors for p160 nuclear receptor coactivators. Journal of Biological Chemistry. 2004;**279**:33799-33805. DOI: 10.1074/jbc.M403997200

[24] Garee JP, Chien CD, Li JV, Wellstein A, Riegel AT. Regulation of HER2 oncogene transcription by a multifunctional coactivator/corepressor complex. Molecular Endocrinology. 2014;**28**:846-859. DOI: 10.1210/me.2013-1379

[25] Long W, Yi P, Amazit L, LaMarca HL, Ashcroft F, Kumar R, et al. SRC-3Δ4 mediates the interaction of EGFR with FAK to promote cell migration. Molecular Cell. 2010;**37**:321-332. DOI: 10.1016/j.molcel.2010.01.004

[26] Yoshida H, Liu J, Samuel S, Cheng W, Rosen D, Naora H. Steroid receptor coactivator-3, a homolog of Taiman that controls cell migration in the Drosophila ovary, regulates migration of human ovarian cancer cells. Molecular and Cellular Endocrinology. 2005;**245**:77-85. DOI: 10.1016/j.mce.2005.10.008

[27] Tilli MT, Reiter R, Oh AS, Henke RT, McDonnell K, Gallicano GI, et al. Overexpression of an N-terminally truncated isoform of the nuclear receptor coactivator amplified in breast cancer 1 leads to altered proliferation of mammary epithelial cells in transgenic mice. Molecular Endocrinology. 2005;**19**:644-656. DOI: 10.1210/me.2004-0106

[28] Nakles RE, Shiffert MT, Díaz-Cruz ES, Cabrera MC, Alotaiby M, Miermont AM, et al. Altered AIB1 or AIB1Δ3 expression impacts ERα effects on mammary gland stromal and epithelial content. Molecular Endocrinology. 2011;**25**:549-563. DOI: 10.1210/me.2010-0114

[29] Louie MC, Revenko AS, Zou JX, Yao J, Chen HW. Direct control of cell cycle gene expression by proto-oncogene product ACTR, and its autoregulation underlies its transforming activity. Molecular and Cellular Biology. 2006;**26**:3810-3823. DOI: 10.1128/MCB.26.10.3810-3823.2006

[30] Li JV, Chien CD, Garee JP, Xu J, Wellstein A, Riegel AT. Transcriptional repression of AIB1 by FoxG1 leads to apoptosis in breast cancer cells. Molecular Endocrinology. 2013;**27**:1113-1127. DOI: 10.1210/me.2012-1353

[31] Ao X, Nie P, Wu B, Xu W, Zhang T, Wang S, et al. Decreased expression of microRNA-17 and microRNA-20b promotes breast cancer resistance to taxol therapy by upregulation of NCOA3. Cell Death and Disease. 2016;**7**:e2463-e2463. DOI: 10.1038/cddis.2016.367

[32] Hossain A, Kuo MT, Saunders GF. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Molecular and Cellular Biology. 2006;**26**:8191-8201. DOI: 10.1128/MCB.00242-06

[33] Wu R-C, Feng Q, Lonard DM, O'Malley BW. SRC-3 coactivator functional lifetime is regulated by a phospho-dependent ubiquitin time clock. Cell. 2007;**129**:1125-1140. DOI: 10.1016/j.cell.2007.04.039

[34] Li C, Liang Y-Y, Feng X-H, Tsai SY, Tsai M-J, O'Malley BW. Essential phosphatases and a phospho-degron are critical for regulation of SRC-3/AIB1 coactivator function and turnover. Molecular Cell. 2008;**31**:835-849. DOI: 10.1016/j.molcel.2008.07.019

[35] Wu R-C, Qin J, Yi P, Wong J, Tsai SY, Tsai M-J, et al. Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic reponses to multiple cellular signaling pathways. Molecular Cell. 2004;**15**:937-949. DOI: 10.1016/j.molcel.2004.08.019

[36] York B, Yu C, Sagen JV, Liu Z, Nikolai BC, Wu R-C, et al. Reprogramming the posttranslational code of SRC-3 confers a switch in mammalian systems biology. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:11122-11127. DOI: 10.1073/pnas.1005262107

[37] Oh A, List H-J, Reiter R, Mani A, Zhang Y, Gehan E, et al. The nuclear receptor coactivator AIB1 mediates insulin-like growth factor I-induced phenotypic changes in human breast cancer cells. Cancer Research. 2004;**64**:8299-8308. DOI: 10.1158/0008-5472.CAN-04-0354

[38] Oh AS, Lahusen JT, Chien CD, Fereshteh MP, Zhang X, Dakshanamurthy S, et al. Tyrosine phosphorylation of the nuclear receptor coactivator AIB1/SRC-3 is enhanced by Abl kinase and is required for its activity in cancer cells. Molecular and Cellular Biology. 2008;**28**:6580-6593. DOI: 10.1128/MCB.00118-08

[39] Heery DM, Kalkhoven E, Hoare S, Parker MG. A signature motif in

transcriptional co-activators mediates binding to nuclear receptors. Nature. 1997;**387**:733-736. DOI: 10.1038/42750

[40] Zhou XE, Suino-Powell KM, Li J, He Y, Mackeigan JP, Melcher K, et al. Identification of SRC3/AIB1 as a preferred coactivator for hormone-activated androgen receptor. The Journal of Biological Chemistry. 2010;**285**:9161-9171. DOI: 10.1074/jbc.M109.085779

[41] Shao W, Keeton EK, McDonnell DP, Brown M. Coactivator AIB1 links estrogen receptor transcriptional activity and stability. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:11599-11604. DOI: 10.1073/pnas.0402997101

[42] Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SAW, et al. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. JNCI Journal of the National Cancer Institute. 2003;**95**:353-361

[43] Lahusen T, Henke RT, Kagan BL, Wellstein A, Riegel AT. The role and regulation of the nuclear receptor co-activator AIB1 in breast cancer. Breast Cancer Research and Treatment. 2009;**116**:225-237. DOI: 10.1007/s10549-009-0405-2

[44] Bautista S, Vallès H, Walker RL, Anzick S, Zeillinger R, Meltzer P, et al. In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with estrogen and progesterone receptor positivity. Clinical Cancer Research. 1998;**4**:2925-2929

[45] Bouras T, Southey MC, Venter DJ. Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. Cancer Research. 2001;**61**:903-907

[46] Gnanapragasam VJ, Leung HY, Pulimood AS, Neal DE, Robson CN. Expression of RAC 3, a steroid hormone receptor co-activator in prostate cancer. British Journal of Cancer. 2001;**85**:1928-1936. DOI: 10.1054/bjoc.2001.2179

[47] Lahusen T, Fereshteh M, Oh A, Wellstein A, Riegel AT. Epidermal growth factor receptor tyrosine phosphorylation and signaling controlled by a nuclear receptor coactivator, amplified in breast cancer 1. Cancer Research. 2007;**67**:7256-7265. DOI: 10.1158/0008-5472.CAN-07-1013

[48] Fereshteh MP, Tilli MT, Kim SE, Xu J, O'Malley BW, Wellstein A, et al. The nuclear receptor coactivator amplified in breast cancer-1 is required for Neu (ErbB2/HER2) activation, signaling, and mammary tumorigenesis in mice. Cancer Research. 2008;**68**:3697-3706. DOI: 10.1158/0008-5472.CAN-07-6702

[49] Laviola L, Natalicchio A, Giorgino F. The IGF-I signaling pathway. Current Pharmaceutical Design. 2007;**13**:663-669

[50] Kuang S-Q, Liao L, Zhang H, Lee AV, O'Malley BW, Xu J. AIB1/SRC-3 deficiency affects insulin-like growth factor I signaling pathway and suppresses v-Ha-ras-induced breast cancer initiation and progression in mice. Cancer Research. 2004;**64**:1875-1885

[51] Torres-Arzayus MI, Yuan J, DellaGatta JL, Lane H, Kung AL, Brown M. Targeting the AIB1 oncogene through mammalian target of rapamycin inhibition in the mammary gland. Cancer Research. 2006;**66**:11381-11388. DOI: 10.1158/0008-5472.CAN-06-2316

[52] Eferl R, Wagner EF. AP-1: A double-edged sword in tumorigenesis. Nature Reviews. Cancer. 2003;**3**:859-868. DOI: 10.1038/nrc1209

[53] Yan J, Yu CT, Ozen M, Ittmann M, Tsai SY, Tsai MJ. Steroid receptor coactivator-3 and activator protein-1 coordinately regulate the transcription of components of the insulin-like growth factor/AKT signaling pathway. Cancer Research. 2006;**66**:11039-11046. DOI: 10.1158/0008-5472.CAN-06-2442

[54] Li LB, Louie MC, Chen HW, Zou JX. Proto-oncogene ACTR/AIB1 promotes cancer cell invasion by up-regulating specific matrix metalloproteinase expression. Cancer Letters. 2008;**261**:64-73. DOI: 10.1016/j.canlet.2007.11.013

[55] Yan J, Erdem H, Li R, Cai Y, Ayala G, Ittmann M, et al. Steroid receptor coactivator-3/AIB1 promotes cell migration and invasiveness through focal adhesion turnover and matrix metalloproteinase expression. Cancer Research. 2008;**68**:5460-5468. DOI: 10.1158/0008-5472.CAN-08-0955

[56] Vassilev A, Kaneko KJ, Shu H, Zhao Y, DePamphilis ML. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes & Development. 2001;**15**:1229-1241. DOI: 10.1101/gad.888601

[57] Stein C, Bardet AF, Roma G, Bergling S, Clay I, Ruchti A, et al. YAP1 exerts its transcriptional control via TEAD-mediated activation of enhancers. PLoS Genetics. 2015;**11**:e1005465. DOI: 10.1371/journal.pgen.1005465

[58] Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. Cancer Cell. 2016;**29**:783-803. DOI: 10.1016/j.ccell.2016.05.005

[59] Liu X, Li H, Rajurkar M, Li Q, Cotton JL, Ou J, et al. Tead and AP1 coordinate transcription and motility. Cell Reports. 2016;**14**:1169-1180. DOI: 10.1016/j.celrep.2015.12.104

[60] Kim T, Hwang D, Lee D, Kim JH, Kim S-Y, Lim D-S. MRTF potentiates TEAD-YAP transcriptional activity causing metastasis. The EMBO Journal. 2017;**36**:520-535. DOI: 10.15252/embj.201695137

[61] Zhang C, Robinson BS, Xu W, Yang L, Yao B, Zhao H, et al. The ecdysone receptor coactivator Taiman links Yorkie to transcriptional control of germline stem cell factors in somatic tissue. Developmental Cell. 2015;**34**:168-180. DOI: 10.1016/j.devcel.2015.05.010

[62] Zanconato F, Forcato M, Battilana G, Azzolin L, Quaranta E, Bodega B, et al. Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nature Cell Biology. 2015;**17**:1218-1227. DOI: 10.1038/ncb3216

[63] Kim T, Yang S-J, Hwang D, Song J, Kim M, Kyum Kim S, et al. A basal-like breast cancer-specific role for SRF-IL6 in YAP-induced cancer stemness. Nature Communications. 2015;**6**:1309. DOI: 10.1038/ncomms10186

[64] Esnault C, Stewart A, Gualdrini F, East P, Horswell S, Matthews N, et al. Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. Genes & Development. 2014;**28**:943-958. DOI: 10.1101/gad.239327.114

[65] O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nature Reviews. Clinical Oncology. 2016;**13**:417-430. DOI: 10.1038/nrclinonc.2016.26

[66] Dimova DK, Dyson NJ. The E2F transcriptional network: Old acquaintances with new faces. Oncogene. 2005;**24**:2810-2826. DOI: 10.1038/sj.onc.1208612

[67] Mussi P, Yu C, O'Malley BW, Xu J. Stimulation of steroid

receptor coactivator-3 (SRC-3) gene overexpression by a positive regulatory loop of E2F1 and SRC-3. Molecular Endocrinology. 2006;**20**:3105-3119. DOI: 10.1210/me.2005-0522

[68] Nikolai BC, Lanz RB, York B, Dasgupta S, Mitsiades N, Creighton CJ, et al. HER2 signaling drives DNA anabolism and proliferation through SRC-3 phosphorylation and E2F1-regulated genes. Cancer Research. 2016;**76**:1463-1475. DOI: 10.1158/0008-5472.CAN-15-2383

[69] O'Hagan RC, Hassell JA. The PEA3 Ets transcription factor is a downstream target of the HER2/Neu receptor tyrosine kinase. Oncogene. 1998;**16**:301-310. DOI: 10.1038/sj.onc.1201547

[70] Fleming FJ, Myers E, Kelly G, Crotty TB, McDermott EW, O'Higgins NJ, et al. Expression of SRC-1, AIB1, and PEA3 in HER2 mediated endocrine resistant breast cancer; a predictive role for SRC-1. Journal of Clinical Pathology. 2004;**57**:1069-1074. DOI: 10.1136/jcp.2004.016733

[71] Long W, Foulds CE, Qin J, Liu J, Ding C, Lonard DM, et al. ERK3 signals through SRC-3 coactivator to promote human lung cancer cell invasion. The Journal of Clinical Investigation. 2012;**122**:1869-1880. DOI: 10.1172/JCI61492

[72] Myers E, Hill ADK, Kelly G, McDermott EW, O'Higgins NJ, Buggy Y, et al. Associations and interactions between Ets-1 and Ets-2 and coregulatory proteins, SRC-1, AIB1, and NCoR in breast cancer. Clinical Cancer Research. 2005;**11**:2111-2122. DOI: 10.1158/1078-0432.CCR-04-1192

[73] Myers E, Hill ADK, Kelly G, McDermott EW, O'Higgins NJ, Young LS. A positive role for PEA3 in HER2-mediated breast tumour progression. British Journal of Cancer. 2006;**95**:1404-1409. DOI: 10.1038/sj.bjc.6603427

[74] Qin L, Liao L, Redmond A, Young L, Yuan Y, Chen H, et al. The AIB1 oncogene promotes breast Cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression. Molecular and Cellular Biology. 2008;**28**:5937-5950. DOI: 10.1128/MCB.00579-08

[75] Wu RC, Qin J, Hashimoto Y, Wong J, Xu J, Tsai SY, et al. Regulation of SRC-3 (pCIP/ACTR/AIB-1/RAC-3/TRAM-1) coactivator activity by I B kinase. Molecular and Cellular Biology. 2002;**22**:3549-3561. DOI: 10.1128/MCB.22.10.3549-3561.2002

[76] Litterst CM, Pfitzner E. Transcriptional activation by STAT6 requires the direct interaction with NCoA-1. Journal of Biological Chemistry. 2001;**276**:45713-45721. DOI: 10.1074/jbc.M108132200

[77] Litterst CM, Pfitzner E. An LXXLL motif in the transactivation domain of STAT6 mediates recruitment of NCoA-1/SRC-1. Journal of Biological Chemistry. 2002;**277**:36052-36060. DOI: 10.1074/jbc.M203556200

[78] Arimura A, vn Peer M, Schröder AJ, Rothman PB. The transcriptional co-activator p/CIP (NCoA-3) is up-regulated by STAT6 and serves as a positive regulator of transcriptional activation by STAT6. Journal of Biological Chemistry. 2004;**279**:31105-31112. DOI: 10.1074/jbc.M404428200

[79] Razeto A, Ramakrishnan V, Litterst CM, Giller K, Griesinger C, Carlomagno T, et al. Structure of the NCoA-1/SRC-1 PAS-B domain bound to the LXXLL motif of the STAT6 transactivation domain. Journal of Molecular Biology. 2004;**336**:319-329

[80] Münz T, Litterst CM, Pfitzner E. Interaction of STAT6 with its co-activator SRC-1/NCoA-1 is regulated by dephosphorylation of the latter

via PP2A. Nucleic Acids Research. 2011;**39**:3255-3266. DOI: 10.1093/nar/gkq1225

[81] Zhou H-J, Yan J, Luo W, Ayala G, Lin S-H, Erdem H, et al. SRC-3 is required for prostate cancer cell proliferation and survival. Cancer Research. 2005;**65**:7976-7983. DOI: 10.1158/0008-5472.CAN-04-4076

[82] Zou JX, Zhong Z, Shi X-B, Tepper CG, deVere White RW, Kung H-J, et al. ACTR/AIB1/SRC-3 and androgen receptor control prostate cancer cell proliferation and tumor growth through direct control of cell cycle genes. Prostate. 2006;**66**:1474-1486. DOI: 10.1002/pros.20477

[83] Tien JC-Y, Liu Z, Liao L, Wang F, Xu Y, Wu Y-L, et al. The steroid receptor coactivator-3 is required for the development of castration-resistant prostate cancer. Cancer Research. 2013;**73**:3997-4008. DOI: 10.1158/0008-5472.CAN-12-3929

[84] Iwase H, Omoto Y, Toyama T, Yamashita H, Hara Y, Sugiura H, et al. Clinical significance of AIB1 expression in human breast cancer. Breast Cancer Research and Treatment. 2003;**80**:339-345. DOI: 10.1023/A:1024916126532

[85] Espina V, Liotta LA. What is the malignant nature of human ductal carcinoma in situ? Nature Publishing Group. 2011;**11**:68-75. DOI: 10.1038/nrc2950

[86] Lesurf R, Aure MR, Mørk HH, Vitelli V, Lundgren S, Børresen-Dale A-L, et al. Molecular features of subtype-specific progression from ductal carcinoma in situ to invasive breast cancer. Cell Reports. 2016;**16**:1166-1179. DOI: 10.1016/j.celrep.2016.06.051

[87] Ory V, Tassi E, Cavalli LR, Sharif GM, Saenz F, Baker T, et al. The nuclear coactivator amplified in breast cancer 1 maintains tumor-initiating cells during development of ductal carcinoma in situ. Oncogene. 2013;**33**:3033-3042. DOI: 10.1038/onc.2013.263

[88] Li AJ, Lerner DL, Gapuzan M-ER, Karlan BY. AIB1 polymorphisms predict aggressive ovarian cancer phenotype. Cancer Epidemiology Biomarkers & Prevention. 2005;**14**:2919-2922. DOI: 10.1158/1055-9965.EPI-05-0540

[89] Henke RT, Haddad BR, Kim SE, Rone JD, Mani A, Jessup JM, et al. Overexpression of the nuclear receptor coactivator AIB1 (SRC-3) during progression of pancreatic adenocarcinoma. Clinical Cancer Research. 2004;**10**:6134-6142. DOI: 10.1158/1078-0432.CCR-04-0561

[90] Kumar S, Das S, Rachagani S, Kaur S, Joshi S, Johansson SL, et al. NCOA3-mediated upregulation of mucin expression via transcriptional and post-translational changes during the development of pancreatic cancer. Oncogene. 2014;**34**:4879-4889. DOI: 10.1038/onc.2014.409

[91] Hsing AW, Chokkalingam AP, Gao Y-T, Wu G, Wang X, Deng J, et al. Polymorphic CAG/CAA repeat length in the AIB1/SRC-3 gene and prostate cancer risk: A population-based case-control study. Cancer Epidemiology Biomarkers & Prevention. 2002;**11**:337-341

[92] Xie D, Sham JST, Zeng W-F, Lin H-L, Bi J, Che L-H, et al. Correlation of AIB1 overexpression with advanced clinical stage of human colorectal carcinoma. Human Pathology. 2005;**36**:777-783. DOI: 10.1016/j.humpath.2005.05.007

[93] Grivas PD, Tzelepi V, Sotiropoulou-Bonikou G, Kefalopoulou Z, Papavassiliou AG, Kalofonos H. Estrogen receptor alpha/beta, AIB1, and TIF2 in colorectal carcinogenesis: Do coregulators have prognostic

significance? International Journal of Colorectal Disease. 2009;**24**:613-622. DOI: 10.1007/s00384-009-0647-9

[94] Mo P, Zhou Q, Guan L, Wang Y, Wang W, Miao M, et al. Amplified in breast cancer 1 promotes colorectal cancer progression through enhancing notch signaling. Oncogene. 2014;**34**:3935-3945. DOI: 10.1038/onc.2014.324

[95] Guo S, Xu J, Xue R, Liu Y, Yu H. Overexpression of AIB1 correlates inversely with E-cadherin expression in pancreatic adenocarcinoma and may promote lymph node metastasis. International Journal of Clinical Oncology. 2014;**19**:319-324. DOI: 10.1007/s10147-013-0549-2

[96] Liu M-Y, Guo H-P, Hong C-Q, Peng H-W, Yang X-H, Zhang H. Up-regulation of nuclear receptor coactivator amplified in breast cancer-1 in papillary thyroid carcinoma correlates with lymph node metastasis. Clinical & Translational Oncology. 2013;**15**:947-952. DOI: 10.1007/s12094-013-1029-x

[97] Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. Clinical Cancer Research. 2005;**11**:865s-870s

[98] Shou J, Massarweh S, Osborne CK, Wakeling AE, Ali S, Weiss H, et al. Mechanisms of tamoxifen resistance: Increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. Journal of the National Cancer Institute. 2004;**96**:926-935

[99] Hurtado A, Holmes KA, Geistlinger TR, Hutcheson IR, Nicholson RI, Brown M, et al. Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. Nature. 2008;**456**:663-666. DOI: 10.1038/nature07483

[100] Dasgupta S, Lonard DM, O'Malley BW. Nuclear receptor coactivators: Master regulators of human health and disease. Annual Review of Medicine. 2014;**65**:279-292. DOI: 10.1146/annurev-med-051812-145316

[101] Alkner S, Jensen M-B, Rasmussen BB, Bendahl P-O, Fernö M, Rydén L, et al. Prognostic and predictive importance of the estrogen receptor coactivator AIB1 in a randomized trial comparing adjuvant letrozole and tamoxifen therapy in postmenopausal breast cancer: The Danish cohort of BIG 1-98. Breast Cancer Research and Treatment. 2017;**166**:481-490. DOI: 10.1007/s10549-017-4416-0

[102] Alkner S, Bendahl P-O, Ehinger A, Lövgren K, Rydén L, Fernö M. Prior adjuvant tamoxifen treatment in breast cancer is linked to increased AIB1 and HER2 expression in metachronous contralateral breast cancer. PLoS One. 2016;**11**:e0150977. DOI: 10.1371/journal.pone.0150977

[103] Wang Y, Lonard DM, Yu Y, Chow D-C, Palzkill TG, Wang J, et al. Bufalin is a potent small-molecule inhibitor of the steroid receptor coactivators SRC-3 and SRC-1. Cancer Research. 2014;**74**:1506-1517. DOI: 10.1158/0008-5472.CAN-13-2939

[104] Song X, Chen J, Zhao M, Zhang C, Yu Y, Lonard DM, et al. Development of potent small-molecule inhibitors to drug the undruggable steroid receptor coactivator-3. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**:4970-4975. DOI: 10.1073/pnas.1604274113

[105] Wang L, Yu Y, Chow D-C, Yan F, Hsu C-C, Stossi F, et al. Characterization of a steroid receptor coactivator small molecule stimulator that overstimulates cancer cells and leads to cell stress and death. Cancer Cell. 2015;**28**:240-252. DOI: 10.1016/j.ccell.2015.07.005

Chapter 5

Oncogenetics of Lung Cancer Induced by Environmental Carcinogens

Victor D. Martinez, Adam P. Sage, Erin A. Marshall, Miwa Suzuki, Aaron A. Goodarzi, Graham Dellaire and Wan L. Lam

Abstract

The molecular landscape of non-tobacco-induced primary lung tumors displays specific oncogenetic features. The etiology of these tumors has been largely associated with exposure to well-established environmental lung carcinogens such as radon, arsenic, and asbestos. Environmental carcinogens can induce specific genetic and epigenetic alterations in lung tissue, leading to aberrant function of lung cancer oncogenes and tumor suppressor genes. These molecular events result in the disruption of key cellular mechanisms, such as protection against oxidative stress and DNA damage-repair, which promotes tumor development and progression. This chapter provides a comprehensive discussion of the specific carcinogenic mechanisms associated with exposure to radon, arsenic, and asbestos. It also summarizes the main protein-coding and non-coding genes affected by exposure to these environmental agents, and the underlying molecular mechanisms promoting their deregulation in lung cancer. Finally, the chapter examines the anticipated challenges in personalized intervention strategies in non-tobacco-induced lung cancer.

Keywords: lung cancer, environmental carcinogens, radon, arsenic, asbestos

1. Introduction

Lung cancer remains the deadliest form of cancer across the globe [1]. While smoking rates have decreased in many areas, it remains to be seen if the incidence and mortality of primary lung cancer will experience a similar shift, particularly in light of the observation that close to 25% of cases arise in individuals who have never smoked [2]. As one of the most environmentally-influenced malignancies, lung tumorigenesis can result from exposure to both physical and chemical carcinogens. Exposure to the mix of compounds present in particulate matter is another well-known factor affecting the development of lung cancer [3]. However, a number of single-agent compounds in the environment have been identified as key lung carcinogens, particularly arsenic, asbestos and radioactive radon (^{222}Rn) gas [4]. These compounds are distributed at varying, potentially-dangerous concentrations in the environment, affecting hundreds of millions of people worldwide.

Exposure to each of arsenic, asbestos, and radon has been shown to induce widespread genetic and epigenetic alterations, which may account for their strong carcinogenicity, independent of smoking status [4]. Interestingly, the molecular aberrations associated with these compounds and the onset of lung cancer in never-smokers follows a mechanism distinct from that of tobacco smoke [5]. While strict guidelines regarding exposure to these compounds have been implemented in some regions, mounting evidence suggests that carcinogenic effects may result from chronic exposure to environmental levels that are well below those currently deemed "safe" [6, 7]. Additionally, individual differences may contribute to varying degrees of susceptibility to the carcinogenic effects of these compounds. For instance, women have been shown to have a higher incidence of lung cancer arising in never-smokers. This inequality can potentially be attributed to a historical bias towards women being more present in the home, resulting in increased exposure to high radon concentrations and polyaromatic hydrocarbons from various home combustion sources [8]. As these genetic and epigenetic aberrations might be indicative of specific molecular damage induced by these carcinogens, they may be able to be used to develop personalized approaches for risk assessment, monitoring and subsequent disease treatment. Thus, it is critical to uncover the extent of these events associated with exposure to environmental carcinogens.

Arsenic is a class I International Agency for Research on Cancer (IARC) carcinogen that threatens global health through its persistent accumulation in drinking water sources, leading to the onset of skin and lung cancers, among other diseases [9]. Asbestos fibers are naturally occurring silicate mineral fibers that have long been used in industry as building insulation, and are closely linked with not only the well-known outcome of mesothelioma, but also to 5–7% of all lung cancer cases [10]. Radon gas accounts for between 3 and 14% of all lung tumors in a given country and is the second most-common cause of lung cancer, behind smoking [11]. While the radioactive gas normally diffuses easily in open air, it can build up in indoor environments and is readily dissolved into water, which can lead to malignancies through radioactive decay and alpha particle emission [11]. Moreover, drinking water may be a particularly prevalent source of exposure to environmental carcinogens, as it is a primary route of exposure for both arsenic and radon, emphasizing the need for a focus on water contamination measurement and remediation. As arsenic, asbestos, and radon exert their carcinogenic effects through different exposure routes, they display similar, yet distinct mechanisms of genetic and epigenetic aberration, which may be useful in the identification and treatment of tumors caused by these agents.

In this chapter we highlight the molecular alterations induced by exposure to arsenic, asbestos, and radon in key lung cancer pathways, and finish with a discussion of the potential translational applications of environmentally-induced molecular damage.

2. Arsenic

2.1 Physiological and molecular impact of exposure

Arsenic exposure largely occurs through contaminated drinking-water sources, but this problem extends well beyond known arsenic-endemic areas. In fact, it is estimated that 200 million individuals are exposed worldwide to levels deemed non-toxic by the WHO, but shown to induce molecular damage [12].

The toxic effects of arsenic are prevalent from ingestion to excretion and are largely attributed to its various metabolites (**Figure 1**). Once ingested, arsenate

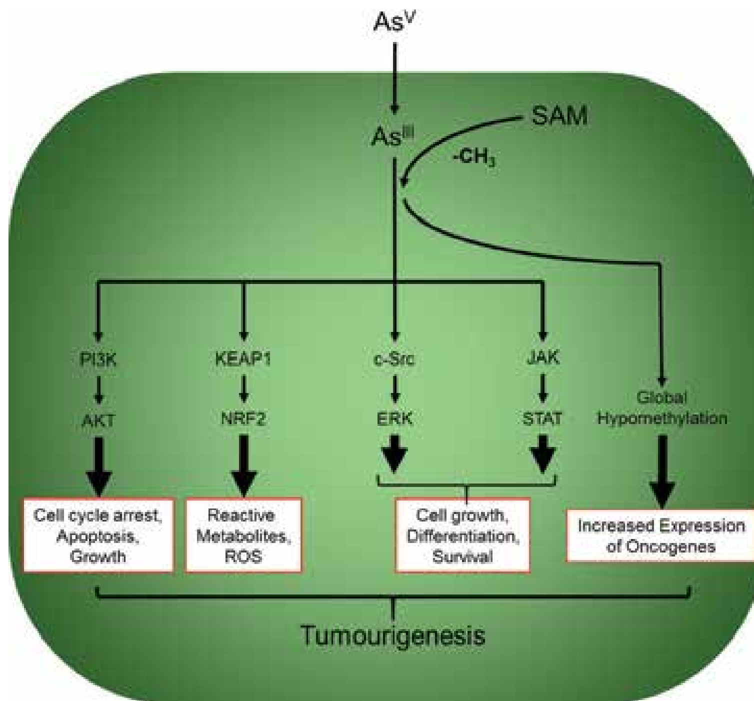


Figure 1.
Molecular mechanisms of arsenic-induced carcinogenesis.

(As^V)—the most common form of the compound in the environment—is taken into cells through membrane transporters, where it is quickly reduced to arsenite (As^{III}) by oxidoreductases including purine nucleotide phosphorylase (PNP) and glutathione-s-transferase omega (GSTO). As^{III} is the most toxic form of arsenic, largely due to its subsequent methylation by methyltransferase enzymes such as arsenic (+3) methyltransferase (As_3MT), a process exploited for promoting the excretion of arsenic [13]. However, methyl groups are provided by *S*-adenosylmethionine (SAM), a key cellular methyl group donor. Methylation of arsenic inside the cell can thus lead to the depletion of the cellular methyl pool through a high demand on SAM, which then promotes global DNA hypomethylation and aberrant histone modification [14–17]. Disruptions in the cellular methyl pool can lead to major disruptions in gene expression, which is known to contribute to malignant transformation [16].

The genomic instability and global changes in gene expression resulting from the exposure and biotransformation of arsenic is exacerbated by the widespread induction of DNA damage from toxic arsenic byproducts. In fact, arsenic has been demonstrated to cause distinct alterations in chromatin, gene expression (both coding and non-coding), as well as splicing, and transcription initiation [18]. In particular, one of the methylated species of arsenic, monomethylarsonic acid (MMA^{III}), can interrupt the electron transport chain in mitochondria, liberating electrons and inducing the formation of reactive oxygen species (ROS) [15, 19, 20]. ROS generated from arsenic exposure result in widespread DNA damage, including single- and double-stranded DNA breaks, DNA base oxidation leading to mutations (largely G>C → T>A transversions), adducts, deletions and even damage to mitochondrial DNA (mtDNA) [20–22]. Unsurprisingly, as oxidative stress is a known driver of tumorigenesis in multiple tissues, the DNA damage induced from arsenic exposure is thought to be a main mechanism of its carcinogenicity [23–25]. The disruption of the electron transport chain produces ROS such as hydroxyl

radicals (OH•), superoxide anion radicals (O_2•−), and hydrogen peroxide (H_2O_2), which can further damage cells through lipid oxidation, protein oxidation, and reduction of the mitochondrial membrane potential [26]. The subsequent liberation of cytochrome c can activate apoptotic pathways through caspases, leading to an abnormal rate of cell death. However in addition to faulty apoptotic signaling, exposure to arsenic can also lead to further aberrations in DNA-repair pathways. Here, arsenic affects the expression of genes involved in both nucleotide- (NER) and base-excision repair (BER) mechanisms, allowing the cell to continue through the cell cycle despite extensive damage and genomic instability [27–30]. Thus, arsenic exposure can induce an array of molecular damage across the genome and epigenome, culminating in malignant transformation.

2.2 Carcinogenic mechanisms

While it is exposure to the methylated metabolic byproducts that yields the largest toxic effects resulting from exposure to environmental arsenic, it is noteworthy that even at very low doses, arsenic may be able to act as a co-mutagen to other known carcinogens, such as ultraviolet light, X-rays, methyl methane sulfonate, and tobacco smoke [15]. ROS are perhaps more immediately damaging to cells, as they can lead to alterations in a variety of lung cancer-specific pathways. As stated previously, arsenic exposure can interfere with DNA damage repair pathways, which exacerbates the effects of ROS generation. In the NER pathway, arsenic can alter the expression of key damage-repair genes, such as XPC, in a process that may be mediated by the proteasome [31].

Collectively, aberrations in cellular DNA-damage repair pathways may not only highlight mechanisms of arsenic toxicity, but also its co-mutagenic effects. One of the most common pathways affected in lung cancer is the constitutive activation of the epidermal growth factor receptor (EGFR), especially in women and individuals who have never smoked [32]. Both amplification and mutation can lead to EGFR activation, which subsequently stimulates cell proliferation. As^{III} can activate proto-oncogene c-Src (c-Src) through vicinal sulfhydryl groups, which then promotes phosphorylation events in intracellular EGFR tyrosine residues (Tyr845) [32]. As tyrosine phosphorylation is a key event in EGFR activation, As^{III} thus promotes EGFR constitutive signaling. Alternatively, arsenic exposure may also indirectly affect downstream members of the EGFR pathway, through arsenic-induced oxidative stress and ROS, a common mechanism of environmentally-induced lung carcinogenesis. In a mechanism similar to that of EGFR activation, arsenic has been shown to induce the phosphorylation of several potential substrates of protein kinase B (Akt), a regulator of epithelial-to-mesenchymal transition (EMT) and metastasis, inducing cell migration [33]. Specifically, arsenic may affect c-Jun N-terminal kinase (JNK) activation and subsequent activation of signal transducer and activator of transcription 3 (STAT3), resulting in Akt growth and migration signaling [34]. Similarly, arsenic may increase the enzymatic activity of phosphoinositide 3-kinase (PI3K) and Akt phosphorylation, a key pathway in lung cancer tumorigenesis and progression [35]. The mechanism of PI3K/AKT activation has proven elusive, yet evidence suggests that ROS may play a mediating role, as well as alterations in histone modifications and activation of other related pathways, such as EGFR, mammalian target of rapamycin (mTOR), or polo-like kinase 1 (PLK1) signaling [35, 36]. Phenotypically, activation of the PI3K/Akt signaling axis by arsenic can result in a variety of changes, including cellular growth and angiogenesis [37]. There are many other lung cancer-specific pathways that may be altered upon exposure to arsenic and its toxic byproducts, including the nuclear

factor (erythroid-derived 2)-like 2/kelch-like ECH-associated protein 1 (NRF2/KEAP1) pathway, the nuclear factor kappa-light-chain-enhancer of activated B cells pathway (NF-κB), and various epigenetic pathways [35, 38]. Further experimental work is required to fully characterize and distinguish the molecular mechanisms of the pathways affected by chronic exposure to arsenic.

2.3 Prominent cancer genes affected by arsenic

As evidenced by its genome-wide effects on cellular physiology and molecular pathways, gene expression alterations cause by arsenic exposure can potentiate negative health outcomes. In fact, there are a growing number of genes that have been observed to have abnormal expression resulting from arsenic exposure, in cell lines, mouse, and human samples. Many of these genes have accepted roles in cancer, both as tumor-suppressors and oncogenes. Most notably, the tumor suppressor gene *TP53* has been shown to be epigenetically inactivated in arsenic-exposed cell lines [39]. Similarly, other cell line studies have suggested that low concentrations of arsenic may upregulate the known lung oncogene *Myc* (also related to the cell cycle) through aberrant expression of miRNAs targeting upstream regulators of its transcription [40].

As previously discussed, the frequent disruption of DNA damage repair and stress response pathways is a common feature of arsenic-induced lung tumors. Notably, arsenic has been associated with stimulation of the DNA damage response through the upregulation of critical genes, such as the gene encoding DNA excision repair protein ERCC1 (*ERCC1*) [41], confirming that DNA damage is prevalent in arsenic-exposed individuals. Alternatively, arsenic may induce repression and decreased activity of main DNA repair enzymes, including poly [ADP-ribose] polymerase 1 (PARP1) inhibition (through ROS) [42], proteasomal degradation of xeroderma pigmentosum, complementation group C (XPC) [31], and widespread hypermethylation of NER genes [43]. Additional lung cancer-related genes affected by arsenic include: *EGFR* [44], *cyclin-dependent kinase inhibitor 1A* (*CDKN1A*) [45], and *B-cell lymphoma 2 (BCL2)* [46]. Despite the mounting evidence of the toxic effects of arsenic, the concentration and identity of key damage-related arsenic compounds varies widely between studies. While different arsenic-based compounds affect similar pathways, specific physiological responses may vary greatly depending on compound type and dose response, necessitating closer examination of these factors in future studies.

However, it is important to note that variations in these genes may exist within individuals prior to arsenic exposure, and that certain genetic polymorphisms may make some individuals more susceptible to the genotoxic effects of arsenic. For instance, a single nucleotide polymorphism (rs238406; C > A) in *ERCC2* (part of the DNA-damage response) leads to the inclusion of an alanine residue in the place of a cysteine in the complete protein, increasing an individual's odds ratio for skin cancer to 2.04 [47]. Additionally, polymorphisms in many of the genes involved in the metabolism and biotransformation of arsenic may result in the production of different metabolic byproducts, conferring differential susceptibility and cancer risk [48]. This is exemplified by the rs1191439 polymorphism of As_3MT, which is correlated with elevated MMA levels in urine [49]. Thus, the landscape of arsenic-induced carcinogenesis is quite complex, with multiple types and outcomes of the molecular aberrations that can result from chronic exposure. A more comprehensive understanding of the mechanisms at play may result in the identification of the underlying causes of lung cancer in never-smokers, and may help to direct the development of novel treatment strategies for these affected individuals.

3. Asbestos

3.1 Physiological and molecular impact of exposure

Asbestos is a term used to define a group of mineral fibers incorporated in a wide variety of products, including talcum powder, brake pads, and construction materials. While more than 50 countries have banned the use of asbestos-containing materials, more than 2 million metric tonnes are still produced every year, which still poses a great public health risk for asbestos-related diseases [50, 51]. There are two main classes of asbestos: chrysotile (spiral-shaped, the most common form) and amphibole (needle-shaped). Other elements such as iron (which can constitute up to 30% of the weight of asbestos fibers) embedded in the surface of fibers can potentiate asbestos-related pathogenic effects [52, 53]. Importantly, all identified forms of asbestos have been classified as carcinogens to humans (Group 1) by the IARC [54].

Exposure to asbestos fibers has been strongly linked to the development of malignant mesothelioma, but it is also a known contributor to the development of lung cancer [55–57]. Between 5 and 7% of all lung cancer cases worldwide have records of high levels of asbestos, mostly derived from occupational exposure (e.g., mining) [10]. Exposure is usually determined by the presence of pleural plaques (areas of fibrosis associated with past exposure to asbestos), or by detection of asbestos fibers in bronchoalveolar lavage (BAL) and lung tissue [58]. The primary source of asbestos exposure comes from inhaled fibers [54]. However, the mechanism of disruption that occurs as a result of asbestos exposure is determined by the efficiency of fiber clearance from airway cells. Longer fibers are cleared at a slower rate than short fibers, and are associated with higher carcinogenic potential [59]. Similarly, thin fibers (width <0.25 μm) are more carcinogenic than thicker ones [60], likely because they can penetrate deeper in airways. Accumulation of asbestos fibers in the lung leads to fibrosis, inflammation, and carcinogenesis, although specific effects depend on the cumulative dose and the type of fiber inhaled [61, 62].

Asbestos-related carcinogenesis is thought to primarily result from the ability of the fibers to induce oxidative stress (**Figure 2**), although the specific mechanisms are not yet fully understood [63]. Asbestos induces the recruitment of alveolar macrophages, followed by an inflammatory reaction [64–66]. Failed phagocytosis of these fibers by macrophages results in the generation of ROS, together with the release of cytokines, chemokines, proteases, and growth factors further amplifying deleterious effects of asbestos [10, 56, 67]. Additionally, the iron contained in asbestos fibers deposits in the lungs and cycles between the reduced and oxidized forms, potentially inducing further oxidative DNA damage in nearby cells via the Fenton reaction which converts H_2O_2 into more reactive ROS [10, 56, 68, 69].

In lungs, oxidative stress following asbestos exposure can activate several signaling pathways including mitogen-activated protein kinases (MAPK), NF-κB, and activator protein 1 (AP1). All of these pathways have been linked to increases in early response genes (e.g., *JUN* and *FOS*) that govern cell proliferation, apoptosis, and inflammatory signaling [55, 56].

3.2 Carcinogenic mechanisms

The most frequent asbestos-induced alterations in cancer-related genes have been reported in tumor suppressor genes (TSGs). Activation of p53 and p21 are frequently described, both in animal models and lung cancer patients with asbestosis (reviewed in [63]). This likely represents the initial DNA-damage response following exposure to asbestos-induced oxidative stress. In lung cancer patients, the frequency of *TP53* gene mutations is similar between asbestos-exposed and

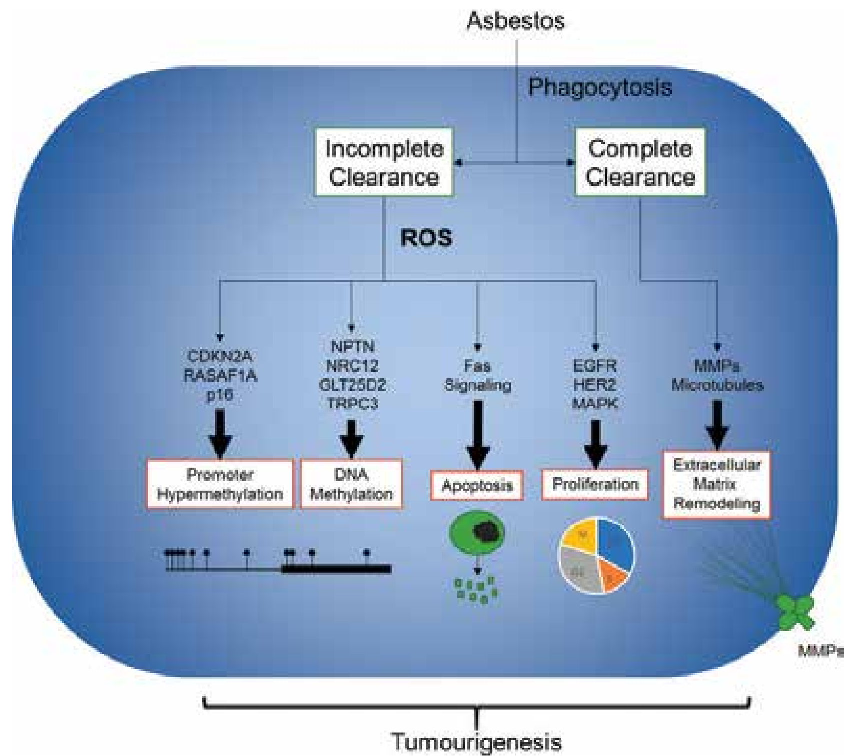


Figure 2.
Molecular mechanisms of asbestos-induced carcinogenesis.

unexposed NSCLC cases; however, a higher frequency of G:C to T:A transversions in the sequence of *TP53* is observed in asbestos-exposed cases [70, 71]. Contrarily, other tumor suppressor genes such as *CDKN2A* are inactivated in asbestos-exposed lung cancer cases, mostly via segmental copy-number losses [72]. In murine models, chrysotile fibers are able to induce the activity of the c-Jun and c-Fos oncogenes and inactivate p53 and p16 tumor suppressors, both at the mRNA and protein levels [73].

Additionally, other well-known lung cancer genes and pathways have been shown to display aberrant functions in response to asbestos exposure. Different mechanisms of asbestos-mediated activation of the EGFR pathway have been described. Asbestos-induced chronic inflammation has been associated with activation of the EGFR-related and extracellular signal-regulated kinase (ERK) signaling pathway that promote lung epithelial cell and fibroblast proliferation [55, 56, 74]. Also, asbestos fibers can induce over-expression of EGFR mRNA and induce protein dimerization, phosphorylation, and subsequent pathway activation by directly interacting with the surface portion of the receptor [63, 75, 76]. On the other hand, DNA mutations affecting EGFR do not seem to be main mechanisms of asbestos-induced EGFR activation. Asbestos-exposed patients displayed a significantly lower rate of *EGFR* mutations compared to non-exposed patients [77]. Moreover, it is unclear if there is a causal relationship between the mutations found in *EGFR* and exposure to asbestos fibers [78, 79].

Other genes, such as *MAP4K3, CEBPZ, QPCT, FANCG, IGFBPL1, CCL19, MELK, FANCM*, and *CDKL1* have shown aberrant gene expression in human epithelial bronchial cell lines (Beas-2B), following asbestos exposure [80]. Asbestos inhalation also causes up-regulation of mRNA levels of matrix metalloproteinase family members in rat lungs, suggesting induction of extracellular matrix remodeling [81].

At the epigenetic level, alterations affecting tumor suppressor genes have been observed in lung cancer cases associated with asbestos exposure, including those in the promoter regions of *RASSF1A* and *CDKN2A* (p16) [82]. Additionally, a genome-wide DNA methylation study identified differentially methylated CpGs in regions nearby the transcription start site of genes such as *NPTN, NRG2, GLT25D2* and *TRPC3* to be significantly associated with asbestos exposure [83].

The effect of asbestos on micro RNA (miRNA) expression has been also investigated. miRNAs are short (~22 nucleotide) RNA transcripts that negatively regulate gene expression through direct interaction with mRNAs. Interestingly, the overexpression of miR-148b has been described in multiple independent studies. This miRNA was part of an asbestos-related signature in lung tumors, also composed of seven other overexpressed (miR-374a, miR-24-1*, let-7d, Let-7e, miR-199b-5p, miR-331-3p, and miR-96) and five miRNAs with decreased expression in tumors (miR-939, miR-671-5p, miR-605, miR-1224-5p, and miR-20) [84]. Additionally, miR-148b was found to be overexpressed in asbestos-related lung cancer compared to tumors in non-exposed individuals, and three of its targets (*GADD45A, LTBP1* and *FOSB*) were down-regulated in asbestos-exposed patients [84].

Despite the known genetic and epigenetic abnormalities resulting from asbestos exposure, a relatively small proportion of exposed individuals develop thoracic malignancies (mesothelioma or lung cancer). It has been hypothesized that specific genetic variants may confer increased risk of developing asbestos-related diseases [85]. Thus, recent studies have investigated the association between genomic variants and risk of lung cancer following asbestos exposure. In a genome-wide association study (GWAS) performed in the Texas lung cancer GWAS dataset, the authors did not find statistical evidence for gene-asbestos interaction in the etiology of lung cancer [86]. However, the Fas signaling pathway (regulation of tissue homeostasis in the immune system by inducing apoptosis) was identified as the most significant pathway associated with asbestos exposure in the etiology of lung cancer. Another study identified three single nucleotide polymorphisms (SNPs) in the *MIRLET7BHG* (*MIRLET7B* host gene located at 22q13.31) significantly associated with increased lung cancer risk among individuals exposed to asbestos [36].

The identification of risk variants linked with asbestos-related lung cancer is a challenging task. Sample sizes for asbestos-related lung cancer cohorts are particularly limited by the number of cases that can be unequivocally attributed to asbestos exposure despite other well-known factors (e.g., smoking). Thus, focusing on the genes and chromosomal regions found by these preliminary studies might be useful for more targeted strategies aiming to validate these results.

3.3 Carcinogenic potential of other fibers

While the oncogenic effects of asbestos have been extensively established, recent evidence indicates that non-asbestos fibers, both natural and synthetic in nature can also cause thoracic cancers. Non-asbestos mineral (natural) fibers include erionite and fluoro-edenite, among others. Erionite is a naturally occurring fibrous mineral that shares some physical properties with asbestos, although it is less widespread. In fact, it has been shown that erionite is a more potent carcinogen in causing malignant mesothelioma [87, 88]. Erionite activates the NLR family pyrin domain containing 3 (*NLRP3, NALP3*) inflammasome, inducing the transcription and production of cytokines critical to cancer initiation [89]. On the other hand, Fluoro-edenite (originating from volcanic activity) can induce ROS that result in DNA damage and increase in lactic dehydrogenase release (a damage and toxicity marker) in human lung adenocarcinoma (A549) and monocyte-macrophage (J774) cell lines [90].

Synthetic graphene-based fibers are widely used in several industries. They have also been explored as a drug delivery system for cancer treatments. Physical similarities to asbestos, particularly its high length-to-width ratio, have raised some concerns about the potential carcinogenicity effects of these fibers [91]. Exposure to carbon nanotubules has been shown to induce oncogenic pathways, such as TGF-β and Akt/GSK-3β, resulting in activation of the SNAIL-1 signaling pathway and epithelial-mesenchymal transition [92]. Additionally, carbon nanotubules can generate ROS, activating MAPKs, AP-1, NF-κB, and Akt in normal and malignant human mesothelial cells [93]. Other genetic alterations, including micronuclei formation, disruption of mitotic spindles, and polyploidy have also been observed in response to carbon nanotubule exposure [94–96]. Moreover, it has been shown that exposure to carbon nanotubules can induce specific methylation changes at the promoter regions several genes, including *DNMT1*, *ATM*, *SKI*, and *HDAC4*, while they seem to have only a marginal effect on miRNA expression [97]. Thus, the oncogenetic factors of natural and synthetic fibers, while similar in morphology, are distinct entities that may collectively culminate in tumor development.

4. Radon

4.1 Physiological and molecular impact of exposure

Radon is the second most common cause of lung cancer in many countries; however, the intricacies of its mechanism of action remain underappreciated. The genotoxicity of radon is largely the result of alpha particle emission during its spontaneous decay into short-lived radioactive progeny (^{218}Po and ^{214}Po) and comparably long-lived radioactive ^{210}Pb, which also induces cellular damage through alpha decay (**Figure 3**) [98].

Alpha decay is the emission of a 4 atomic mass unit helium ion (two protons and two neutrons), which can liberate electrons from water molecules and result in the generation of several types of ROS [15]. Much like the mechanisms of arsenic and asbestos toxicity, ROS generated as a consequence of radon exposure can lead to widespread molecular aberrations, especially base oxidation (leading to mismatches and mutagenesis), DNA strand breaks, chromosomal aberrations, and deletions. For example, chromatid deletions in blood lymphocytes may be a result of radon exposure, which may in part explain the associations between radon exposure and blood malignancies [8]. These events may occur at levels well below those currently deemed safe in many countries, exemplified by the observation of chromosomal abnormalities in lymphocytes at very low doses of polonium-214, a radioactive progeny of radon [99].

Beyond the molecular events resulting from ROS generation, alpha radiation from radon exposure can induce bystander responses in cells that have not been directly affected by alpha particles [100]. The bystander effect of radiation exposure can occur through the release of signals from nearby irradiated cells, generating a physiological response in non-irradiated cells, even at relatively low doses of radiation [101]. The effect requires direct contact between adjacent cells, such as through gap junctions, as well as compounds in the surrounding medium, including cytokines [102]. One of these compounds, nitric oxide (NO), has been shown to be an important factor for the cell-killing effects of the bystander response, largely through the direct interaction with and damage of DNA [103]. Moreover, NO byproducts such as dinitrogen trioxide (N_2O_3) can promote nitrosation of other amines, such as those of DNA bases, leading to cross-linking and DNA alkylation [102]. Another compound that may be relevant to the bystander effect of cellular

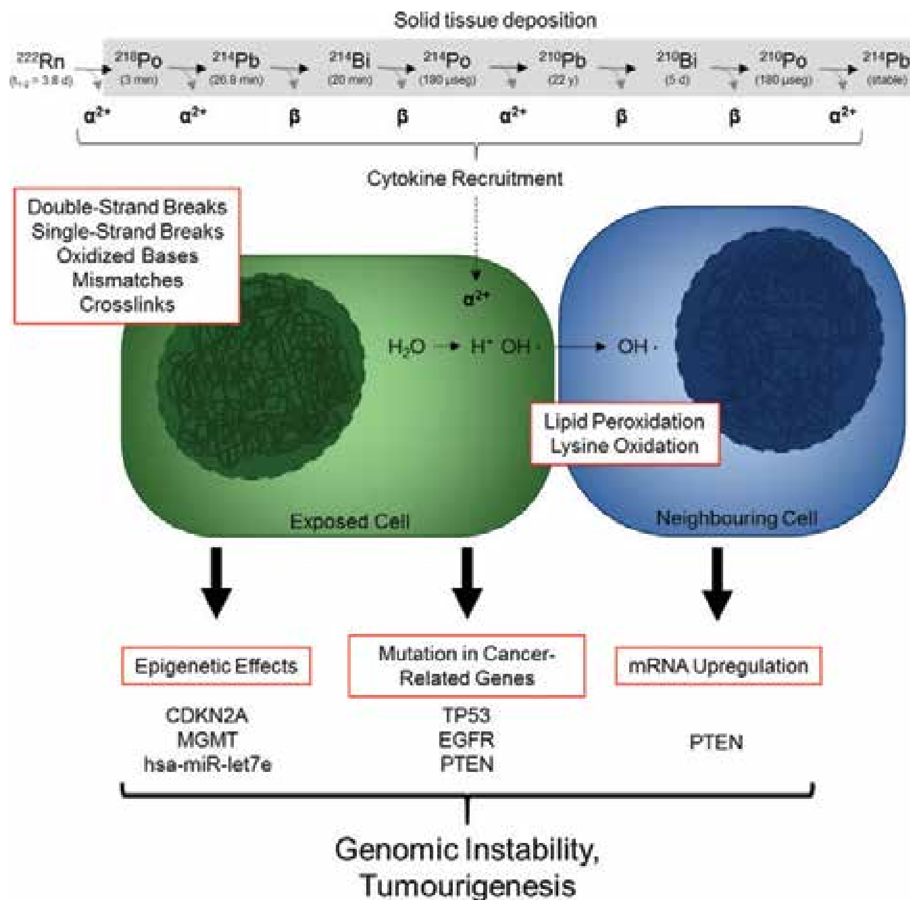


Figure 3.
Molecular mechanisms of radon-induced carcinogenesis.

radiation exposure is cyclooxygenase 2 (COX-2), which is related to the NF-κB pathway, an effect that is attenuated upon COX-2 inhibition [103, 104]. Finally, this response may be dependent on *TP53* status, which will be discussed in Section 4.3.

4.2 Carcinogenic mechanisms

Despite differences in the details of exposure, the molecular mechanisms contributing to carcinogenesis in individuals exposed to arsenic, asbestos, and radon converge in that they all produce ROS. Radon has a half-life of 3.8 days, and as previously mentioned, commonly generates alpha particles and polonium decay products, which themselves emit further alpha radiation [105]. Alpha particles have a high linear energy transfer (LET) despite having relatively low penetration capability, meaning that they interact readily with DNA, especially in regions close to their site of exposure, such as the bronchial epithelium [105]. Thus, it is not surprising that lung malignancies are the most common type of radon-induced cancer. High LET radiation is distinct from low LET radiation (such as x-rays or gamma rays) in that it produces a substantially greater proportion of clustered damage, meaning the occurrence of ≥2 lesions of ≥1 different types within 1–2 helical turns of DNA. Clustered DNA damage is typically repaired with slower kinetics and has a greater likelihood of producing sequence alterations, as repair pathways converge and conflict with one another [106–108].

The largest radon-induced mechanisms of carcinogenesis include DNA damage, ROS, and alpha particle generation; likewise, pathways associated with these functions are also known to be associated with lung cancer. In fact, patients positive for rearrangements in the gene encoding anaplastic lymphoma kinase (*ALK*)—an event frequently found to drive lung tumorigenesis—were found to have two-fold increases in residential radon levels than those without these rearrangements [109, 110]. While a synergistic effect between radon and smoking has been suggested [11], the G:C to T:A transversions associated with tobacco-related molecular damage are not as commonly observed in individuals exposed to radon, suggesting a unique molecular signature in radon-associated lung tumors [15]. Again, it is important to note that a number of the pathways affected by radon exposure, including gene expression alterations and apoptotic disturbances, may actually be from cells neighboring those that are irradiated [104]. In fact, pro-inflammatory and ROS-generating cytokines such as tumor necrosis factor alpha (TNF-α) may be released upon radiation exposure, which may perpetuate the damage enacted by ROS [111]. Thus, key pathways such as DNA repair, proliferation, and cell death can be altered in cells beyond those that are irradiated [111].

4.3 Prominent cancer genes affected by radon

ROS-induced DNA damage is a large factor in radon-induced carcinogenesis, thus, many of the examinations into genes affected by radon are relevant to DNA-repair and apoptotic pathways. Naturally, a heavy focus is placed on *TP53.* Many investigations into *TP53* examine whether hotspot mutations in *TP53* can act as a molecular signal for radon-induced genotoxicity in at-risk populations. Although *TP53* is observed to be altered in high exposure populations, there are limited observations available to suggest a consistent mutational landscape [112]. However, the role of *TP53* in the molecular response to radon exposure may be relevant to the bystander effect, wherein *TP53* may mediate the inhibition of response signals coming from irradiated cells [103]. Additionally, other key lung cancer-related genes may also be mutated by radon exposure, including *EGFR* and phosphatase and tensin homolog (*PTEN*), but the exact mechanisms remain to be characterized [113].

As previously discussed, radon may also exhibit its carcinogenic effects epigenetically, as evidenced by the promoter hypermethylation of the tumor suppressor genes *CDKN2A* and *MGMT.* In normal human lung cell lines, miRNAs shown to be primarily involved in cell proliferation, differentiation, and adhesion displayed aberrant expression upon radon exposure [114]. Moreover, the miRNA *let-7e*—an epigenetic regulator of the RAS oncogene—was found to be upregulated upon low radon exposure [115]. In this study, the upregulation of miRNAs targeting tumor suppressor genes was also noted, including *PTEN*, which may present an alternative mechanism of radon-induced carcinogenesis.

Finally, a number of studies have examined the effect of genetic polymorphisms of DNA damage repair genes in the outcome of individuals exposed to radon. For instance, individuals with a polymorphism leading to the Asp1104His substitution of DNA repair gene *ERCC5* (XpG) displayed a higher frequency of micronuclei in their lymphocytes, representative of elevated cytogenetic damage and decreased radiosensitivity [116]. Alternatively, the absence of *GSTM1* and *GSTT1*, members of the glutathione-s-transferase enzyme family—critical to detoxification and excretion—is associated with an increased risk of lung cancer development [117, 118]. When radon exposure is considered, individuals with null alleles show a doubly increased odds ratio of lung cancer development [118]. Notably, this enzyme is relevant in the biotransformation and excretion of arsenic, suggesting similar carcinogenic pathways between these two environmental agents.

Taken together, the molecular landscape of radon-induced carcinogenesis is complex and diverse, with effects being observed at the genetic, epigenetic and extracellular level. Future studies may examine the underlying molecular events common to radon-induced lung cancer, to aid in diagnosis and perhaps novel treatment strategies.

5. Common oncogenic features exhibited by environmental carcinogens

The landscape of the genomic disruptions induced by environmental carcinogens is extensive. It has been demonstrated that these compounds can induce alterations such as chromosomal abnormalities, DNA double-strand breaks, gene expression dysregulation, and epigenetic aberrations. While each agent presents a unique mechanism and clinical challenge, a number of parallels can be seen. The molecular effects of exposure to arsenic, asbestos, and radon converge in that each compound can result in DNA damage induced by ROS and inflammation. As these events occur early during tumor development, the identification of the underlying genomic and epigenomic abnormalities caused by these compounds is extremely relevant in identifying early oncogenic events and individual susceptibility differences.

Although the intricacies of the molecular mechanisms of alteration may differ between the various toxic agents, ROS generation is a common outcome of exposure that can lead to extensive DNA damage and further perturbations in various cellular compartments and processes [119]. As mitochondria are one of the primary sources of ROS, they are also key targets of oxidative toxicity [120]. Arsenic exposure is associated with dysfunction of the mitochondria, through the ability of its metabolites to disrupt the mitochondrial membrane potential and reduce mitochondrial ATP levels, as well as ROS-induced mitochondrial damage [121, 122]. Mitochondrial damage induced by arsenic can then lead to numerous alterations in key signaling pathways, such as the decreased expression of apoptotic regulator protein Bcl-2 [122]. Regardless of the molecular mechanism, mitochondrial insult culminates in apoptosis and increased inflammation, in addition to the exacerbation of reactive species generation; events that commonly precede tumorigenesis [121, 123].

Another frequently observed early consequence of exposure to environmental carcinogens is an inflammatory response. Indeed, inflammation caused by infiltrating immune cells underlies numerous hallmarks of cancer biology by providing key molecules for tumor survival and growth, as well as the promotion of genomic aberrations, again through the generation of ROS [124]. Asbestos-induced carcinogenesis is thought to rely heavily on the inflammatory response, where the macrophages of the innate immune system attempt to clear the carcinogenic fibers through phagocytosis [125]. However, these fibers are inherently difficult to digest, leading to the eventual death of the macrophage and subsequent release of pro-inflammatory cytokines, ROS, and other growth factors [126]. Interestingly, many malignancies have noticeable local immune responses prior to tumor development, highlighting the complex and dichotomous role of host immune cells in both pro- and anti-tumor functions [127]. Thus, exposure to environmental carcinogens threatens the genetic and epigenetic landscape of oncogene expression in the development of malignancies, and subsequently changes cellular and systemic processes.

The intertwined role of genetic and epigenetic aberrations resulting from exposure to these compounds highlights the complexity of environmentally-induced lung cancer. However, the carcinogenic mechanisms associated with exposure to these agents have been mainly identified using a “one-agent-at-a-time” approach. Further, we have yet to understand how these factors interplay with one another in cases of

combined exposure and how individual genomes modulate the molecular events that arise following exposure. For example, it is difficult to accurately assess the relative risk of lung cancer in an individual who is exposed to occupational asbestos, arsenic-contaminated water, and high levels of domestic indoor air radon. Whether these factors synergize in terms of their molecular effects is not clearly understood and has critical implications to patient monitoring and disease management.

Recently, the idea of the human exposome has sought to provide a method for analyzing individual risk factors by integrating the effects of factors ranging from DNA-level alterations to geographic location. The human exposome is defined as the sum of every exposure to which an individual is subjected to from conception to death [128]. The exposome is dynamic: the nature, amount, and conditions of exposure change over time. It also includes exposure from internal (e.g., metabolism, endogenous hormones, gut microflora, inflammation, oxidative stress, etc.) and external (e.g., radiation, infectious agents, chemical contaminants and environmental pollutants, among others) sources [129]. The lungs are one of the organs at the highest risk of disease development from environmental exposures as the lung exposome can be comprised of an array of molecules and environmental insults. Arsenic, asbestos, and radon, together with air pollution and tobacco smoke, constitute a fraction of the complex mix of environmental carcinogens posing risks for developing thoracic malignancies in humans. However, understanding the oncogenic events following exposure to these agents may allow for the identification of key intervention points to minimize environmentally-induced lung cancer in at-risk populations.

6. Translational outlook for environmentally-induced cancer

As the molecular mechanisms of environmentally-induced carcinogenesis continue to emerge, a need to characterize the clinical utility of these findings should be underscored. This need is further emphasized by the complex interplay between the numerous features of the lung exposome. Many of the single cancer-associated genes that are affected by exposure to these environmental agents are promising therapeutic intervention points. For instance, targeted inhibitors of EGFR (e.g., erlotinib, afatinib)—a protein transcribed from a gene commonly up-regulated upon exposure to arsenic—are used in lung cancer treatment to interfere with the aberrant growth pathways activated by the upregulation of this signaling receptor [130]. Additionally, the association between radon exposure and *ALK* gene rearrangements in lung cancer patients may be amenable to therapy with inhibitors of the ALK protein (e.g., crizotinib, ceritinib) [131]. However, patients that do not present with alterations in genes that are clinically actionable remain extremely difficult to treat beyond standard regimes. Thus, it is critical to analyze the oncogenetic alterations induced by environmental carcinogens, to not only identify the contribution of each of these widely-distributed agents to tumorigenesis, but also to direct the development of novel treatment and risk-management strategies. Concurrent analysis of altered genes, transcripts, and proteins may help to parse out the risk associated with the varying molecular aberrations that have been observed to be induced by these compounds [132]. This approach, while difficult in terms of scale, necessitates the use of geographic, demographic, and exposome level data, which can be scarce in areas where environmental carcinogen levels are especially concerning. **Table 1** summarizes the currently available sources of information for carcinogens found in the environment that are associated with lung cancer. Overall, future mitigation of the environmental risk factors that lead to lung cancer will rely on the integration of information from the genomic to epidemiological levels.

Name	Website	Description
The IARC Monographs, International Agency for Research on Cancer	http://monographs.iarc.fr/	Compilation of factors that increase the risk of human cancer: occupational exposures, physical agents, biological agents, and lifestyle
Carcinogens, American Cancer Society	http://www.cancer.org/Cancer/CancerCauses/OtherCarcinogens/index	Environmental carcinogens from different sources (e.g., indoor, pollution, medical tests)
Cancer-Causing Substances in the Environment, National Cancer Institute	https://www.cancer.gov/about-cancer/causes-prevention/risk/substances	Information of environmental carcinogens to affect human health.
Chemicals of Public Health Concern, World Health Organization (WHO)	http://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/index.html	Information on the 10 chemicals or groups of chemicals of major public health concern
Radon and Health, WHO	http://www.who.int/mediacentre/factsheets/fs291/en/	Health effects and guide line of Radon.
Arsenic Fact Sheet, WHO	http://www.who.int/mediacentre/factsheets/fs372/en/	Contents include health effects, prevention, and control on Arsenic.
Elimination of asbestos-related diseases, WHO	http://www.who.int/mediacentre/factsheets/fs343/en/	Information about asbestos related diseases.
Science and Technology: Health, Environmental Protection Agency (EPA)	http://www.epa.gov/gateway/science/humanhealth.html	Information on human health impacts associated with environmental exposures
Work-Related Lung Disease (WoRLD) Surveillance System, National Institute for Occupational Safety and Health (NIOSH)	http://www2.cdc.gov/drds/WorldReportData/	Contents on occupationally-related respiratory disease surveillance data.
U.S. Geological Survey (USGS)	http://www.usgs.gov/	Organization that provides impartial information on the health of U.S. environment and the natural hazards
CARcinogen EXposure Canadian Surveillance Project (CAREX)	http://www.carexcanada.ca/	Project that combines academic expertise and government resources to generate an evidence-based carcinogen surveillance program
Lung Cancer and the Environment, Centers for Disease Control and Prevention (CDC)	https://ephtracking.cdc.gov/showCancerLcEnv.action	Information about exposure to environmental carcinogen and the risk for lung cancer.

Table 1.
Sources of information on environmental carcinogens associated with lung cancer.

7. Conclusions and future directions

The geographical conditions facilitating human exposure to environmental lung carcinogens such as arsenic, asbestos and radon occur commonly across the globe. While millions of individuals are known to be exposed to potentially damaging doses of these carcinogens, another significant part of the population is unaware of its exposure. Despite the worldwide impact of the public health risk posed by these compounds, the genomic and epigenetic consequences of these exposures are drastically understudied. Barriers such as: (i) availability of individual-level exposure data; (ii) collection of genomic, epigenomic, and transcriptomic readouts following acute and chronic exposure to carcinogens; and (iii) obtaining enough samples to reach statistical power; impose even further challenges to determining the true extent of environmentally-induced health effects.

Understanding these mechanisms could have a significant impact on the establishment of safe exposure limits for each of these agents. For instance, most of the current frameworks used to regulate arsenic exposure in drinking water have been derived from studies performed in specific populations exposed to high levels of arsenic, such as Bangladesh, Chile, and China [9, 133, 134]. However, an increased risk of arsenic-related health effects (including cancer) has been documented at levels below current safety thresholds that are commonly found in water sources throughout North America and Europe [7]. Thus, characterizing the effects of these agents at the genomic/epigenomic level will not only aid in determining the oncogenes that are perturbed in environmentally-induced lung cancers, but may also uncover early molecular events that can be used as diagnostic and prognostic markers.

The fraction of lung cancer patients who have never smoked or have ceased smoking is likely to increase in the coming years. Exposure to environmental carcinogens, such as arsenic, asbestos, and radon will play a key role in their etiology. Further elucidation of the detailed mechanisms driving environmentally-induced lung tumors will provide the much-needed insight to define specific detection methods and intervention strategies. Collectively, uncovering these carcinogen-specific mechanisms, as well as the affected genes driving malignant transformation, will greatly contribute to the development of personalized approaches to provide better support to lung cancer patients.

Acknowledgements

This work was supported by grants from the Canadian Institutes for Health Research (CIHR FDN-143345). VDM, APS, and EAM are supported by scholarships from the University of British Columbia. APS is further supported by the Frederick Banting and Charles Best Scholarship from CIHR. EAM is also supported by the Vanier Canada Graduate Scholarship from CIHR. AAG is the Canada Research Chair for Radiation Exposure Disease and this work was undertaken, in part, thanks to funding from the Canada Research Chairs program. The AAG laboratory is supported by the Canadian Institutes of Health Research.

Conflict of interest

Authors declare no conflict of interest.

Author details

Victor D. Martinez[1,6,†*], Adam P. Sage[1,6,†], Erin A. Marshall[1,6], Miwa Suzuki[1], Aaron A. Goodarzi[2,3,6], Graham Dellaire[4,5,6] and Wan L. Lam[1,6]

1 Department of Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada

2 Department of Biochemistry & Molecular Biology, Robson DNA Science Centre, Arnie Charbonneau Cancer Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

3 Department Oncology, Robson DNA Science Centre, Arnie Charbonneau Cancer Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

4 Department of Pathology, University of Dalhousie, Halifax, Nova Scotia, Canada

5 Department of Biochemistry & Molecular Biology, University of Dalhousie, Halifax, Nova Scotia, Canada

6 Canadian Environmental Exposures in Cancer (CE2C) Network, Dalhousie University, Halifax, Nova Scotia, Canada

† These authors contributed equally to this work.

*Address all correspondence to: vmartinez@bccrc.ca

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Global Burden of Disease Cancer. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. JAMA Oncology. 2017;**3**(4):524-548

[2] Pallis AG, Syrigos KN. Lung cancer in never smokers: Disease characteristics and risk factors. Critical Reviews in Oncology/Hematology. 2013;**88**(3):494-503

[3] Shahadin MS et al. Challenges and future direction of molecular research in air pollution-related lung cancers. Lung Cancer. 2018;**118**:69-75

[4] Hubaux R et al. Arsenic, asbestos and radon: Emerging players in lung tumorigenesis. Environmental Health. 2012;**11**:89

[5] Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—A different disease. Nature Reviews. Cancer. 2007;**7**(10):778-790

[6] Stanley FK et al. Comprehensive survey of household radon gas levels and risk factors in southern Alberta. CMAJ Open. 2017;**5**(1):E255-E264

[7] Saint-Jacques N et al. Estimating the risk of bladder and kidney cancer from exposure to low-levels of arsenic in drinking water, Nova Scotia, Canada. Environment International. 2018;**110**:95-104

[8] Teras LR et al. Residential radon exposure and risk of incident hematologic malignancies in the cancer prevention study-II nutrition cohort. Environmental Research. 2016;**148**:46-54

[9] World Health Organization Arsenic. 2016. Available from: http://www.who.int/mediacentre/factsheets/fs372/en/ [Accessed: Feb 15, 2017]

[10] Huang SX et al. Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. Journal of Toxicology and Environmental Health. Part B, Critical Reviews. 2011;**14**(1-4):179-245

[11] World Health Organization. Radon and Health. Geneva, Switzerland: WHO; 2016. Available from: http://www.who.int/mediacentre/factsheets/fs291/en/ [cited 2018]

[12] Naujokas MF et al. The broad scope of health effects from chronic arsenic exposure: Update on a worldwide public health problem. Environmental Health Perspectives. 2013;**121**(3):295-302

[13] Lin S et al. A novel S-adenosyl-L-methionine: Arsenic(III) methyltransferase from rat liver cytosol. The Journal of Biological Chemistry. 2002;**277**(13):10795-10803

[14] Ren X et al. An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. Environmental Health Perspectives. 2011;**119**(1):11-19

[15] Hubaux R et al. Molecular features in arsenic-induced lung tumors. Molecular Cancer. 2013;**12**:20

[16] Reichard JF, Puga A. Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. Epigenomics. 2010;**2**(1):87-104

[17] Howe CG et al. Sex-specific associations between one-carbon metabolism indices and posttranslational histone modifications in arsenic-exposed Bangladeshi adults. Cancer Epidemiology, Biomarkers & Prevention. 2017;**26**(2):261-269

[18] Riedmann C et al. Inorganic arsenic-induced cellular transformation is coupled with genome wide changes in

chromatin structure, transcriptome and splicing patterns. BMC Genomics. 2015;**16**:212

[19] Naranmandura H et al. Mitochondria are the main target organelle for trivalent monomethylarsonous acid (MMA(III))-induced cytotoxicity. Chemical Research in Toxicology. 2011;**24**(7):1094-1103

[20] Kligerman AD, Malik SI, Campbell JA. Cytogenetic insights into DNA damage and repair of lesions induced by a monomethylated trivalent arsenical. Mutation Research. 2010;**695**(1-2):2-8

[21] Martinez VD et al. Whole-genome sequencing analysis identifies a distinctive mutational spectrum in an arsenic-related lung tumor. Journal of Thoracic Oncology. 2013;**8**(11):1451-1455

[22] Zhao CQ et al. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:10907-10912

[23] Bhattacharjee P, Banerjee M, Giri AK. Role of genomic instability in arsenic-induced carcinogenicity. A review. Environment International. 2013;**53**:29-40

[24] Kessel M et al. Arsenic induces oxidative DNA damage in mammalian cells. Molecular and Cellular Biochemistry. 2002;**234-235**(1-2):301-308

[25] Matsui M et al. The role of oxidative DNA damage in human arsenic carcinogenesis: Detection of 8-hydroxy-2′-deoxyguanosine in arsenic-related Bowen's disease. The Journal of Investigative Dermatology. 1999;**113**(1):26-31

[26] Turrens JF. Mitochondrial formation of reactive oxygen species. The Journal of Physiology. 2003;**552**(Pt 2):335-344

[27] Melis JP, van Steeg H, Luijten M. Oxidative DNA damage and nucleotide excision repair. Antioxidants & Redox Signaling. 2013;**18**(18):2409-2419

[28] Hanawalt PC, Spivak G. Transcription-coupled DNA repair: Two decades of progress and surprises. Nature Reviews. Molecular Cell Biology. 2008;**9**(12):958-970

[29] Banerjee M et al. Polymorphism in the ERCC2 codon 751 is associated with arsenic-induced premalignant hyperkeratosis and significant chromosome aberrations. Carcinogenesis. 2007;**28**(3):672-676

[30] Ahsan H et al. DNA repair gene XPD and susceptibility to arsenic-induced hyperkeratosis. Toxicology Letters. 2003;**143**(2):123-131

[31] Holcomb N et al. Inorganic arsenic inhibits the nucleotide excision repair pathway and reduces the expression of XPC. DNA Repair (Amst). 2017;**52**:70-80

[32] Andrew AS et al. Arsenic activates EGFR pathway signaling in the lung. Toxicological Sciences: An Official Journal of the Society of Toxicology. Oxford UK: Oxford University Press; 2009:**109**

[33] Li L et al. Filamin A phosphorylation by Akt promotes cell migration in response to arsenic. Oncotarget. 2015;**6**(14):12009-12019

[34] Liu J et al. JNK-dependent Stat3 phosphorylation contributes to Akt activation in response to arsenic exposure. Toxicological Sciences. 2012;**129**(2):363-371

[35] Carpenter RL, Jiang BH. Roles of EGFR, PI3K, AKT, and mTOR in heavy metal-induced cancer. Current Cancer Drug Targets. 2013;**13**(3):252-266

[36] Li Z et al. Low-dose arsenic-mediated metabolic shift is associated with activation of polo-like kinase 1 (Plk1). Cell Cycle. 2015;**14**(19):3030-3039

[37] Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. Molecular BioSystems. 2015;**11**(7):1946-1954

[38] Chen C et al. MicroRNA-155 regulates arsenite-induced malignant transformation by targeting Nrf2-mediated oxidative damage in human bronchial epithelial cells. Toxicology Letters. 2017;**278**:38-47

[39] Al-Eryani L et al. Differentially expressed mRNA targets of differentially expressed miRNAs predict changes in the TP53 axis and carcinogenesis related pathways in human keratinocytes chronically exposed to arsenic. Toxicological Sciences. 2018;**162**:645-654

[40] Pratheeshkumar P et al. Oncogenic transformation of human lung bronchial epithelial cells induced by arsenic involves ROS-dependent activation of STAT3-miR-21-PDCD4 mechanism. Scientific Reports. 2016;**6**:37227

[41] Mo J et al. Elevated ERCC1 gene expression in blood cells associated with exposure to arsenic from drinking water in Inner Mongolia. Anticancer Research. 2009;**29**(8):3253-3259

[42] Zhou X et al. S-nitrosation on zinc finger motif of PARP-1 as a mechanism of DNA repair inhibition by arsenite. Oncotarget. 2016;**7**(49):80482-80492

[43] Zhang A et al. Aberrant methylation of nucleotide excision repair genes is associated with chronic arsenic poisoning. Biomarkers. 2017;**22**(5):429-438

[44] Michailidi C et al. Involvement of epigenetics and EMT-related miRNA in arsenic-induced neoplastic transformation and their potential clinical use. Cancer Prevention Research (Philadelphia, PA). 2015;**8**(3):208-221

[45] Komissarova EV, Rossman TG. Arsenite induced poly(ADP-ribosyl)ation of tumor suppressor P53 in human skin keratinocytes as a possible mechanism for carcinogenesis associated with arsenic exposure. Toxicology and Applied Pharmacology. 2010;**243**(3):399-404

[46] Lee HY et al. Formation of biofilm by listeria monocytogenes ATCC 19112 at different incubation temperatures and concentrations of sodium chloride. Brazilian Journal of Microbiology. 2013;**44**(1):51-55

[47] Hsu LI et al. Association of environmental arsenic exposure, genetic polymorphisms of susceptible genes, and skin cancers in Taiwan. BioMed Research International. 2015;**2015**:892579

[48] Minatel BC et al. Environmental arsenic exposure: From genetic susceptibility to pathogenesis. Environment International. 2017;**112**:183-197

[49] Antonelli R et al. AS3MT, GSTO, and PNP polymorphisms: Impact on arsenic methylation and implications for disease susceptibility. Environmental Research. 2014;**132**:156-167

[50] The International Ban Asbestos Secretariat. Current Asbestos Bans. 2018. Available from: http://www.ibasecretariat.org/alpha_ban_list.php [cited Apr 1, 2018]

[51] Virta RL. Worldwide Asbestos Supply and Consumption Trends from 1900 through 2003 Circular 1298. Reston, Virginia: U.S. Geological Survey; 2006. p. 80. Available from: http://pubs.usgs.gov/circ/2006/1298/c1298.pdf

[52] Ghio AJ, Churg A, Roggli VL. Ferruginous bodies: Implications in the mechanism of fiber and particle toxicity. Toxicologic Pathology. 2004;**32**(6):643-649

[53] Fubini B, Barcelo F, Otero Arean C. Ferritin adsorption on amosite fibers: Possible implications in the formation and toxicity of asbestos bodies. Journal of Toxicology and Environmental Health. 1997;**52**(4):343-352

[54] Global Burden of Disease Cancer Collaboration et al. The global burden of cancer 2013. JAMA Oncology. 2015;**1**(4):505-527

[55] Heintz NH, Janssen-Heininger YM, Mossman BT. Asbestos, lung cancers, and mesotheliomas: From molecular approaches to targeting tumor survival pathways. American Journal of Respiratory Cell and Molecular Biology. 2010;**42**(2):133-139

[56] Mossman BT et al. Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. Journal of Toxicology and Environmental Health. Part B, Critical Reviews. 2011;**14**(1-4):76-121

[57] Sankpal UT et al. Environmental factors in causing human cancers: Emphasis on tumorigenesis. Tumour Biology. 2012;**33**(5):1265-1274

[58] American Thoracic S. Diagnosis and initial management of nonmalignant diseases related to asbestos. American Journal of Respiratory and Critical Care Medicine. 2004;**170**(6):691-715

[59] Loomis D et al. Increased lung cancer mortality among chrysotile asbestos textile workers is more strongly associated with exposure to long thin fibres. Occupational and Environmental Medicine. 2012;**69**(8):564-568

[60] Stayner L et al. An epidemiological study of the role of chrysotile asbestos fibre dimensions in determining respiratory disease risk in exposed workers. Occupational and Environmental Medicine. 2008;**65**(9):613-619

[61] Xu A et al. Genotoxic mechanisms of asbestos fibers: Role of extranuclear targets. Chemical Research in Toxicology. 2007;**20**:724-733

[62] Choe N, Tanaka S, Kagan E. Asbestos fibers and interleukin-1 upregulate the formation of reactive nitrogen species in rat pleural mesothelial cells. American Journal of Respiratory Cell and Molecular Biology. 1998;**19**:226-236

[63] Liu G, Cheresh P, Kamp DW. Molecular basis of asbestos-induced lung disease. Annual Review of Pathology. 2013;**8**:161-187

[64] Pinkerton KE et al. Alveolar type II cell responses to chronic inhalation of chrysotile asbestos in rats. American Journal of Respiratory Cell and Molecular Biology. 1990;**3**(6):543-552

[65] Mossman BT et al. Asbestos: Scientific developments and implications for public policy. Science. 1990;**247**(4940):294-301

[66] Brody AR, Overby LH. Incorporation of tritiated thymidine by epithelial and interstitial cells in bronchiolar-alveolar regions of asbestos-exposed rats. The American Journal of Pathology. 1989;**134**(1):133-140

[67] Kamp DW et al. The role of free radicals in asbestos-induced diseases. Free Radical Biology & Medicine. 1992;**12**(4):293-315

[68] Shukla A et al. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free Radical Biology & Medicine. 2003;**34**(9):1117-1129

[69] Pascolo L et al. The interaction of asbestos and iron in lung tissue

revealed by synchrotron-based scanning X-ray microscopy. Scientific Reports. 2013;**3**:1123

[70] Andujar P et al. Differential mutation profiles and similar intronic TP53 polymorphisms in asbestos-related lung cancer and pleural mesothelioma. Mutagenesis. 2013;**28**(3):323-331

[71] Wang X et al. Mutations in the p53 gene in lung cancer are associated with cigarette smoking and asbestos exposure. Cancer Epidemiology, Biomarkers & Prevention. 1995;**4**(5):543-548

[72] Andujar P et al. p16INK4A inactivation mechanisms in non-small-cell lung cancer patients occupationally exposed to asbestos. Lung Cancer. 2010;**67**(1):23-30

[73] Cui Y et al. Chrysotile effects on the expression of anti-oncogene P53 and P16 and oncogene C-jun and C-fos in Wistar rats' lung tissues. Environmental Science and Pollution Research International. 2017;**25**:22378-22388

[74] Shukla A et al. ERK2 is essential for the growth of human epithelioid malignant mesotheliomas. International Journal of Cancer. 2011;**129**(5):1075-1086

[75] Shukla A et al. The gamma-glutamylcysteine synthetase and glutathione regulate asbestos-induced expression of activator protein-1 family members and activity. Cancer Research. 2004;**64**(21):7780-7786

[76] Zanella CL et al. Asbestos-induced phosphorylation of epidermal growth factor receptor is linked to c-fos and apoptosis. The American Journal of Physiology. 1999;**277**(4 Pt 1):L684-L693

[77] Paris C et al. Association between lung cancer somatic mutations and occupational exposure in never-smokers. The European Respiratory Journal. 2017;**50**(4)

[78] Kradin RL, Iafrate J, Christiani DC. Pulmonary adenocarcinoma with epidermal growth factor receptor-mutations in asbestos exposed non-smokers: A case series. American Journal of Industrial Medicine. 2017;**60**(3):306-309

[79] Maki-Nevala S et al. Epidermal growth factor receptor mutations in 510 Finnish non-small-cell lung cancer patients. Journal of Thoracic Oncology. 2014;**9**(6):886-891

[80] Nymark P et al. Gene expression profiles in asbestos-exposed epithelial and mesothelial lung cell lines. BMC Genomics. 2007;**8**:62

[81] Shukla A et al. Transcriptional up-regulation of MMP12 and MMP13 by asbestos occurs via a PKCdelta-dependent pathway in murine lung. The FASEB Journal. 2006;**20**(7):997-999

[82] Dammann R et al. CpG island methylation and expression of tumour-associated genes in lung carcinoma. European Journal of Cancer. 2005;**41**(8):1223-1236

[83] Kettunen E et al. Asbestos-associated genome-wide DNA methylation changes in lung cancer. International Journal of Cancer. 2017;**141**(10):2014-2029

[84] Nymark P et al. Integrative analysis of microRNA, mRNA and aCGH data reveals asbestos- and histology-related changes in lung cancer. Genes, Chromosomes & Cancer. 2011;**50**(8):585-597

[85] Tunesi S et al. Gene-asbestos interaction in malignant pleural mesothelioma susceptibility. Carcinogenesis. 2015;**36**(10):1129-1135

[86] Wei S et al. Genome-wide gene-environment interaction analysis for asbestos exposure in lung cancer susceptibility. Carcinogenesis. 2012;**33**(8):1531-1537

[87] Carbone M et al. A mesothelioma epidemic in Cappadocia: Scientific developments and unexpected social outcomes. Nature Reviews. Cancer. 2007;**7**(2):147-154

[88] Carbone M, Yang H. Molecular pathways: Targeting mechanisms of asbestos and erionite carcinogenesis in mesothelioma. Clinical Cancer Research. 2012;**18**(3):598-604

[89] Hillegass JM et al. Asbestos and erionite prime and activate the NLRP3 inflammasome that stimulates autocrine cytokine release in human mesothelial cells. Particle and Fibre Toxicology. 2013;**10**:39

[90] Cardile V et al. Behaviour of the new asbestos amphibole fluor-edenite in different lung cell systems. The International Journal of Biochemistry & Cell Biology. 2004;**36**(5):849-860

[91] Shvedova AA et al. Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: One year postexposure comparisons. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2014;**306**(2):L170-L182

[92] Polimeni M et al. Multi-walled carbon nanotubes directly induce epithelial-mesenchymal transition in human bronchial epithelial cells via the TGF-beta-mediated Akt/GSK-3beta/SNAIL-1 signalling pathway. Particle and Fibre Toxicology. 2016;**13**(1):27

[93] Pacurari M et al. Raw single-wall carbon nanotubes induce oxidative stress and activate MAPKs, AP-1, NF-kappaB, and Akt in normal and malignant human mesothelial cells. Environmental Health Perspectives. 2008;**116**(9):1211-1217

[94] Asakura M et al. Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with chrysotile A fibers. Journal of Occupational Health. 2010;**52**(3):155-166

[95] Yamashita K et al. Carbon nanotubes elicit DNA damage and inflammatory response relative to their size and shape. Inflammation. 2010;**33**(4):276-280

[96] Kisin ER et al. Single-walled carbon nanotubes: Geno- and cytotoxic effects in lung fibroblast V79 cells. Journal of Toxicology and Environmental Health. Part A. 2007;**70**(24):2071-2079

[97] Ghosh M et al. Single-walled and multi-walled carbon nanotubes induce sequence-specific epigenetic alterations in 16 HBE cells. Oncotarget. 2018;**9**(29):20351-20365

[98] Mohery M et al. Radon decay products in realistic living rooms and their activity distributions in human respiratory system. Radiation Protection Dosimetry. 2014;**162**(3):394-399

[99] Pohl-Ruling J et al. Chromosomal aberrations of blood lymphocytes induced in vitro by radon-222 daughter alpha-irradiation. Mutation Research. 2000;**449**(1-2):7-19

[100] Jovanovic B, Nikezic D. Probability of bystander effect induced by alpha-particles emitted by radon progeny using the analytical model of tracheobronchial tree. Radiation Protection Dosimetry. 2010;**142**(2-4):168-173

[101] Jalal N et al. Radiation induced bystander effect and DNA damage. Journal of Cancer Research and Therapeutics. 2014;**10**(4):819-833

[102] Tomita M, Maeda M. Mechanisms and biological importance of photon-induced bystander responses: Do they

have an impact on low-dose radiation responses. Journal of Radiation Research. 2015;**56**(2):205-219

[103] Tomita M et al. Dose response of soft X-ray-induced bystander cell killing affected by p53 status. Radiation Research. 2013;**179**(2):200-207

[104] Hei TK et al. Radiation induced non-targeted response: Mechanism and potential clinical implications. Current Molecular Pharmacology. 2011;**4**(2):96-105

[105] Robertson A et al. The cellular and molecular carcinogenic effects of radon exposure: A review. International Journal of Molecular Sciences. 2013;**14**(7):14024-14063

[106] Moore S, Stanley FK, Goodarzi AA. The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation—No simple task. DNA Repair (Amst). 2014;**17**:64-73

[107] Rothkamm K et al. Radiation-induced HPRT mutations resulting from misrejoined DNA double-strand breaks. Radiation Research. 2008;**169**(6):639-648

[108] Kuhne M, Rothkamm K, Lobrich M. No dose-dependence of DNA double-strand break misrejoining following alpha-particle irradiation. International Journal of Radiation Biology. 2000;**76**(7):891-900

[109] Tsao MS, Hirsch FR, Yatabe Y, editors. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. 2nd ed. Aurora, CO, USA: IASLC; 2016

[110] Ruano-Ravina A et al. Residential radon, EGFR mutations and ALK alterations in never-smoking lung cancer cases. The European Respiratory Journal. 2016;**48**(5):1462-1470

[111] Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: A review. Radiation Research. 2012;**178**(6):505-523

[112] Ruano-Ravina A, Faraldo-Valles MJ, Barros-Dios JM. Is there a specific mutation of p53 gene due to radon exposure? A systematic review. International Journal of Radiation Biology. 2009;**85**(7):614-621

[113] Choi JR et al. Novel genetic associations between lung cancer and indoor radon exposure. Journal of Cancer Prevention. 2017;**22**(4):234-240

[114] Cui FM et al. Radon-induced alterations in micro-RNA expression profiles in transformed BEAS2B cells. Journal of Toxicology and Environmental Health. Part A. 2013;**76**(2):107-119

[115] Chaudhry MA et al. Micro RNA responses to chronic or acute exposures to low dose ionizing radiation. Molecular Biology Reports. 2012;**39**(7):7549-7558

[116] Sinitsky MY et al. Associations of DNA-repair gene polymorphisms with a genetic susceptibility to ionizing radiation in residents of areas with high radon (222Rn) concentration. International Journal of Radiation Biology. 2015;**91**(6):486-494

[117] Bonner MR et al. Radon, secondhand smoke, glutathione-S-transferase M1 and lung cancer among women. International Journal of Cancer. 2006;**119**(6):1462-1467

[118] Ruano-Ravina A et al. Genetic susceptibility, residential radon, and lung cancer in a radon prone area. Journal of Thoracic Oncology. 2014;**9**(8):1073-1080

[119] Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: Initiators, amplifiers or an Achilles' heel? Nature Reviews. Cancer. 2014;**14**(11):709-721

[120] Yang Y et al. Mitochondria and mitochondrial ROS in cancer: Novel targets for anticancer therapy. Journal of Cellular Physiology. 2016;**231**(12):2570-2581

[121] Pace C et al. Monomethylarsonous acid, but not inorganic arsenic, is a mitochondria-specific toxicant in vascular smooth muscle cells. Toxicology In Vitro. 2016;**35**:188-201

[122] Flora SJ. Arsenic-induced oxidative stress and its reversibility. Free Radical Biology & Medicine. 2011;**51**(2):257-281

[123] Pulido MD, Parrish AR. Metal-induced apoptosis: Mechanisms. Mutation Research. 2003;**533**(1-2):227-241

[124] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5):646-674

[125] Murphy FA et al. The mechanism of pleural inflammation by long carbon nanotubes: Interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells. Particle and Fibre Toxicology. 2012;**9**:8

[126] Matsuzaki H et al. Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis. Journal of Biomedicine & Biotechnology. 2012;**2012**:492608

[127] Diakos CI et al. Cancer-related inflammation and treatment effectiveness. The Lancet Oncology. 2014;**15**(11):e493-e503

[128] Wild CP. Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiology, Biomarkers & Prevention. 2005;**14**(8):1847-1850

[129] Wild CP. The exposome: From concept to utility. International Journal of Epidemiology. 2012;**41**(1):24-32

[130] Bartholomew C et al. EGFR targeted therapy in lung cancer: An evolving story. Respiratory Medicine Case Reports. 2017;**20**:137-140

[131] Sullivan I, Planchard D. ALK inhibitors in non-small cell lung cancer: The latest evidence and developments. Therapeutic Advances in Medical Oncology. 2016;**8**(1):32-47

[132] Vargas AJ, Harris CC. Biomarker development in the precision medicine era: Lung cancer as a case study. Nature Reviews. Cancer. 2016;**16**(8):525-537

[133] Martinez VD et al. Emerging arsenic threat in Canada. Science. 2013;**342**(6158):559

[134] Martinez VD et al. Arsenic-related DNA copy-number alterations in lung squamous cell carcinomas. British Journal of Cancer. 2010;**103**(8):1277-1283

Edited by Pinar Erkekoglu

Oncogenes are mutated and/or overexpressed at high levels in tumor cells. Tumors of the lung, breast, pancreas, and colon may display specific oncogenetic features. These tumors have been largely associated with exposure to environmental carcinogens and a variety of biological agents, including viruses. These carcinogens can induce specific genetic and epigenetic alterations in these tissues, leading to aberrant functioning of oncogenes and tumor suppressor genes. On the microRNAs (miRNAs) there are significant modifiers of both transcription and translation of oncogenes in carcinogenesis. In the last 50 years, several oncogenes and microRNAs related to these oncogenes have been identified in different types of human cancers. It is now clear that high expression of oncogenes, DNA damage response, and regulation of the cell cycle are related to the circadian clock. This book will mainly focus on the expressions of different oncogenes in breast, colon, and lung cancers. Moreover, readers will gain qualified scientific knowledge of the alterations in miRNAs in different types of cancers and the effects of the circadian clock on the expression of oncogenes in carcinogenesis.

Published in London, UK

© 2019 IntechOpen
© ktsimage / iStock

IntechOpen