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Recent Developments in Myelodysplastic Syndromes

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Published in London, United Kingdom







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http://dx.doi.org/10.5772/intechopen.73936
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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

Recent Developments in Myelodysplastic Syndromes
Edited by Ota Fuchs
p. cm.
Print ISBN 978-1-78985-477-0
Online ISBN 978-1-78985-478-7
eBook (PDF) ISBN 978-1-83962-040-9

Meet the editor

Ota Fuchs graduated from the Chemical Technological University, Prague, Czech Republic, in 1971. He obtained his PhD in Biochemistry from the Faculty of Natural Sciences, Charles University, Prague, in 1981. He is employed as a senior scientist at the Institute of Hematology and Blood Transfusion, Prague. He undertook a visiting scientist short-term affiliation in the Beatson Institute for Cancer Research, Glasgow, UK, the Institute of Experimental Medicine of the Russian Academy of Medical Sciences in St. Petersburg, Russia, and in the Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada. He was the principal investigator of five projects for the Internal Grant Agency of the Ministry of Health of the Czech Republic and of one grant project for the Grant Agency of the Czech Republic. He is a member of the Czech Medical Society J.E. Purkyne and the Czech MDS Group.

Contents

Preface

Genome and exome sequencing together with targeted deep-sequencing studies have defined several gene mutations in almost every myelodysplastic syndrome (MDS) patient. These mutations can impact disease phenotype, prognosis and disease progression. However, there are many non-pathogenic mutations, and most mutations are not specific for MDS. Applying this information clinically is in progress even if the clinical impact of some mutations remains controversial. Several mutations will likely be incorporated into future prognostic scoring systems. Great progress has also been reached in the studies of non-coding RNAs in MDS. It is possible that they will be used in MDS diagnostics and prognosis. Big advancements in the studies of immune mechanisms in MDS have been achieved and translated into clinical studies of immunotherapies in MDS. Innate and adaptive immune pathways are excessively active in the niche of hematopoietic cells in MDS. Common etiological mechanisms of MDS and autoimmune diseases is possible because these diseases are often associated with MDS. Unfortunately, no new drugs have been approved for MDS since approval by the Food and Drug Administration of azacitidine in 2004, lenalidomide in 2005, and decitabine in 2006. The European Medicines Agency approved azacitidine in 2008 and lenalidomide in 2013. This progress in many aspects of MDS warrants this new book about this heterogeneous group of clonal neoplasms arising from hematopoietic stem cells, and characterized by inefficient hematopoiesis, peripheral cytopenias, frequent karyotypic abnormalities, risk of clonal evolution, and transformation to acute myeloid leukemia, three years after the last book about MDS published by IntechOpen.

This book provides a review of several fields of MDS not only for research workers and clinicians, but also for medical students with an interest in MDS. The first introductory chapter deals with recent progress in this area. The second chapter provides a review of diagnosis and classification of MDS. Chapter 3 discusses the association of MDS and autoimmune disorders. Chapter 4 introduces immune dysregulation in MDS. The fifth chapter analyzes an update on pathophysiology and management of MDS. The last chapter discusses non-coding RNAs in MDS.

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Chapter 1

Introductory Chapter: Progress in Myelodysplastic Syndrome Area

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1. Advances in our knowledge of cytogenetic abnormalities and gene mutations

Myelodysplastic syndromes (MDS) constitute a group of age-associated heterogeneous clonal hematopoietic disorders characterized by ineffective hematopoiesis with peripheral cytopenias, dysplasia, and an increased risk of progression to acute myeloid leukemia (AML) [1–6]. About 50% of cases of MDS are characterized by the presence of cytogenetic abnormalities. Losses of chromosomal material as del(5q), del(20q), monosomy 7 or del(7q), and del(Y) are most common cytogenetic abnormalities and are more frequent than gains of chromosomal material as trisomy 8 or trisomy 21 [7].

MDS are caused by abnormalities in many genes. The great progress in analysis of these mutations and in elucidation of relationships between gene mutations and clinical phenotypes of these disorders was achieved. Somatic mutations were found in more than 90%. Next-generation sequencing (NGS) detected about 10 different mutations in almost every patient with MDS. The majority of these mutations are nonpathogenic passenger mutations. However, one or more driver mutations in most patients with MDS are associated with the pathogenesis of MDS. Gene mutations affect proteins involved in various important cell processes as RNA-splicing, DNA methylation, histone and chromatin modifications, signal transduction, transcription (transcription factors), tumor suppressor (*TP53*), *RAS* pathway, and separation of sister chromatids during cell division (cohesion complex) [4, 8–10].

RNA-splicing and DNA methylation mutations occur early and are known as founding mutations. Other mutations are called subclonal mutations. No MDS-specific mutations exist. Strongly represented mutations in genes coding for proteins involved in DNA methylation, such as *TET2*, *DNMT3A*, and *ASXL1*, are common also in older individuals with normal blood count (clonal hematopoiesis of indeterminate potential/CHIP/) [11, 12]. Until now, mutations in *TP53*, *EZH2*, *RUNX1*, and *SF3B1* predict independently overall survival (OS) of MDS patients. The first three mutations are associated with shorter OS but the last mutation is connected with better survival in refractory anemia with ring sideroblast (MDS-RS) and with thrombocytosis (RARS-T) [13, 14]. *SF3B1* mutations are present in about 80% of MDS-RS and correlates with its development. *SF3B1* mutations could alter the expression of the gene for ABCB7 transporter and abnormally regulate iron homeostasis in mitochondria mediating the phenotype of acquired MDS-RS [15]. Effects of other mutations are not clear up to now and results are often controversial.

We lack clinical methods to stop clonal development from relatively benign state of CHIP to malignancy. Especially, *TP53*-mutant clones induce progress to therapy-related MDS/AML. Therapy-related myeloid neoplasms have mutations in *TP53* and

epigenetic modifying genes, instead of mutations in tyrosine kinase and spliceosome genes [16]. The possible treatments are now the use of hypomethylating agents or in future anti-inflammatory therapy and clonally selective immunotherapies.

MDS are associated with genomic instability and extensive DNA damage caused by deficient repair mechanisms. Aberrations in DNA damage response/repair genes other than *TP53* and some genes involved in DNA damage checkpoints are rare. Differential expression of homologous recombination DNA repair-associated genes during MDS progression was detected and could be confirmed as new biomarkers related to pathogenesis and poor prognosis in MDS [17, 18].

2. Advance in our understanding of del(5q) myelodysplastic syndrome pathogenesis and its treatment with lenalidomide

The greatest progress was achieved in the study of molecular pathogenesis of del(5q) MDS disease phenotype and its treatment by immunomodulatory or cereblon-binding drug lenalidomide [2, 19–35]. Ebert et al. described that impaired ribosome biosynthesis due to *RPS14* (ribosomal protein 14 of the small ribosome subunit) gene haploinsufficiency leads to the E3 ubiquitin ligase HDM2 (human homolog to mouse double minute 2, major negative regulator of p53) inactivation by free ribosomal proteins, particularly RPL11 [36]. HDM2 degradation drives p53-mediated apoptosis of erythroid cells carrying the del(5q) aberration. This p53-mediated apoptosis of erythroid cells is a key effector of hypoplastic anemia in MDS patients with del(5q) [36]. *RPS14* haploinsufficiency causes a block in erythroid differentiation mediated by calprotectin (the heterodimeric S100 calcium-binding proteins S100A8 and S100A9) [37]. Proinflammatory proteins, S100A9 and tumor necrosis factor-α, suppress the effect of erythropoietin in MDS [38]. Some patients originally considered as MDS patients without del(5q) can have a phenotype of atypical 5q− syndrome and can be sensitive to lenalidomide therapy because they have diminutive somatic deletions in the 5q region. These deletions were not identified by fluorescence in situ hybridization or cytogenetic testing but by single nucleotide polymorphism array genotyping [39]. Low RPS14 expression in 50–70% MDS patients without del(5q) confers higher apoptosis rate of nucleated erythrocytes and predicts prolonged survival [40, 41].

What is the mechanism of lenalidomide in del(5q) MDS based on what has been achieved and elucidated to date? Lenalidomide stabilizes E3 ubiquitin ligase HDM2, thereby accelerating p53 degradation [42, 43]. Lenalidomide inhibits phosphatases PP2a and Cdc25c (coregulators of cell cycle which genes are very commonly deleted in del(5q) MDS) with consequent G2 arrest of del(5q) MDS progenitors and their apoptosis. PP2a and Cdc25c inhibition by lenalidomide suppress HDM2 autoubiquitination and subsequent degradation. Thus, lenalidomide has been shown to not only reverse apoptosis within the erythroid compartment, but also directly induce apoptosis of the myeloid clone in del(5q) MDS [44, 45]. Lenalidomide upregulates expression of other two haploinsufficient genes located on chromosome 5q, genes for microRNAs (miR-145 and miR-146a) [46]. These miRs are involved in Toll-like receptor pathway, IL-6 induction, and regulation of megakaryopoiesis [20].

Ito et al. discovered that thalidomide (founding member of immunomodulatory drugs/IMiDs/) binds cereblon (CRBN) in the terminal C-region (parts of exons 10 and 11 of the *CRBN* gene code this IMiD binding region) [47]. Several researchers confirmed CRBN as target of lenalidomide in multiple myeloma (MM), lymphoma, chronic lymphocytic leukemia, and del(5q) MDS [48]. CRBN is the ubiquitously expressed 51 kDa protein with a putative role in cerebral development, especially memory and learning [49, 50].

Our group found that del(5q) MDS patients (the so-called 5q minus syndrome) have higher levels of full-length CRBN mRNA than other patients with lower risk MDS, linking higher levels of a known lenalidomide target CRBN and del(5q) MDS subgroup known to be especially sensitive to lenalidomide [51].

CRBN is a member and substrate receptor of the cullin 4 RING E3 ubiquitin ligase complex (CRL4). CRBN recruits substrate proteins to the CRL4 complex for ubiquitination and the subsequent degradation in proteasomes. IMiDs binds to CRBN in CRL4 complex and block normal endogenous substrates (CRBN and the homeobox transcription factor MEIS2 in multiple myeloma/MM/) from binding to CRL4 for ubiquitination and degradation [52]. After IMID binding to CRBN, CRL4 complex is recruiting transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) for ubiquitination and degradation in MM cells [53]. Degradation of these transcription factors explains lenalidomide's growth inhibition of MM cells and increased interleukin-2 (IL-2) release from T cells. However, it is unlikely that degradation of IKZF1 and IKZF3 accounts for lenalidomide's activity in MDS with del(5q). Fink et al. identified a novel target casein kinase1A1 (CSNK1A1) by quantitative proteomics in the myeloid cell line KG-1 [54]. CSNK1A1 is encoded in the del(5q) commonly deleted region and the gene is haploinsufficient. Lenalidomide treatment leads to increased ubiquitination of the remaining CSNK1A1 and decreased protein abundance. CSNK1A1 negatively regulates β-catenin which drives stem cell self-renewal, and *CSNK1A1* haploinsufficiency causes the initial clonal expansion in patients with the del(5q) MDS and contributes to the pathogenesis of del(5q) MDS. The further inhibition of CSNK1A1 in del(5q) MDS is associated with del(5q) failure and p53 activation. The inhibition of CSNK1A1 reduced RPS6 phosphorylation, induced p53 expression and growth inhibition, and triggered myeloid differentiation program. TP53-null leukemia did not respond to CSNK1A1 inhibition, strongly supporting the importance of the p53 expression for the yield of CSNK1A1 inhibition. *CSNK1A1* mutations have been recently found in 5–18% of MDS patients with del(5q) [55]. These mutations are associated similarly to the effect of *TP53* mutations with rise to a poor prognosis in del(5q) MDS [56]. Other studies did not find impact of *CSNK1A1* mutations on lenalidomide treatment in del(5q) MDS [57, 58].

Even if the treatment of del(5q) MDS patients with lenalidomide is very efficient, 50% of treated patients relapse after 2–3 years. Martinez-Hoyer et al. found that low platelet count and occurrence of additional mutations, mainly *TP53* mutations induce lenalidomide resistance [59–61]. They used whole genome sequencing and observed in several resistant patients mutations in *RUNX1* gene or decreased amount of *RUNX1* transcript without aberration in *TP53* [59]. Results were verified in model system of two human del(5q) lines, MDS-L and KG-1a. *RUNX1* knock-out or RUNX1 shRNA increased proliferation and reduced apoptosis in lenalidomide-treated cells with decreased *RUNX1* transcript. Therefore, effect of lenalidomide in del(5q) requires functional RUNX1. Similar results were obtained with *TP53* knock-out cells. Both *RUNX1* and *TP53* transcripts cooperate and alter the activity of GATA2 transcriptional complex [59].

3. Studies on lenalidomide use also in lower risk non-del(5q) MDS treatment and new possible therapies

While CSNK1A1 is $CRL4^{CRBN}$ target in del(5q) MDS, $CRL4^{CRBN}$ targets in lower risk non-del(5q) remain to be determined. The mechanism of action of lenalidomide is still unclear in non-del(5q) MDS cells. Recent evidence shows that lenalidomide directly improves erythropoietin receptor (EPOR) signaling by EPOR upregulation mediated by a posttranscriptional mechanism [62]. Lenalidomide

stabilizes the EPOR protein by inhibition of the E3 ubiquitin ligase RNF41 (ring finger protein 41, also known as neuregulin receptor degradation protein-1/Nrdp1/ and fetal liver ring finger/FLRF/) responsible for EPOR polyubiquitination and next degradation [62] and induces lipid raft assembly to enhance EPOR signaling in MDS erythroid progenitors [63, 64].

After failure of ESAs, lenalidomide yields red blood cell transfusion independence in 20–30% of lower risk non-del(5q) MDS. Indeed, several observations suggest an additive effect of ESA and lenalidomide in this situation [65, 66] and also in del(5q) MDS patients [67]. Synthetic corticosteroids (dexamethasone and prednisone) are also able to potentiate the effect of lenalidomide or combination of lenalidomide and erythropoietin [67–69].

Basiorka et al. and Sallman et al. reported activation of the NLRP3 inflammasome in MDS [70, 71]. NRLP3 drives clonal expansion and pyroptotic cell death. Independent of genotype, MDS hematopoietic stem and progenitor cells (HSPCs) overexpress inflammasome proteins. Activated NLRP3 complexes direct then activation of caspase-1, generation of interleukin-1β (IL-1β) and IL-18, and pyroptotic cell death. Mechanistically, pyroptosis is triggered by the alarmin S100A9 that is found in excess in MDS HSPCs and bone marrow plasma. Further, like somatic gene mutations, S100A9-induced signaling activates NADPH oxidase (NOX) and increasing levels of reactive oxygen species (ROS). ROS initiate cation influx, cell swelling, and β-catenin activation. Knockdown of NLRP3 or caspase-1, neutralization of S100A9, and pharmacologic inhibition of NLRP3 or NOX suppress pyroptosis, ROS generation, and nuclear β-catenin in MDSs and are sufficient to restore effective hematopoiesis. Thus, alarmins and founder gene mutations in MDSs cause a common redox-sensitive inflammasome circuit. They are new candidates for therapeutic intervention.

Not only apoptosis and pyroptosis are involved in increased cell death in MDS. Recently, possible further mechanism of cell death, necroptosis, in MDS has been described [72, 73]. Necroptosis is like pyroptosis associated with membrane permeabilization and the release of damage-associated molecular patterns (DAMPs) such as alarmins. Alarmins bind Toll-like receptor 4 (TLR4) and activate the transcription factor NF-κB and inflammation [74].

The effects of lenalidomide in non-del(5q) are thought to be secondary to modulation of the immune system. Hyperactivated T cells inhibit hematopoiesis. Immunosuppressive therapies with antithymocyte globulin alone and in combination with prednisone or cyclosporine show response rates between 25 and 40% [75, 76].

The studies discussed in this and other chapters of this book will help to translate our knowledge of genetic aberrations and of pathophysiological mechanisms in MDS into clinical use in diagnosis, prognosis, and therapy. Novel agents developed on the basis of this knowledge (luspatercept, rigosertib, immune checkpoint inhibitors, venetoclax, and others) are in clinical trials and will help in relapsed/refractory MDS.

The work of our group in this area was supported by the research project for conceptual development of research organization (00023736; Institute of Hematology and Blood Transfusion, Prague) from the Ministry of Health of the Czech Republic.

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Chapter 2

Diagnosis and Classification of Myelodysplastic Syndrome

Gamal Abdul Hamid, Abdul Wahab Al-Nehmi and Safa Shukry

Abstract

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by morphological dysplastic changes in one or more of the major hematopoietic cell lines. MDS can present with varying degrees of single or multiple cytopenias including neutropenia, anemia and thrombocytopenia. Presentation of MDS can range from asymptomatic to life threatening. MDS diagnosis and classification present important challenges, particularly in the distinction from benign conditions. French-American-British (FAB) classification proposed a classification based on easily obtainable laboratory information and was recommended in early and as modified by guidelines of new classification of World Health Organization (WHO). The strategy of diagnostic laboratory in MDS depends on morphological changes and is based on existence of dysplastic changes in the peripheral blood and bone marrow including peripheral blood smear, bone marrow aspirate smear and bone marrow trephine biopsy. The correct morphological interpretation and the use of cytogenetics, immunophenotyping, immunohistochemistry and molecular analysis will give valuable information on diagnosis and prognosis.

Keywords: myelodysplasia, cytopenia, diagnostic criteria, classification

1. Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders with a relatively heterogeneous spectrum, characterized by morphological dysplasia in hematopoietic cells and by bone marrow failure and varying degrees of peripheral blood cytopenias. MDS have been recognized for more than 70 years and named refractory anemia, oligoblastic leukemia and smoldering acute leukemia.

The risks of MDS include infection, anemia, bleeding and transformation to acute myeloblastic leukemia (AML) in approximately 30% of cases. MDS incidence increased from less than 5/100,000 for patients less than 60 years to 36.2 per 100,000 in patients more than 80 year old and more common among men.

In the last 20 years, different MDS classification and prognostic scoring systems have been proposed [1]. French-American-British (FAB) classification was recommended in early and as modified by the World Health Organization (WHO). The WHO classification system uses percentages of blasts in bone marrow, ring sideroblasts and dysplastic changes to differentiate MDS subtypes. The International Prognostic Scoring System (IPSS) is based on a multivariate to evaluate the prognosis. The updated and recent scoring system combine with WHO classification

for identification transfusion need was modified by Malcovati and co-workers, the so-called WPSS (World Prognostic Scoring System). This score suggests that patients with unilineage erythroid dysplasia do not need transfusion [2].

2. Diagnosis

The risk of MDS increased with advancing age; approximately 86% of patients with newly diagnosed MDS predominate in the elderly, with a median age at diagnosis 65 years [3]. The age of MDS patients at diagnosis was different according to residency; the results of some studies on patients show that the median age of diagnosis in German, Japan, and Korea were 74, 60, and 57 years, respectively [4].

The chosen diagnostic criterion of MDS is the dysplasia in ≥10% of total count, this morphology features can point to underlying pathological cytogenetic changes which suggestive MDS diagnosis according to the World Health Organization (WHO) 2016 revision [5].

The minimal prerequisites diagnostic guidelines for MDS according to an International Working Group (IWG) are: (1) stable cytopenia for >6 months unless accompanied a specific chromosomal analysis (Karyotype) or bilineage dysplasia [6]; (2) the exclusion of other potential disorders as a primary reason for dysplasia or cytopenia or both.

3. Diagnostic workup

MDS diagnosis based on morphological characteristics of bone marrow dysplasia in patients with clinical manifestations evidence of hematopoiesis impairments by different combinations of anemia, leukopenia, neutropenia and thrombocytopenia. The National Comprehensive Cancer Network (NCCN) recommend specific guidelines for evaluation of MDS include physical examination; peripheral blood examination, bone marrow examination with iron stain and cytogenetic, RBC folate and vitamin B12 and serum ferritin [7]. The combination peripheral cytopenias despite of bone marrow hypercellularity is the hallmark of MDS, and is a consequence of bone marrow dysfunction with an increase apoptosis rate of bone marrow cells.

According to NCCN the diagnosis of MDS requires ≥1 of MDS-related criteria: (1) dysplasia (≥10% in ≥1 of bone marrow cell line); (2) presence of 5–19% blast cells; and (3) presence of a specific MDS-linked chromosomal abnormalities like del(5q), del(20q), +8, or −7/del(7q) [8].

4. Differential diagnosis

Before treatment, the major role is to distinguish MDS from other causes of cytopenia and dysplastic changes and from other clonal stem cell disorders [9]. The investigations work-up is important to rule the possible differential diagnosis and pre-MDS conditions (**Table 1**).

4.1 Cytopenic causes

1. Chronic liver diseases

2. Drug induced cytopenia

	Characteristics
IDUS	Mild cytopenias for >6 months (Hb ≥ 11/dl, neutrophils ≥ 1500/μl, platelets ≥ 100,000/μl, all below lower limit of normal) or no cytopenias but marked dysplasia in >10% of cell lineages and no clonal cytogenetic/molecular markers
ICUS	Mild cytopenias (hemoglobin <11.0 g/dl, neutropenia <1500/μl and/or thrombocytopenia <100,000/μl and lack of significant dysplasia in the bone marrow but exclusion of other diseases and/or no clonal cytogenetic/molecular markers
CCUS	Hemoglobin, <11 g/dl, ANC <1500/μl, platelet count <100,000/μl, ≥10% dysplasia in the granulocytic, erythroid, or megakaryocytic lineage, myeloblasts comprise ≥5% of total cellularity. Common mutations; TET2, DNMT3A, ASXL1, SRSF2, TP53
CHIP	The presence of clonal hematopoiesis in the absence of cytopenias and dysplastic changes. The incidence of CHIP increases with age. Common mutations; TET2, DNMT3A, ASXL1, PPM1D, JAK2, TP53, *SF3B1*

ICUS, idiopathic cytopenia of uncertain significance; ICUS, idiopathic dysplasia of unknown significance; CCUS, clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential.
An absence of mutation and unexplained cytopenias are criteria do not meet World Health Organization (WHO)-defined requirements for myelodysplastic syndrome.

Table 1.
Differentiation of ICUS, IDUS, CCUS and CHIP [8].

3. Excessive alcohol intake
4. Cytotoxic therapy
5. B12/folate deficiency
6. Autoimmune cytopenia
7. Anemia of chronic disorders
8. Parasitic manifestation (hypersplenism in malaria and leishmaniasis)
9. Human immunodeficiency virus infection (HIV)
10. Other stem cell disorder

4.2 Idiopathic cytopenia of uncertain significance (ICUS)

4.3 Idiopathic dysplasia of unknown significance (IDUS)

4.4 Clonal cytopenia of undetermined significance (CCUS)

4.5 Clonal hematopoiesis of indeterminate potential (CHIP)

5. Clinical presentation

Clinical presentation of MDS is nonspecific and varies considerably depending on subtypes and severity of cytopenias. This should include family history,

tobacco, alcohol intake, pesticides, heavy metals, prior chemotherapy, irradiation, radioiodine, radioimmunotherapy, concomitant medication including "alternative medication", infection, tendency for bleeding/bruising, and a complete physical examination including spleen size. Symptoms can include general weakness, pallor, shortness of breathing, bleeding manifestations; gum bleeding and petechiae.

6. Blood tests

Complete blood count (CBC) includes white blood cell count (WBC) with differential blood count including erythrocyte morphology, hemoglobin, platelet count, red blood cell indices, mean corpuscular volume (MCV), and reticulocyte count.

Serum tests of erythropoietin, protein electrophoresis, folic acid, cobalamin, iron, total iron binding capacity (TIBC), ferritin, lactate dehydrogenase (LDH), bilirubin, Coombs test, alanine aminotransferase (ALT) test, aspartate aminotransferase (AST), alkaline phosphatase, albumin, uric acid, creatinine (S-immunoglobulins), B2 microglobulin and thyroid function tests.

Also some investigations are mandatory to exclude viral infection especially; anti-HIV, anti-Parvovirus B19 (hypoplastic MDS), hepatitis C antibody, hepatitis B surface antigen (HBsAg) and cytomegalovirus test (CMV) in transfusion dependent patients.

Cytogenetic study for BCR-ABL and JAK2 (Janus kinase 2) are important for differential diagnosis of myeloproliferative disorders.

6.1 Interpretation of peripheral blood

The WHO recommendations for the definition of cytopenia are the same reported in the International Prognostic Scoring System (IPSS), when the hemoglobin less than 10 g/dl, the leukocyte count 3000/mm^3, an absolute neutrophil less than 1800/mm^3 and platelets less than 100,000/mm^3. These thresholds have been a matter of debate, and as a result, any cytopenia should be differentiated from MDS in case of clear morphologic or the result of genetic features consistent with MDS [5, 6]. Anemia is represent in most patients, the mean corpuscular volume (MCV) is often increased and an increased erythrocyte distribution width (RDW) which the erythropoiesis disturbances. A dimorphic red blood cell (RBC) population (macrocytes and microcytes), anisocytosis, poikilocytosis, nucleated red blood cells, basophilic stippling and Howell-Jolly bodies are also indications that the erythrocyte has undergone abnormal development [10]. Peripheral blood may reveal very abnormal nuclei such as Pelger-Huet anomalies and hypo-or hypersegmentation and ring forms nuclei also occur in neutrophils are important morphological features in MDS/MPN peripheral blood when diagnosing and distinguishing MDS/MPN is important to understand the similarities and differences in pathologic mechanism from similar diseases (AML, infectious diseases and other causes of cytopenia). The platelet morphological changes include giant platelets and platelets hypogranulation or agranulation. Some platelets may possess large fused granules. Circulating micromegakaryocytes (dwarf cells), multiple small nuclei separated by strands of nuclear material, and large mononuclear cells with dysmorphic nuclear features have been described in peripheral blood from patients with MDS [11].

The diagnosis of MDS requires a careful light microscopic examination of optimally stained peripheral blood and bone marrow smear and trephine biopsy sections with presence of 1% blast in peripheral blood, with <5% BM blasts and uni- or multilineage dysplasia is defined as unclassifiable MDS. Monocytic hyperplasia accounting for >10% of the white blood cells is a common finding in chronic myelomonocytic leukemia (CMML) and is a common finding in dysplastic marrows and can be dominant manifestation of the hematopoietic abnormality in CMML for months and years.

Cell line	Peripheral blood	Bone marrow
Erythroid	Dimorphic; macrocytic; anisocytosis; polychromatic; hypochromasia, tear drop cells Pappenheimer bodies; basophilic stippling	Erythroid hyperplasia; megaloblastic changes; dyserythropoiesis; multinuclearity, nuclear bridges, nuclear budding, atypical mitosis, ring sideroblasts
Granulocytes	Hypersegmented neutrophils; hypogranular or agranular neutrophils; pseudo-Pelger cells	Shift to left, promyelocytes with absent or spares azurophilic granules; hypogranular myelocytes, metamyelocytes and neutrophils, Auer rods, pseudo-Pelger cells, nuclear anomalies
Monocytes	Mature monocytes	Increase and morphological abnormalities of monocytes
Megakaryocytes	Thrombocytosis; giant platelets	Increase or decrease; mononuclear megakaryocytes, micromegakaryocytes, hypersegmented megakaryocytes
Blasts	**Figure 2**	

The highly specific dysplastic changes in granulocytes of patients with MDS are hypoagranularity and nuclear abnormality of neutrophils in peripheral blood smear and positive on peroxidase reaction or Sudan black. The highly specific dysplastic changes in erythropoiesis are sideroblastic rings and megaloblastoid changes. The highly specific for dysmegakaryopoiesis are micromegakaryocytes.

Table 2.
MDS morphology abnormalities on peripheral blood and bone marrow [12, 13].

7. Bone marrow aspirate and biopsy

A diagnosis of MDS often requires repeated bone marrow aspiration/biopsy examinations a few weeks or months, or even years apart in order to firmly establish the diagnosis and to identify cases with rapid disease progression. Bone marrow morphology evaluation and dysplasia in blood and bone marrow follow guidelines in the WHO 2016 classification. A good quality diagnostic bone marrow analysis includes marrow aspirate May-Grunewald Giemsa (MGG)/equivalent and bone marrow iron stain and a bone marrow biopsy either decalcified/paraffin embedded or plastic embedded. Degree of fibrosis should be estimated. The cytochemistry staining should include iron staining, Peroxidase-Staining, in addition to hematoxylin-eosin/equivalent [12].

The cell counting of bone marrow and blood smear should include at least 200 cells in blood smear, 500 cells in bone marrow and 25 megakaryocytes and at least 100 erythroblasts should be evaluated. An optimal staining of blood and marrow slides prepared from freshly drawn aspirates is important for evaluation of dysplasia (**Table 2**) [12–15].

8. Dysplastic features

Dysplastic changes are the most important diagnostic features of myelodysplastic syndrome. A marrow cell lineage is considered picture of MDS if >10% of cells are affected.

8.1 Dyserythropoiesis

Dyserythropoiesis is the presence of oval macrocytes and erythroblast may resemble megaloblasts that have nuclear-cytoplasmic maturation asynchrony, nuclear fragmentation, or cytoplasmic nuclear remnants. This pattern is referred to as megaloblastoid erythropoiesis [14–16]. A dimorphic red blood cell population,

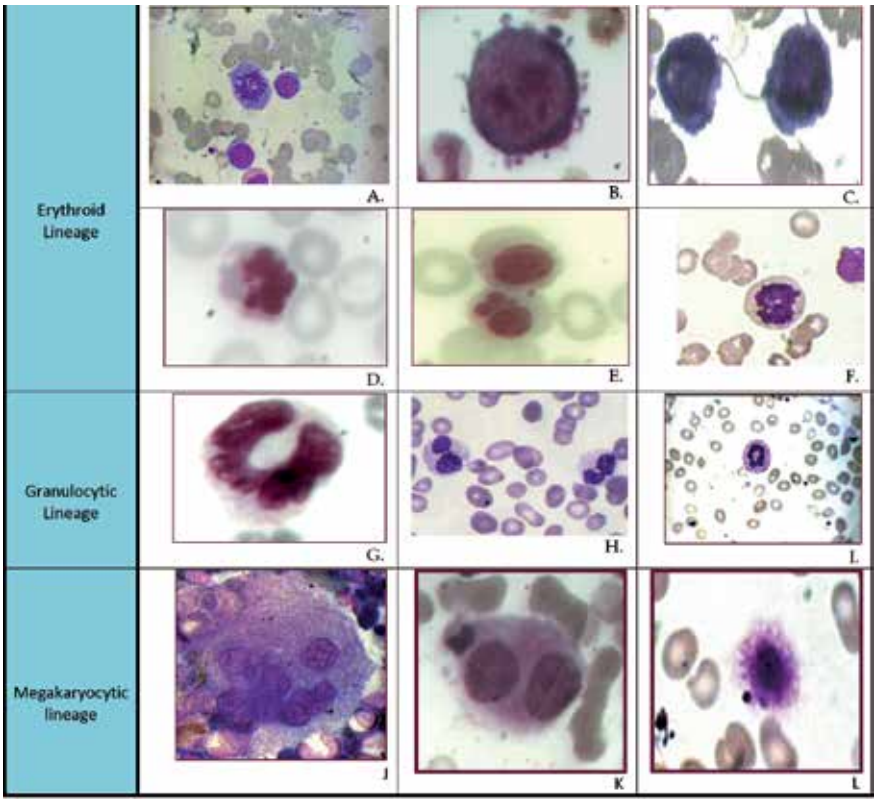

Figure 1.
Morphological abnormalities of myelodysplastic syndrome: Leishman stain. (I) Erythroid dysplastic changes; (A) megaloblastic changes, (B) cytoplasmic fraying, (C) internuclear bridging, (D) nuclear lobulation, (E) nuclear lobulation (F) karyorrhexis (II) granulocytic dysplastic changes; (G) hypogranulation band neutrophil, (H) pseudo-Pelger anomaly, (I) nucleus ring or doughnut shaped (III) megakaryocytic dysplastic changes; (J) hypersegmented, (k) micromegakaryocyte, (L) giant abnormal platelet.

anisocytosis, poikilocytosis, nucleated red blood cells, Howell-Jolly bodies and basophilic stippling are indications that the erythrocyte has undergone abnormal development. The RBC with abnormally round nucleus may have lobes or buds, internuclear bridging, nuclear fragments and abnormal mitosis are occasionally present. Pathologic sideroblast may be identified when the marrow treated with Prussian blue stain (**Figure 1**) [14, 15].

8.2 Dysmyelopoiesis

The most striking abnormalities are hypogranulated neutrophils. The defect in granulation may be seen in myelocytes early in the course of disease. Very abnormal nuclei, such as Pelger-Huet anomalies and hypo- or hypergranulation, and ring shaped nuclei in neutrophils. Monocytic hyperplasia is a common finding in dysplastic marrows and can be the dominant manifestation of the hematopoietic abnormalities of CMML for months or years (**Figure 1**) [11]. Cytoplasmic changes may include uneven staining such as a dense ring of basophilia around the periphery with a clear unstained area around the nucleus [14, 15]. Occasionally there are Auer rods, either in circulating or BM blast cells, entails an unfavorable prognosis and this could lead to misclassification the disease in the AML. Myeloperoxidase and the study of specific immunophenotypic markers are helpful to differentiate between MDS and other types of AML [17].

8.3 Dysmegakaryopoiesis

The common changes include giant platelets and abnormal platelet granulation, either hypogranulation or agranulation. Some platelets may possess large fused granules. Circulating micromegakaryocytes, multiple small nuclei separated by stands of nuclear material and large mononuclear cell with dysmorphic nuclear features have been described in peripheral blood of patients with MDS (**Figure 1**) [13–15].

For significant dysplasia, dysplastic features should be present in at least 10% of the nucleated cells in the lineage in consideration.

9. Blast cells

9.1 Counting blasts

Myeloblast cell should be differentiated from promyelocyte. The promyelocyte is larger than myeloblast and characterized by clear Golgi zone and azurophilic granulations. Myeloblast was defined in terms of several nuclear characteristics, including a high nuclear/cytoplasmic ratio, easily visible nucleoli and usually contain fine nuclear chromatin and viable nuclear shape. The International Working Group (IWGM) recommended that myeloblast in MDS should be classified as agranular or granular [12]. The agranular blast corresponds to the type I blast of the FAB classification. Type II have scanty granules [18] and type III blast with more than 20 fine azurophilic granules as defined by Goasguen et al. [13]. The nuclear characteristic of promyelocytes included an eccentric or central nucleus and intermediate or fine chromatin and azurophilic granulation (**Figure 2**) [12].

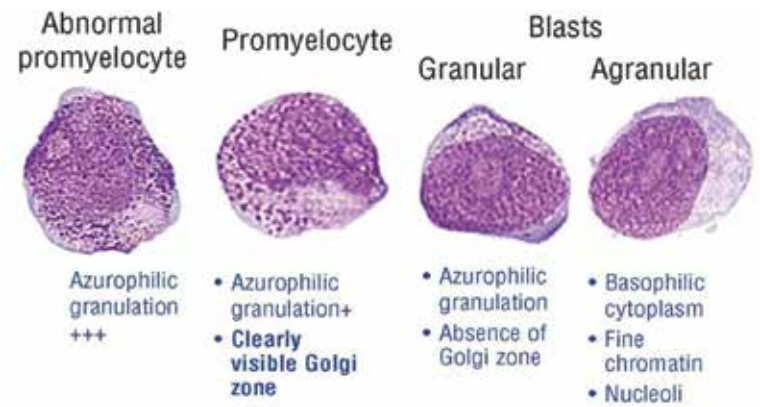

Figure 2.
Blasts, promyelocytes, abnormal promyelocytes, modified of Mufti et al. [12]. In MDS, the granular and agranular blasts have azurophilic granules and Auer rods; both exhibit visible nucleoli, scant basophilic cytoplasm, lack Golgi zone and exist of fine nuclear chromatin, while promyelocytes have eccentric nucleus with visible nucleoli and Golgi zone and exist of azurophilic granules.

10. Cytogenetic analysis

The cytogenetic study of bone marrow aspirate has a major role in determining clonality in patients with MDS. Karyotyping should be done in all patients, at least 25 metaphases, whenever possible, and described according to International System recommendations. Chromosomal abnormalities are reported in more

Prognostic category	Chromosomal categories	Median survival (months)
Very good	Del(11q), −Y	60
Good	Normal, del(5q), double aberrations including del(5q), del(12p), del (20q)	40.5
Intermediate	Del(7q), +8.i(17q), +19, any other	25.0
Poor	Inv(3)/t(3q), −7, −7/7q, double aberrations including −7/7q−, complex karyotypes with 3 abnormalities	15.0
Very poor	Complex karyotypes with >3 abnormalities	5.7

IPSS-R, Revised International Prognostic Scoring System.
MDS prognosis is calculated by utilizing the International Prognostic Scoring System (IPSS) score, which includes cytogenetics analyzed categories, in addition to number of cytopenias and counting of blast percentage. The present of >3 chromosomal abnormalities indicate very poor prognosis.

Table 3.
Cytogenetic prognosis in MDS according IPSS-R [21, 22].

than 50% of patients with MDS by counting 25 metaphase cytogenetic analysis, but not by fluorescence in situ hybridization (FISH) or sequencing technologies. The technique by using FISH method may be helpful to detect monosomy 7 and to clarify complex aberrations. Screening FISH (5q−, −7, +8) from peripheral blood may be performed in patients of dry tap bone marrow and this may influence management of the patient [19]. Different cytogenetic abnormalities are considered MDS-defining [20]. The presence monosomy 5, 7, or 13; 5q, 7q and 13q deletions; i(17p) and t(17p); 11q deletion; 9q or 12p deletion or t(12p), idic (X)(q13) allows for the diagnosis of MDS even in the absence of dysplastic changes. Cytogenetic is strongly correlated with not only the calculation prognosis but also selection of the most effective therapy; thus, a complete BM karyotype remains the standard work up evaluation procedure of the patient with MDS according to IPSS-R (**Table 3**) [21, 22].

11. Molecular genetic testing

The most important mutated genes for MDS prognostication involved in epigenetic regulation are acquired mutation have been detected in several genes: (*ASXL1*, *EZH2*, *DNMT3A*, *TET2*, *IDH1/2*, *pre-mRNA* splicing factors *(SF3B1*, *SRSF2*, *U2AF1) transcription (RUNX1*, *TP53*) and signaling transduction are seen in MDS and can demonstrate clonal disease [23]. According to the new *2016* World Health Organization (WHO) Classification of MDS, the analysis of the SF3B1 considered the only important diagnostic method for diagnosis of MDS-RS. The prognosis of MDS-RS is favorable in presence of SF3B1 mutations [24]. Mutations in the ASXL1, TP53, ETV6, RUNX1 and EZH2 are reported as independently associated with decreased overall survival in cases of MDS [25].

12. Immunophenotyping

By flow cytometry and immunohistochemistry, immunophenotyping of the blast population can be useful for emerging pathological CD34 and or CD117 and myeloperoxidase (MPO) positive populations are suggestive of transformation.

According to WHO classification 2016, the best method for diagnosis of MDS is the percentages of blast cells in bone marrow. The immunophenotyping can be useful to study the expression of maturation and anomalies as marker of dysplasia of a particular lineage [26].

Multiple aberrant features (>3) in maturation patterns of erythroid and myeloid lineage are highly specific for MDS, but single aberrancies are not diagnostic [27]. The role of flow cytometry can be useful in the diagnostic work-up of MDS, and to detect minimal residual disease after treatment according to the European Leukemia Net (ELN) work package for flow cytometry [28]. For prognostic follow up, the increase expression of CD33, CD34, CD13, HLA-DR/human leukocyte antigen-DR and decreased reactivity for CD11b in the bone marrow have been associated with shorter survival and high risk of transformation to acute leukemia.

13. Classification

13.1 FAB classification

Several classifications have been developed to predict the transformation of MDS to acute myeloid leukemia (AML). In 1982, the FAB system, was introduced based on percentage of blasts and morphological features in blood and bone marrow, namely medullary and peripheral blast cell count, ringed sideroblasts, number of monocytes in peripheral blood, and Auer rods. According to this classification, patients are diagnosed with MDS when dysplastic changes in bone marrow are present and/or myeloblast cells are between 5 and 30% of all bone marrow cells. Five subgroups with significantly different prognoses were established: refractory anemia (RA) with blasts <5% in BM, refractory anemia with ringed sideroblasts (RARS) with blasts <5% and ring sideroblasts >15%, refractory anemia with excess of blasts between 5 and 20% (RAEB), RAEB in transformation to acute leukemia and blast cells ranged between 20 and 30% (RAEB-T) and chronic myelomonocytic leukemia characterized by increase of peripheral blood monocytes (CMML) (**Table 4**) [20, 29]. For more than 20 years this classification served as the standard for the evaluation of MDS [30]. Hypercellular MDS, and MDS with bone marrow fibrosis were not recognized by the FAB classification [31].

Type	Blasts in blood	Blasts in bone marrow
1. Refractory anemia (RA)	<1%	Blasts <5%, ring sideroblastic <15%
2. Refractory anemia with ring sideroblastic (RARS)	<1%	Blasts <5%, ring sideroblasts >15%
3. Refractory anemia with excess of blast (RAEB)	<5%	Blasts 5–20%
4. Refractory anemia with excess blast in transformation (RAEB-t)	<30%	Blasts 20–30%
5. Chronic myelomonocytic leukemia (CMMoL)	<5% with increase monocytes	Blasts 0–20%
AML	>30%	>30%

Modified of Ref. [20].
CMML, chronic myelomonocytic leukemia blast cells <20% and monocytes ≥1000/μl); RA, refractory anemia <1% in PB and <5% blasts in BM; RAEB, RA with excess blasts in PB <5% and 5–20% blasts in BM; RAEB-t, RAEB with excess blasts in transformation between 20 and 30%; RARS, RA with ringed sideroblasts >15%.

Table 4.
Myelodysplastic syndrome (MDS) according to FAB classification [20].

13.2 WHO classification

The World Health Organization (WHO) classification of MDS revised in 1999 and redefine subtypes of MDS [32]. The definitions of refractory anemia (RA) and refractory anemia with ring sideroblastic (RARS) unchanged became more consistent and characterized by the presence of dysplastic morphology in the erythroid cell line. Refractory anemia with ring sideroblastic (RARS) is morphologically similar to *RA* with the presence of ≥15% ring sideroblasts.

Refractory anemia with excess of blasts (RAEB) recognized by the World Health Organization (WHO) classification in all versions and remains unchanged but distinguishes between two categories of RAEB: RAEB-1 with 5–10% blast cells and RAEB-2 with 11–20% blasts in the bone marrow.

The other new subgroups of MDS were incorporated: (1) refractory cytopenia with multilineage dysplasia (RC1Dys), is a frequent subtype of MDS, which is equivalent to RA or RARS in the FAB classification with the presence of dysplasia but lacking an increase in blast cells with no Auer rods or increase of monocytes; (2) del (5q) syndrome is a myelodysplastic disorder characterized by macrocytic anemia, dysplastic changes in the erythroid cell line only, thrombocytosis and increase of hypolobulated micromegakaryocyte; (3) MDS unclassifiable; myelodysplastic syndromes that do not meet criteria of a specific WHO entity.

RAEB-T and CMML subgroups were removed from the new MDS classification: RAEB-T, because of distinctive biologic features and similarities in treatment strategies with acute myeloid leukemia (AML), and CMML, because of having overlapping dysplastic and proliferative features and its close relation to myeloproliferative diseases [33].

2008 WHO classification	2016 WHO classification
Refractory cytopenia with unilineage dysplasia (RCUD) encompassing refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT)	MDS with single lineage dysplasia (MDS-SLD)
Refractory cytopenia with multilineage dysplasia (RCMD)	MDS with multilineage dysplasia (MDS-MLD)
Refractory anemia with ringed sideroblasts (RARS)	**MDS with ring sideroblasts (MDS-RS)**
	MDS-RS with single lineage dysplasia (MDS-RS-SLD)
	MDS-RS with multilineage dysplasia (MDS-RS-MLD)
Myelodysplastic syndrome associated with isolated del(5q)	MDS with isolated del(5q)
	MDS with excess blasts (MDS-EB)
Refractory anemia with excess blasts-1 (RAEB-1)	MDS-EB-1
Refractory anemia with excess blasts-2 (RAEB-2)	MDS-EB-2
Myelodysplastic syndrome, unclassified (MDS-U)	**MDS, unclassifiable (MDS-U)**
	With 1% blood blasts
	With single lineage dysplasia and pancytopenia
	Based on defining cytogenetic abnormality
Refractory cytopenia of childhood	Refractory cytopenia of childhood

WHO, World Health Organization; MDS, myelodysplastic syndromes; RS, ring sideroblasts; RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; MDS-EB, MDS with excess blasts; MDS-U, MSD, unclassifiable; RCC, refractory cytopenia of childhood.

Table 5.
World Health Organization (WHO) classifications of myelodysplastic syndromes [20, 22].

In 2001, the WHO proposed an alternative classification for MDS that was modified from the original French-American-British (FAB) definitions [18]. Since then, the WHO classification has been updated twice (2008 and 2016) (**Table 5**) [22, 33].

The last edition of WHO classification guidelines identify 6 types of MDS: MDS with single lineage dysplasia (MDS-SLD); MDS with ring sideroblasts (MDS-RS); MDS with multilineage dysplasia; MDS with excess blasts (MDS-EB); MDS with isolated del(5q); and MDS unclassifiable (MDS-U). There is an additional provisional entity termed "refractory cytopenia of childhood." MDS-SLD includes refractory anemia (unilineage erythroid dysplasia), refractory neutropenia (unilineage dysgranulopoiesis), and refractory thrombocytopenia (unilineage dysmegakaryocytopoiesis). The latter 2 were previously classified as MDS-U in 2001 but were reclassified in the 2008 update [34].

According to 2016 WHO classification guidelines identify MDS subtypes based on the results of blood and bone marrow test. The classification of 2016 WHO of MDS was according to factors that differ from those of the FAB system and defined by precise criteria including: (1) dysplastic changes (2) number of cytopenia in peripheral blood (3) percentage of sideroblastic rings (**Table 6**) [5].

13.2.1 MDS with single lineage dysplasia (MDS-SLD)

One dysplastic lineage with dysplasia in at least 10% of the early cells of 2 or 3 cell types (red blood cells, white blood cells, and/or megakaryocytes in the bone marrow. No Auer rodes blast cells less than 5% in BM and <1% in PB. Sideroblastic ring less than 15% in BM and <5% in PB.

Subtype	Blood	Bone marrow
(1) MDS with single lineage dysplasia (MDS-SLD)	Single of bicytopenia	Dysplasia in ≥10 of one cell line, <5% blasts
(2) MDS with ring sideroblasts (MDS-RS)	Anemia, no blasts	≥15% of erythroid precursors w/ ring sideroblasts, or ≥5% of ring sideroblasts, <5% blasts
(3) MDS with multilineage dysplasia (MDS-MLD)	Cytopenia(s*), <1 or 10^9/l monocytes	Dysplasia in ≥10 of cells in ≥2 hematopoietic lineages, ±15% ring sideroblasts, <5% blasts
(4.1) MDS with excess blasts-1 (MDS-EB-1)	Cytopenia(s), ≤2–4% blasts#, <1 × 10^9/l monocytes	Unilineage or multilineage dysplasia, 5–9% blasts, no Auer rods
(4.2) MDS with excess blasts-2 (MDS-EB-2)	Cytopenia(s), 5–19% blasts#, <1 × 10^9/l monocytes	Unilineage or multilineage dysplasia, 10–19% blasts, ±Auer rods
(5) MDS with isolated del(5q)	Anemia, platelets normal or increased	Unilineage erythroid dysplasia, isolated del(5q), <5% blasts
(6) MDS, unclassifiable (MDS-U)	Cytopenia(s), +1% blasts on at least 2 occasions	Unilineage dysplasia or no dysplasia but characteristic MDS cytogenetics, <5% blasts
(7) Refractory cytopenia of childhood	Cytopenias, <2% blasts	Dysplasia in 1–3 lineages, <5% blasts

**Cytopenias defined as: hemoglobin, 10 g/dl; absolute neutrophil count, 1800/mm^3 and platelet count less than 100,000/mm^3; S; bicytopenia may be observed in most cases of MDS.*
#Present of 5–9% myeloblast in BM and 2–4% myeloblasts in the blood, the diagnostic is MDS-EB-1 and 10–19% myeloblast in BM and 5–19% myeloblasts in the blood, the diagnostic is MDS-EB-2. Cases with pancytopenia with unilineage or absent dysplasia with 1% myeloblasts in the blood should be classified as MDS-U.

Table 6.
Peripheral blood and bone marrow findings according to 2016 WHO classification of MDS [5, 20].

13.2.2 MDS with ring sideroblasts (MDS-RS)

MDS-RS previously named as refractory anemia with ring sideroblasts (RARS). In this type of MDS, there is increased sideroblastic rings of nucleated red blood and for diagnosis, ring sideroblasts seen in nucleated red blood cells or at least 5% if the cells also have high of *SF3B1* mutations [35]. Mutations in SF3B1 are seen in ≥80% of cases.

MDS-RS include 2 subtypes based on dysplastic bone marrow:

- MDS-RS with single lineage dysplasia (MDS-RS-SLD): one dysplastic lineage, one or two PB cytopenia, sideroblastic rings >15% in BM or 5% in cases with SF3B1 mutation, blast cells <5% in BM and <1% in PB and no Auer rods.

- MDS-RS with multilineage dysplasia (MDS-RS-MLD)**:** dysplasia in more than one lineage, one to three PB cytopenias, sideroblastic ring in BM 15 and 5% if SF3B1 mutation is present. Blast cells in BM <5% and in PB <1% without Auer rods.

This type of MDS is not common. It rarely turns into AML, and the outcome for people with this type is generally better than for some other types of MDS.

13.2.3 MDS with multilineage dysplasia (MDS-MLD)

Dysplastic changes in two or three lineages and PB cytopenia in one to three lineages, sideroblastic ring in 15% in BM or 5% in cases with SF3B1 mutation, blast cell without Auer rods <5% in BM and <1% in PB.

13.2.4 MDS with excess blasts (MDS-EB)

In this type of MDS, the blasts are present in the bone marrow and/or peripheral blood. Dysplastic changes present in one to three lineage and cytopenia in one to three lines. Sideroblastic ring not present.

There are 2 types, based on how many of the cells in the bone marrow or blood are blasts:

- *MDS* with excess blasts-1 (MDS-EB1): *blast* cells make up 5–9% in the bone marrow aspirate, or 2–4% of blast cells in peripheral blood and absent of Auer rods.

- *MDS* with excess blasts-2 (MDS-EB2): previously named *refractory anemia with excess blasts* (RAEB), characterized by excess blasts 10–19% of bone marrow aspirate cells, or 5–19% blast cells in peripheral blood and/or present of Auer rods.

13.2.5 MDS with isolated del(5q)

"5q– syndrome" is a specific type of myelodysplastic syndrome (MDS). Is not common and it occurs most often in older women. It is characterized by missing part of chromosome number 5. The patient also has cytopenia in one or two blood cell lines with common manifestations including severe anemia, typical dysmegakaryopoiesis, frequent thrombocytosis and favorable outcome [5]. The median survival of patients with isolated 5q– syndrome of 9 years and they have good prognosis and rarely transform to develop AML [35].

13.2.6 MDS, unclassifiable (MDS-U)

This type of MDS is uncommon. For MDS-U, the pathological findings in bone marrow more than in peripheral blood. We observe that, one or more cytopenias are a standard feature of MDS-U but other clinical features are variable. Dysplastic changes in bone marrow in less than 10% but typical cytogenetic abnormality was reported [5].

13.2.7 Refractory cytopenia of childhood

Usually hypocellular with similar picture of aplastic anemia. The mutations are less common than in adult MDS (24% of patients) and have a different profile NRAS/KRAS, SETBP1, ASXL1, RUNX1, BCOR/BCORL, PTPN.

14. Chronic myelomonocytic leukemia (CMML)

14.1 Definition

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder classified by the WHO as an overlapping feature of myelodysplastic syndromes and myeloproliferative neoplasms (MPN). It is characterized by peripheral blood monocytosis, dysplastic features in at least 1 hematopoietic cell line and increased risk of progression to AML [36].

The disease annual incidence became stable at around 0.4 per 100,000 population in Western countries [37]. CMML is occurring in elderly patients whose median age at diagnosis is 71–75 years. The incidence of CMML was higher in men than in women whose origin remains unclear [37].

14.2 Diagnosis of CMML

Diagnosis is based on the presence of sustained (>3 months) peripheral blood monocytosis ($\geq 1 \times 10^9$/l; monocytes ≥10%), along with bone marrow dysplastic changes. Bone marrow and BCR-ABL are recommended to exclude acute leukemia and a classic myeloproliferative neoplasms. Atypical monocytes differ from promonocytes and monoblasts. They contain no nucleolus, exhibit swelling, abnormally folded nuclei, aggregated chromatin, nucleus-cytoplasm asynchrony. Their presence is usually associated with increase of neutrophils and shift to left picture with increase of platelet count but the association of macrocytic anemia and thrombocytopenia are the most common [38]. The CMML classified into three groups/categories for precise prognostication include: CMML0; a group with <2% blasts in PB and <5% blast in BM, the second group CMML1 include patients with 2–5% blasts in PB and 5–9% blasts in BM and third group include patients with 5–9% blasts in PB and 10–19% blasts in BM (**Table 7**) [5, 39].

14.2.1 Immunophenotyping of CMML

An international nomenclature has been used to help diagnose CMML [40]. Human monocytes can be divided into three subsets; MO1, CD14+/CD16− (classical), MO2, CD14+/CD16+ (intermediate) and MO3, CD14−/CD16+ (nonclassical). CMML is characterized by the accumulation of classical monocytes with an MO1 threshold to 94% of total circulating monocytes and with different gene expression profiles, chemokine receptor expressions and phagocytic activities [41].

Persistent monocytosis ≥1 × 10^9/l and monocytes ≥10% of WBC in peripheral blood
<20% blasts in peripheral blood and bone marrow aspiration*
No criteria and no previous history of CML, ET**, PV, and PMF
If eosinophilia, no *PDGFRA*, *PDGFRB*, *FGFR1* rearrangement, no *PMC1-JAK2* fusion gene
≥1 following criteria: 1. Acquired clonal cytogenetic or molecular abnormality in hematopoietic cells*** 2. Dysplasia in ≥1 myeloid lineage 3. Monocytosis persistent for at least 3 months, with other causes excluded

CML, chronic myeloid leukemia; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; WBC, white blood cell.

**Total blast cells include monoblast, promonoblasts and myeloblasts.*

***Exclude of myeloproliferative neoplasms (MPN) associated with monocytosis by bone marrow cytology and/or of MPN-associated mutations (JAK2, calreticulin gene "CALR", or myeloproliferative leukemia mutation "MPL") tend to confirm the diagnosis of MPN with monocytosis rather than chronic myelomonocytic leukemia (CMML).*
****The mutations associated with CMML which may support confirmation of diagnosis like ASXL1, SRSF2, SETBP1, TET2.*

Table 7.
Diagnostic criteria of CMML modified according to Daniel Arber of 2016 WHO classification Blood 2016 [5].

14.2.2 Cytogenetic abnormalities of CMML

Clonal cytogenetic abnormalities identify non-specific chromosomal abnormalities in 30–40% of CMML patients. Peripheral blood/bone marrow for BCR-ABL rearrangement for all patients should be done to exclude any pathological disorder related to myeloproliferative disorders and PDGFRA, PDGFRB, FGFR1 rearrangements or PCM1-JAK2 (**Table 7**) [5, 42]. The most common alterations include; trisomy 8 (4–11%),—Y (5–20%), abnormalities of chromosome 7 (monosomy 7 and del7q) in 2–14%, trisomy 21, and complex karyotypes [43].

15. Conclusions

Myelodysplastic syndrome diagnosis based on data accumulated since the 2008 WHO classification of MDS, much of which relates to adequate medical information, cytomorphology and dysplastic assessment and new molecular genetic information about these neoplasms. The revised WHO classification is the more accurate classification introduces refinements in morphologic interpretation and cytopenia assessment and addresses the influence of genetic information in MDS diagnosis and classification of patients and will allow for better guidance of treatment.

The evaluation of cytogenetic results is important for the classification and determination of the prognosis according to the revised International Prognostic Scoring System (IPSS-R). Immunophenotyping and molecular analysis will provide valuable information on diagnosis and prognosis.

DOI: http://dx.doi.org/10.5772/intechopen.82532

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Chapter 3

Myelodysplastic Syndrome and Autoimmune Disorders: Causal Relationship or Coincidence?

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Abstract

Myelodysplastic syndromes are heterogeneous group of clonal hematologic malignancies characterized by peripheral blood cytopenias secondary to the ineffective hematopoiesis. ADs are frequently reported in MDS, the incidence ranging from 10 to 30%, and particularly ADs are more frequently seen at CMML. ADs may prone patient to MDS, especially when immune suppressors such as azathioprine are used for the underlying AD. Both innate and adaptive immune systems, and different cytokines including interleukins, TNF-α, and C-X-C motif chemokine 10 (CXCL10) contribute in immune dysregulation of MDS. Vasculitis, seronegative rheumatoid arthritis, SLE, Behçet's disease, RP, and AIHA are just some of the ADs occurring concomitantly with MDS. Although hematopoietic growth factors are recommended by the American Society of Clinical Oncology (ASCO), it has been recognized from several case reports that treatment of the underlying MDS may resolve the associated autoimmune disorders. The heterogeneity and complexity of pathology, clinical manifestations, response to therapy, and prognosis of MDS and its immune dysregulation make the prognosis of MDS with autoimmune diseases a matter of debate. Better understanding of the immune dysregulation of MDS in the molecular level may help to design prospective, double blind clinical trials to find the best treatment options for autoimmune disorders associated with MDS.

Keywords: autoimmune disorders (ADs), myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), Behçet's disease, systemic lupus erythematosus (SLE), azathioprine, azacitidine, tumor necrosis factor alpha (TNF-α), regulatory T-cells (Treg), autoimmune hemolytic anemia (AIHA), vasculitis, chronic inflammatory demyelinating polyneuropathy (CIDP), neutrophilic dermatosis, Henoch-Schonlein purpura, relapsing polychondritis (RP), granulomatosis with polyangiitis (GPA), giant cell arteritis (GCA), polyarteritis nodosa (PAN)

1. Introduction

Myelodysplastic syndromes (MDS) are characterized by peripheral blood cytopenias secondary to the ineffective hematopoiesis, and represent a heterogeneous group of clonal hematologic malignancies in which abnormal multipotent progenitor cells are involved. As a result, there is an increased risk of bleeding diathesis and anemia requiring frequent transfusions, infections, and progression to

acute myeloid leukemia [1–4]. It is a very well-known fact that a large spectrum of genetic mutations is involved in MDS pathogenesis that may affect clinical outcome and response to the treatment. These genetic mutations may control cell cycle by affecting key proteins of spliceosome, DNA repair, kinase signaling, tumor suppressor genes, and transcription factors, changing bone marrow micro environment, resulting in hypercellular bone marrow with peripheral cytopenias through enhanced programmed cell death (PCD) and bone marrow dysfunction [5, 6]. To overcome programmed cell death, hematopoietic growth factors such as erythropoiesis stimulating agents (ESAs) and granulocyte colony stimulating factor (G-CSF) are the 1st step in management of the low-grade MDS recommended by the American Society of Clinical Oncology (ASCO) to reduce early apoptosis [1].

Thrombopoietic stimulating agents (TSAs), ESAs, G-CSF, antithymocyte globulin (ATG) [7], lenalidomide [8], and hypomethylating agents are some of the non-transplantation options for management of the MDS patients suggesting that immune dysregulation plays a pivotal role in MDS pathogenesis [1]. Although its etiology is not clear, it has been shown that natural killer (NK) cell activity and its response to chemokines is decreased in MDS, and natural killer cells will be progressively more dysfunctional with MDS progression [9]. It has been shown that although dysfunctional regulatory T-cells (Treg), cells in charge of suppressing T helper (Th) activity, contribute in early stages of MDS, Th expands in the later stages of MDS, and there function is significantly reduced with treatment [10].

Tumor necrosis factor alpha (TNF-α) level is higher in bone marrow and peripheral blood plasma of MDS patients, and may reflect an unfavorable outcome [11, 12]. Study shows that plasma level of 19 cytokines are significantly altered compared with normal individuals, among all of them, C-X-C motif chemokine 10 (CXCL10) and interleukin 6 were associated with shortened survival [13]. The relationship between shorter survival and interleukin 6 levels is very well known, and high producing genotypes of both TNF-α and interleukin 6 are highly associated with transfusion dependency for both anemia and thrombocytopenia, and severity of the bicytopenias [14]. Interferon regulatory factor-1 (IRF-1), a transcriptional activator of interferon system, has anti oncogenic properties, inhibits tumor formation, and regulates innate immune response. It has been shown that IRF-1 mRNA is 10 fold decreased in MDS patients, while it is increased in MDS patients with autoimmune disorders, showing that IRF-1 may promote inflammation and autoimmunity and has a protective roll in MDS patient's [15].

Although it has been reported that MDS is significantly associated with autoimmune disorders, it may occur secondary to the autoimmune disorders per se [16] or exposure to the therapeutic agents used for treatment of autoimmune disorders. A retrospective study in Sweden on 1662 MDS patients and 42,878 matched controls revealed that underlying autoimmune disorder increased risk of MDS by 2.1 with highest risks observed with prior autoimmune hemolytic anemia (AIHA), polyarteritis nodosa (PAN), granulomatosis with polyangiitis (GPA), giant cell arteritis (GCA) and aplastic anemia. It was speculated that chronic stimulation of the immune system may act as a trigger and prone the patient to MDS [17]. In a retrospective study of 2471 patients, it was found that MDS occurred subsequent to autoimmune disorders, most commonly rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, polyarteritis nodosa, discoid lupus erythematosus, and pernicious anemia were associated with MDS [18]. In a case control study of 80 for MDS patients, it was found that there is strong and statistically significant evidence that MDS occurred after autoimmune disorders with Grave's disease and Hashimoto's thyroiditis were among the most important disorders [19]. There is a case report of limited granulomatosis with polyangiitis (GPA) treated with corticosteroids, who did develop myelodysplastic syndrome, papillary thyroid carcinoma, and gastric adenocarcinoma [20].

There are several reports regarding therapy related MDS in population with autoimmune disorders who underwent disease modifying antirheumatic drugs (DMARDs) therapy. In a retrospective case-control study of 40,011 patients with 27 kinds of autoimmune disorders, 311 patients met the inclusion criteria, 86 of them had MDS, and found azathioprine exposure has increased 7 fold myeloid neoplasms in a median of 8 years. In this study, methotrexate, and mycophenolate mofetil did not elevate the risk of myeloid neoplasms [21]. However, there is a case report regarding association of low-dose oral methotrexate therapy with MDS in a rheumatoid arthritis patient [22]. In another study, from 370 rheumatologic patients who received azathioprine for at least 1 year, 59 patients underwent bone marrow examination and 2 of them found to have MDS. This study revealed that risk of secondary MDS is 100 fold higher in patients who received azathioprine for their rheumatologic disorders. Chromosomal examination of the patients with MDS secondary to the azathioprine showed abnormalities of chromosome 7 in majority of them (8 out of 10) [23]. There is also evidence that MDS patients who have autoimmune disorders might respond to hypomethylating agents such as azacitidine and decitabine [24].

2. Rheumatologic manifestations of MDS

It is a very well-known fact that about 10–30% of MDS patients may present with a variety of autoimmune or laboratory manifestations or develop an autoimmune disorder. These manifestations are polymorphic and include leukocytoclastic vasculitis, clubbing, peripheral neuropathy, autoimmune hemolytic anemia, polyarthritis, myositis, acute or systemic vasculitis, Raynaud's phenomenon, polyarteritis nodosa, vitiligo, iritis, colonic ulcerations, pulmonary involvement, and reported autoimmune disorders include rheumatoid arthritis, Sjogren's disease, giant cell arteritis, polymyalgia rheumatica, relapsing polychondritis, Behçet's disease, and systemic lupus erythematosus. Although autoimmune manifestations are mostly seen during the course of MDS, autoimmune disorders may occur before MDS diagnosis. Laboratory manifestations of MDS include hypergammaglobulinemia, hypogammaglobulinemia, monoclonal gammopathy, positive direct antiglobulin test (DAT), positive ANA, rheumatoid factor, cryoglobulinemia, and anti-double-stranded DNA.

3. Autoimmune disorders in myelodysplastic syndrome

Several studies support that autoimmune disorders may occur in the setting of MDS, and treating MDS with immunosuppressors may improve the autoimmune disorder. Although there were several case reports of MDS preceding autoimmune disorders, the first comprehensive retrospective study in 1986 on 104 MDS patients revealed two patients had pernicious anemia, two had hypothyroidism, and one had both pernicious anemia and hypothyroidism [23]. Later, in a retrospective study in 1994, five patients with MDS reviewed and revealed polyarthritis with positive rheumatoid factor (RF) and necrotizing vasculitis [25]. In another retrospective study in 1995, 221 patients with MDS reviewed and found 30 patients with autoimmune disorders, and categorized patient to three categories of acute systemic vasculitis or autoimmune disorder, chronic or isolated autoimmune phenomena, and classic connective tissue disorders. Skin vasculitis, arthritis, and fever were among the most common autoimmune manifestations [26].

A case series in 2002 showed various autoimmune paraneoplastic disorders including vasculitis, pyoderma gangrenosum, Coombs negative autoimmune

hemolytic anemia, autoimmune thrombocytopenia, and chronic inflammatory demyelinating polyneuropathy (CIDP) with good response to immunosuppressive therapy [27]. In a retrospective review of 235 MDS patients autoimmune manifestations such as skin vasculitis (24%), noninfectious fever (13%), arthralgia and arthritis (13%), peripheral neuropathy (10%), and pulmonary infiltrates (8%) were more common than systemic vasculitis [28]. In a cohort of 1408 patients with MDS, 391 (28%) had autoimmune disorders, with hypothyroidism (44%), as the most prevalent, and idiopathic thrombocytopenic purpura (12%), rheumatoid arthritis (10%), and psoriasis (7%) were among the common manifestations of autoimmune diseases [29].

In a retrospective study of 67 MDS patients with autoimmune diseases, neutrophilic dermatosis was the most common autoimmune disease (35.8%), followed by Behçet's disease (14.9%), and rheumatoid arthritis (13.4%) [30].

3.1 Vasculitis

The association between MDS and vasculitis is rare, but more common than solid tumors, and has been described for decades. Cutaneous vasculitis presents by palpable purpura mainly in lower extremities that involves small vessels and is characterized by perivascular inflammation and vessel wall damage by infiltrating neutrophils. Although both cutaneous and systemic vasculitis has been reported in MDS patients, at times they can be seen together in MDS patients. For instance, in a case series of 6 biopsy proven cutaneous vasculitis patients with MDS, 3 patients had evidence of systemic vasculitis [31]. In one case, MDS patient with biopsy proven cutaneous vasculitis developed acute myeloid leukemia within 4 months of vasculitis diagnosis [32]. Henoch-Schonlein purpura, a small vessel vasculitis with IgA dominant immune deposits has been described in MDS patients [33].

The association of MDS and polyarteritis nodosa (PAN) type medium-vessel vasculitis has been reported [34]. In a retrospective study of 8 patients with chronic myelomonocytic leukemia (CMML), with vasculitis involved the medium-vessel, fulfilling the criteria for classic PAN, the presentation was non-specific, and patients developed atypical manifestations [35]. There is a case report of 43-year-old man who qualified for a diagnosis of PAN and developed systemic vasculitis at the time of chronic myelomonocytic leukemia (CMML) diagnosis [36]. There is a report of two cases with CMML who presented with PAN-like systemic vasculitis with bilateral perirenal hemorrhage and negative antineutrophil cytoplasmic antibody with improvement of vasculitis with systemic steroids [37].

Although most MDS associated vasculitis described as leukocytoclastic vasculitis, published case reports documented large vessel vasculitis as autoimmune complication of MDS. Aortitis has been reported as an autoimmune manifestation of an MDS patient at presentation [38]. There is a report of two MDS cases that presented with acute large vessel vasculitis with rapid improvement with systemic steroids [39]. In a retrospective analysis of 271 temporal arteritis patients, it was found that 20 patients had malignancy, of which 11 patients had MDS, favoring a relationship between large vessel vasculitis and MDS [40]. There is a case report of Takayasu's arteritis diagnosed shortly after diagnosis of MDS, with progression to AML regardless of improvement of vasculitis with immunosuppressive treatment [41]. There is a case report of a 71-year-old woman presenting with fever, neck pain, anemia, and thrombocytopenia, with positive positron emission tomography (PET)/CT scan of the aorta and carotid arteries with negative temporal artery biopsy who received the diagnosis of MDS after a bone marrow aspiration analysis [42, 43].

3.2 Behçet's disease

A multisystem, chronic inflammatory disease of unknown etiology, Behçet's disease (BD) is characterized by recurrent oral and genital ulcers, uveitis, arthritis, and vascular involvement of several organs including pulmonary, central nervous system and gastrointestinal tract. In a retrospective study of 805 BD patients, 16 patients had MDS, from which 43.8% had BD prior to MDS, 18.7% diagnosed after MDS and 37.5% had concurrent BD and MDS. It has been shown that trisomy 8 has been accumulated in all of BD patients with MDS, and these patients more likely to be female, older age, and have fever and ileocecal ulcerations [44]. In a retrospective study of 46 MDS patients, 8 patients had trisomy 8, 5 of them had multiple intestinal ulcers, a common feature of BD. Two of the MDS patients with trisomy 8 and multiple intestinal ulcers were treated with granulocyte-colony stimulating factor (G-CSF), aggravating their symptoms, suggesting G-CSF should be used cautiously in this subgroup of MDS patients [45]. There is several case reports of BD associated with MDS [46–49]. In a case report of two patients with BD and MDS, it has been suggested that PET/CT may help diagnosis of both BD and MDS with high uptake by bone marrow in MDS patients and genital and gastrointestinal aphthous ulcers in BD patients [50]. It has been suggested that the frequency of gastrointestinal involvement is more common in MDS-associated BD patients than general BD population [51].

3.3 Inflammatory arthritis

There are several case reports and small series of inflammatory arthritis and MDS co-occurrence. In a retrospective study of 28 MDS patients, 8 had acute seronegative inflammatory arthritis with good response of arthritis to steroids [52]. In a French multicenter retrospective study of 22 patients with MDS, 77% of patients had polyarthritis, and 68% had symmetric joint involvement. Radiologic erosions are rare, and MDS associated arthritis is more frequent in refractory anemia with excess blast (RAEB) [53].

3.4 Miscellaneous

There are several case reports of relapsing polychondritis (RP) presenting as a paraneoplastic disorder in the setting of MDS [54, 55]. In a retrospective study of hematological changes in 19 patients with relapsing polychondritis, MDS was found in three RP patients [56]. Autoimmune hemolytic anemia has been reported in association with MDS [57–59]. Association of systemic lupus erythematosus and MDS has been reported [60–62].

3.5 Immunological abnormalities

It is a very well-known fact that a spectrum of immunological abnormalities occur in MDS. In a retrospective study of 104 MDS patients, 12.5% had monoclonal gammopathy, 19% had low immunoglobulin levels, 32% had polyclonal rise in serum immunoglobulin level, and 8.1% had positive direct antiglobulin test (DAT) [25]. In a case series of 142 patients with MDS and CMML, 23.2% had non-organ specific autoantibody ANA as the most frequent serologic finding [25]. Thrombocytopenia is a common finding in MDS, and can be seen in up to two third of the patients. In a study of 54 MDS patients with no treatment of transfusions, direct platelet immunofluorescence test for platelet associated IgG was positive in 28 patients. Patients with higher amount of platelet associated IgG, had significantly higher mean platelet volume (MPV), thrombocytopenia and worse outcome [63].

4. Prognosis

It has been suggested that appearance of skin vasculitis in MDS patients may reflect acute myeloid leukemia transformation. In a prospective study of 157 MDS patients for a median of 44 months, 15 patients (9.55%) experienced skin lesions, and neutrophilic dermatosis (7, 4.46%), specific lesions (5, 3.18%), cutaneous vasculitis (2, 1.27%) and Behçet's disease (1, 0.63%) were reported. This study revealed that neutrophilic dermatosis was more prevalent in MDS patients, may confer the higher risk of acute myeloid leukemia transformation [64]. In another study of 84 newly diagnosed MDS patients, correlation of cutaneous findings with immunologic parameters and prognostic features of MDS examined, and revealed that 21 patients had skin lesions at presentation, and skin manifestations were a significant predictor of the high-risk MDS subgroup [65].

In a retrospective study of 153 MDS patients, 12% had autoimmune diseases, and 63% has at least one immunological abnormality in test results. In this study, the survival of patients without autoimmune diseases was better than patients with autoimmune disease [66]. However, in a 4 year prospective study of 70 MDS patients, 53 patients without and 13 patients with autoimmune disease, there was no particular difference concerning prognosis between two groups. And patients with autoimmune diseases were not statistically different in survival compared with MDS patients without autoimmune disease [67].

5. Treatment

Autoimmune disorders associated with MDS may predate or occur after MDS diagnosis, and their treatment many be associated with significant side effects in MDS patients. It has been recognized from several case reports that treatment of the underlying MDS may resolve the associated autoimmune disorders. In a transfusion dependent MDS patient who was receiving G-CSF and erythropoietin, neutrophilic dermatosis did not improve with G-CSF withholding. Two months after starting 5-azacitidine, a hypomethylating agent, the skin rash completely resolved, and did not recur after 2 years [68]. In another case series of 3 MDS patients with autoimmune disorders, 5-azacitidine improved both MDS and autoimmune disorders although long term steroid could not be tapered [69].

In a retrospective study of 123 MDS patients with autoimmune disorders, 118 patients (96%) were treated with steroids, and 48% of patients were required a second line treatment for refractory disease or relapse. Although autoimmune disorder treatment did not improve MDS, MDS treatment with 5-azacitidine improved the autoimmune disorder in 9 out of 11 (80%) of patients [70]. In another retrospective study of 123 MDS/CMML patients with autoimmune disorders, 28 patients received at least 5 cycles of azacitidine, 20 of them did not respond to steroids. In 86% of MDS/CMML patients, clinical autoimmune syndromes improved by azacitidine, and prednisone dose tapered in 64% of patients [71].

The overall effect of biologic medications efficacy in MDS patients who presented with autoimmune disorder is not clear. As of today, there is only one retrospective study of MDS patients with autoimmune disorders and biologic medications. In this study of at least one biologic medication, 29 patients followed for at least 3 years. 89% of patients received a biologic after failure or intolerance of two disease modifying anti rheumatic agents (DMARDs), however, 11% of patients received biologics as a first line treatment. Except rituximab, a CD-20 blocker, mainly for vasculitis (58% response), there was partial or insufficient response to

TNF-α antagonists, and their efficacy is much less in autoimmune disorders associated with MDS than autoimmune disorders without MDS. Overall, response rate to 5-azacitidine in MDS-associated autoimmune disorders was 67% in favor of a causality relationship between MDS and autoimmune disorders [72].

6. Conclusion

Myelodysplastic syndromes are a heterogeneous group of progressive clonal hematopoietic stem cell disorders characterized by a varying degree of peripheral cytopenia, and increased probability of transformation to acute myeloid leukemia. MDS and particularly CMML are frequently associated with a variety of autoimmune disorders that can be diagnosed concomitantly with MDS or before or after MDS. The heterogeneity and complexity of pathology, clinical manifestations, response to therapy, and prognosis of MDS and its immune dysregulation makes the prognosis of MDS with autoimmune diseases a matter of debate. Prospective, randomized studies are required to confirm the autoimmune diseases role in MDS prognosis.

Acknowledgements

The authors would like to thank Mr. Dixon Bennet for his technical assistance.

Disclosure

None.

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Chapter 4

Immune Dysregulation in MDS: The Role of Cytokines and Immune Cells

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Abstract

Myelodysplastic syndrome (MDS) is a hematopoietic stem cell disorder affecting individuals over the age of 60 years. It is characterized by ineffective hematopoiesis and extensive apoptosis of hematopoietic cells. MDS patients are at a high risk of transforming in to acute myeloid leukemia. The main cause of apoptosis and escape from immune surveillance in MDS is immune dysregulation caused by a number of factors such as aberrant cytokine production and influence of various immune cells. In the past decade various pro-inflammatory cytokines and a number of immune cells such as Natural Killer cells, regulatory T cells, cytotoxic T cells, mesenchymal stem cells, myeloid derived suppressor cells and dendritic cells have been implicated in immune dysregulation leading to MDS pathogenesis. In this review we focus on the current data available on the role of these immune factors.

Keywords: myelodysplastic syndrome, cytokines, natural killer cells, dendritic cells, mesenchymal derived stem cells

1. Introduction

Myelodysplastic syndrome (MDS) is a group of clonal hematopoietic stem cell disorders with ineffective hematopoiesis, cytopenias and risk of transformation to acute myeloid leukemia (AML). Incidence of MDS is higher in older individuals (>70 years of age) [1, 2]. The severity of MDS could range from indolent disease in which no blood transfusion is required, to borderline AML. An individual can get MDS either de novo, secondary to other myeloid disorders or MDS related to therapy. There are several point mutations associated with MDS [3–5]. Few of the individuals carrying these point mutations have a risk of developing MDS in the future but the factors involved in progression of the disease have not yet been identified [6]. MDS is a multifactorial disorder arising due to factors such as genetic alterations, epigenetic changes, gene transcription dysregulation and immune dysregulation [7]. This chapter is a review of the current data available indicating the role of immune cells in immune dysregulation related to MDS pathogenesis.

2. Classification of MDS

In 1982, the French-American-British (FAB) classification was established for categorizing various MDS cases. According to this classification, all MDS patients were classified into one of the five groups based on the number of myeloblasts present in the bone marrow: refractory anemia, refractory anemia with ring sideroblasts, chronic myeloid leukemia, refractory anemia in excess of blasts (RAEB), and RAEB in transformation (RAEB-T) [8]. This was then later modified into the World Health Organization (WHO) classification which consists of the following groups: Refractory cytopenia with unilineage dysplasia (RCUD), Refractory anemia with ringed sideroblasts (RARS), Refractory anemia with multilineage dysplasia(RAMD), Refractory anemia with excess blasts-1 (RAEB-1), Refractory anemia with excess blasts-2 (RAEB-2), Unclassified myelodysplastic syndrome (MDS-U), MDS associated with isolated deletion 5q, and Refractory cytopenia of childhood (RCC). Various prognostic scoring systems are used in clinical practice to predict overall survival and risk of transformation to AML in MDS patients. Currently, the International Prognostic Scoring System (IPSS) is used for risk stratification and treatment decision making. This system divides MDS patients into 4 different risk categories: low risk, intermediate-1 risk, intermediate-2 risk and high risk, based on the number of cytopenias, the percentage of blasts and cytogenetics [9].

3. Immune dysregulation in MDS

Immune cells are typically involved in immune surveillance, however, they also play a role in disease progression. Many studies have highlighted the role of different immune cells in immune dysregulation leading to pathogenesis of MDS and progression of disease to AML (**Table 1**). These factors could range from aberrant cytokine levels, increased T helper and cytotoxic cells, lower number of regulatory T cells, and dysfunctional Natural Killer (NK) cells among others.

In MDS there is an imbalance in cell production and apoptosis of aberrant cells. The main feature of MDS is high rate of apoptosis leading to cytopenia. The disturbed immune system leads to cytopenia by not only killing the tumor cells but also normal hematopoietic precursors. The immune dysregulation mechanisms vary between the low-risk and high-risk MDS patients, wherein, low risk patients show a high rate of apoptosis resulting from an immune system that is in an activated proinflammatory state, and in high risk patients there is increase in clonality of the tumor cells which escape immune surveillance resulting in a more immunosuppressive environment [7].

Innate immune cells such as cytokines and NK cells and T cells of the adaptive immunity play a major role in immunosurveillance. Since, immunosurveillance maintains the homeostasis as well as removes the aberrant cells any error in the immune regulatory pathways can lead to cancer [31]. The major cause of escape of immune surveillance in MDS is dysregulation of the immune mechanisms which involves various immune cells (**Figure 1**).

3.1 Role of cytokines in immune dysregulation in MDS

Cytokines and chemokines are soluble low-molecular-weight proteins secreted by immune cells that mediate inflammatory responses and regulate hematopoiesis by modulating bone marrow microenvironment. These are essential for the viability, proliferation and differentiation of hematopoietic stem cells. The lymphoid tissues host the effector lymphocytes in their nascent form. Upon stimulation by

chemokines released by the macrophages, the lymphocytes secrete pro-inflammatory cytokines such as IL-2, IFN-gamma, IL-17 and TNF-alpha. Additionally, cytokines such as IL-10 and TGF-Beta are also secreted which down regulate the immune

Sr. No.	Immune factor	Levels	Results	References
1	TNF-α	High	Increased rate of apoptosis	[10]
2	IL2	High	Higher levels in advanced stage MDS as compared to early stage	[11]
3	IL6, GM-CSF	High	Higher levels in advanced stage MDS as compared to early stage	[12]
4	TNF-α	High	Lower overall survival and lower event free survival	[13]
5	TNF-α	Low (<10 pg./ml)	Better overall survival and better progression free survival	[14]
6	CD_4^+ and CD_8^+	Low	Response to immunosuppressive therapy	[15]
7	CD_3^+ and CD_8^+	Low	frequent infections, lower overall survival and transformation into AML	[16]
8	CD_8^+ T cells	High	Inhibition of hematopoiesis	[17, 18]
9	CTLA-4	High	Suppression of T cell activity, escape from immune surveillance, resistance to immunotherapy	[19]
10	CTL's	High	Impairment of immune surveillance and disease progression	[20]
11	MDSC's	High	Inefficient hematopoiesis	[21]
12	MSC's	High	Increased apoptosis, immunosuppression and reduced hematopoiesis	[22]
13	MSC's	High	Poor potential for inhibition of DC differentiation and maturation in low-risk MDS patients	[10]
14	DC's	Abnormalities	Escape of tumor cell from immune recognition, Higher rate of relapse after HSCT	[23]
15	IFN-γ producing NK Cells	Low	Decreased cytolytic function leading to increased tumor load	[24]
16	$CD56^{dim}CD16^+$ NK cells	High	Dysregulation of immune surveillance and ineffective hematopoiesis	[25]
17	Tregs	Low	Defective immune activation and decreased immune surveillance	[26, 27]
18	Tregs	Low	T cell cytotoxicity leading to increased apoptosis in low risk MDS	[28]
19	Tregs	High	Impaired antineoplastic immunity and immune suppression in High risk MDS	[15, 28, 29]
20	Th22 (IL22 producing T cells)	High	Increased apoptosis in high risk MDS patients	[30]

TNF-α: tumor necrosis factor-alpha; IL-2: interleukin-2, IL6: interleukin 6, GM-CSF: granulocyte macrophage colony-stimulating factor, CD4+: cluster of differentiation 4, CD8+: cluster of differentiation 8, CD3+: cluster of differentiation 3, CTLA-4: cytotoxic T-lymphocyte-associated antigen 4, CTL's: cytotoxic T lymphocytes, MDSC's: marrow derived stem cells, MSC's: mesenchymal stem cells, DC's: dendritic cells, IFN-γ: interferon gamma, NK: natural killer, CD56: cluster of differentiation 56, CD16: cluster of differentiation 16, Tregs: T regulatory cells.

Table 1.
The role of various immune cells in MDS pathogenesis.

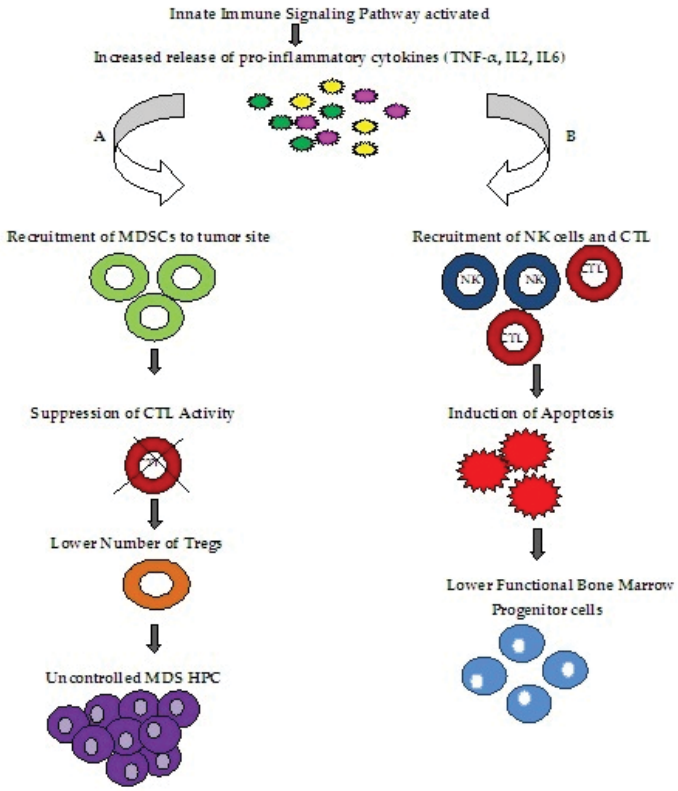

Figure 1.
The role of various immune cells in MDS pathogenesis. **A**. *The abnormal proliferation of MDS hematopoietic cells. The increased release of various pro-inflammatory cytokines directs the recruitment of MDSC's at the tumor site. These kill the erythroid precursor cells and also lead to suppression of CTL activity. Lower number of Tregs enables the tumor cells to escape immune surveillance and leads to accumulation of uncontrolled proliferation of MDS cells.* **B**. *The decrease in normal hematopoiesis. The release of cytokines also leads to increase in the number of natural killer and cytotoxic T cells, which induces apoptosis in hematopoietic stem cells. This in turn results in lower number of functional bone marrow progenitor cells. TNF-α: tumor necrosis factor-alpha, IL2: interleukin-2, IL6: interleukin-6, MDSC: marrow derived stem cells, CTL: cytotoxic T cells, Tregs: regulatory T cells, MDS: mesenchymal stem cells, HPC: hematopoietic cells, NK: natural killer.*

response and contribute in healing the tissue involved. TGF-Beta is also an inducer of regulatory T cells (Tregs). Cytokines such as TNF-alpha have been associated with pathogenesis of high risk MDS, however, their prognostic value is unknown.

One of the causes of cytopenia in MDS is abnormal apoptosis. The increased expression of cytokines such as TNF-alpha, upregulates the expression of cell surface protein Fas receptor. This Fas receptor when bound to its cognate Fas ligand triggers apoptosis of the cell carrying the Fas receptor via the Fas associated death domain (FADD). Hence higher levels of TNF-alpha have been associated with rate of apoptosis in MDS patients [27].

Abnormal levels of cytokines, chemokines and growth factors in MDS patients have been reported by various researchers [32–34]. The levels of pro-inflammatory cytokines such as TNF-alpha, IFN-gamma, TGF-Beta, IL6, IL8, and granulocyte-CSF have been shown to be increased in MDS patients, highlighting the role of these immune cells in immune dysregulation related to MDS [33, 35–39]. Kornblau et al. [40] reported that MDS patients have lower levels of pleiotropic cytokines IL10 and IL4 as compared to healthy controls. Zoumbos et al. [11] have reported higher serum IL2 levels in patients with advanced MDS as compared to early stage patients. Similarly, higher serum IL6 and GM-CSF levels have been reported in patients with

advanced MDS [12]. Verhoef et al. [34] have also reported higher levels of IL-3, IL-6 and G-CSF in patients with MDS as compared to controls.

Aberrant cytokine levels have also been associated with MDS clinical outcomes. Tsimberidou et al. [13] have associated higher levels of leukocytes and lower levels of hemoglobin with lower overall survival and event free survival in high-risk MDS patients having higher TNF-alpha levels. Meyers et al. [41] have associated higher levels of TNF-alpha, IL-6, IL-1R with fatigue in MDS patients. Low TNF-alpha (<10 pg./ml) have been associated with better overall survival and better progression free survival [14]. Pardanani et al. [42] suggested the levels of IL6, IL7 and CXCL10 to be an independent prognostic factor for overall survival, wherein, patients with normal levels of these cytokines show better overall survival as compared to those with elevated levels of at least 1.

Lopes et al. [43] reported difference in cytokine profiles between low and high risk MDS cases. Also an inverse relation between IL10 and CD_8^+ T cells has been reported and IL10 expression is much higher in patients suffering from MDS. When comparing the MDS according to the risk, patients in the low risk group show elevated levels of type I cytokines such as IL-1Beta, IL7, IL8 and IL12, whereas high-risk MDS patients show elevated levels of inhibitory factors such as IL10 and sIL2 [13, 14]. Since low-risk MDS is associated with higher apoptotic rates, the levels of cytokines such as TNF-alpha, IFN-gamma and IL-6 are higher in these patients as compared to high-risk MDS patients [35]. High risk MDS is associated with escape of tumor clones from immune recognition and hence serum of these patients have higher amounts of immunosuppressive cytokines like IL10 [44]. IFN-gamma and IL-6 are involved in apoptosis induction in BM. Hence, when they are present in higher levels, it will result in a low risk MDS [27, 32].

4. Role of immune cells

4.1 Helper and cytotoxic T cells

Cytotoxic T cells (CTLs) are cells that are capable of killing cancer cells and abnormal cells. Most of these cells have a T cell receptor (TCR) that recognizes specific antigens, hence, stimulating an immune response. Functionally stable TCRs are associated with both CD_4^+ and CD_8^+ glycoproteins. CD_4^+ glycoproteins are found on cells such as dendritic cells (DC), T helper cells, etc. and are involved in communicating with antigen presenting cells (APCs). The CD_8^+ glycoprotein is present in various cytotoxic cells such as CTL, NK cells etc. Function of CD_8^+ glycoprotein is to recognize and destroy foreign/infectious particles. Only when the TCR is associated with CD_8^+ glycoprotein can it bind to the specific antigens and enable presentation and destruction of these foreign antigens [45, 46].

Growth of both malignant and non-malignant cells in MDS is inhibited by CD_8^+ T cells that target MHC class I molecules on the hematopoietic precursors. This response forms part of the immune surveillance. Majority of MDS patients have peripheral blood lymphopenia, which results in reduction of CD_4^+ and CD_8^+ cell, leading to an inverted CD_4/CD_8 ratio in these patients. In younger patients there is a decrease in both naïve CD_4^+ and CD_8^+ T cells which is also correlated with response to immunosuppressive treatments [15].

The distribution of CD_8^+ T cells differs between low and high risk MDS patients. Low risk MDS patients usually show downregulated Tregs and upregulated CD_8^+ T cells, whereas, high risk MDS patients have upregulated Tregs and downregulated CD_8^+ T cells and NK cells. Symeonidis et al. [16] have reported association of CD_3^+ and CD_8^+ lymphopenia in MDS patients with frequent infections, lower overall

survival and transformation into AML. Smith et al. [17] demonstrated inhibition of hematopoiesis by CD_8^+ T cells. This inhibition due to type-I polarization of CD_4^+ and CD_8^+ T cells was also confirmed by Wu et al. in 2008 [18].

Recently, a few epitopes on CD_8^+ T cells such as Wilms tumor 1 protein (WT1), MHC Class I etc., have been implicated in activation of the CD_8^+ T cells. Sloand et al. [47] reported that in MDS patients with trisomy 8, WT1 is highly overexpressed on CD_{34}^+ T cells making these patients more responsive to immunosuppressive therapies. However, these immunosuppressive therapies hinder tumor surveillance by T cells resulting in disease progression.

Further, molecules such as programmed death 1 (PD1), its ligand PD1L, and T cell associated antigen CTLA-4 suppress T cell activity leading to escape of tumor cells from immune surveillance and resistance to immunotherapy [19]. Sand et al. [20] have reported a higher number of CTL's in high-risk MDS patients, however, these CTL's have a lower TCR mediated cytotoxicity, which results in impairment of immune surveillance and leads to disease progression.

A Japanese study revealed lower marrow T cells in MDS-RAEB patients and low $CD_4^+CD45RA^+$ naïve cells in MDS patients indicating impaired immune surveillance and expansion of the tumor clone [48]. Hamdi et al. [49] reported higher Th1/Th2 ratios in patients with lower risk prognostic score. Li et al. [50] have reported higher T cell count in low-risk MDS patients as compared to high-risk patients and controls.

These studies highlight the role of abnormal helper and cytotoxic T cells in autoimmunity towards hematopoietic progenitors.

4.2 Myeloid derived suppressor cells (MDSC's)

Myeloid derived suppressor cells (MDSC's) are cells belonging to the innate immunity involved in suppression of the immune system. These cells express CD33 immune receptor [51]. They are generally in few numbers in healthy individuals but increase in response to stress stimuli. They have been reported in higher frequencies in various cancers.

MDSC's are one of the cells that inhibit anti-tumor immunity. These can be differentiated based on the surface markers as monocytic (MO-MDSC) and granulocytic (PMN-MSDC). The surface markers on monocytic MDSC's are $CD11b^+CD14^+CD15^-CD33^+$ and HLA-DR^{low}, whereas, those on granulocytic MDSC's are $CD11b^+CD14^-CD15^+CD33^+$ and HLA-$DR^{low/-}$. MDSC's are generally induced by the pro-inflammatory nature of the tumor cells. Their function is to inhibit both adaptive as well as the innate immune system by either cell–cell contact or by releasing cytokines, which results in the inhibition of T cell proliferation and activation, induction of Tregs and NK cell impairment. MDSC's are also involved in inhibiting T cells to settle in the lymph nodes and inflammation sites. MDSC's derived from monocytes and granulocytes support tumor by releasing growth factors.

There is sparing data available regarding the role of MDSC in MDS. The increase in MDSC's in MDS patient results in suppression of normal hematopoiesis and leads to progression of the disease. Chen et al. [21], reported an association of inefficient hematopoiesis with increased MDSC's in the bone marrow of patients with MDS. Graft versus host disease (GvHD) is the number one cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation. Low levels of MO-MDSC's have been reported in association with onset of aGvHD [52]. The levels of both MO-MDSCs and PMN-MDSCs are increased in the peripheral blood soon after transplant and are reported to lower the risk of acute GvHD (aGvHD) as well as response to steroid therapy [53].

Lechner et al. [54], reported the role of various cytokines in the development of MDSC's. In MDS there are irregular levels of cytokines which indicates that targeting the cytokine levels can lead to loss of immunosuppression by the MDSC's. Jiang et al. [55], reported increased levels of circulating MDSC's in MDS patients. The immature myeloid cells induce secretion of cytokines such as IL-10 and TGF-Beta which increase the levels of MDSC's.

4.3 Mesenchymal stem cells

MSC's are self-renewing cells with the ability to differentiate into a number of cells such as adipocytes, and chondrocytes. The main role of MSC's is maintaining normal hematopoiesis, however, they are also implicated in MDS pathogenesis. The role of MSC's in MDS is different in low risk and high-risk patients. In high risk patients MSC's induce apoptosis and suppress the immune system by secreting various cytokines like TGF-B, whereas, in low risk patients they reduce the efficiency for inhibiting DC differentiation. Since MSC's have an immunomodulatory role, they have been investigated for their role in MDS disease progression. However, most of the studies have reported contradictory results and a consensus on the involvement of MSC's in MDS is not yet sought.

Han et al. [56] have demonstrated MSC's with normal morphology and phenotype, but with deficient immunomodulatory function that could not inhibit in vitro T cell activation and proliferation. Klaus et al. [57], did not find any difference in the differentiation potential and immunosuppressive potential of MSC's in MDS patients and controls. In high-risk MDS patients, Zhao et al. [22] reported MSC's with an increase in apoptosis, immunosuppressive rate and reduced hematopoiesis, whereas, Wang et al. [10] reported MSC's with poor potential for inhibition of DC differentiation and maturation in low-risk MDS patients.

4.4 Dendritic cells

The site of origin of DC's is the bone marrow. There are four different types of dendritic cells present in the peripheral blood: plasmacytoid DCs (pDCs), two types of myeloid DCs and slan DC's. The main function of DC's is pathogen and tumor recognition and antigen presentation [58, 59]. Hence, any defect in DC's could mean that the tumor cells will escape immune recognition [28, 29]. DC's upregulate and activate co-receptors on T cells. In MDS patients the ability of DC's to activate T cells is much lower as compared to healthy controls [60]. Ma et al. [61], reported similarities in cytogenetic abnormalities in DC's of MDS patients and in their malignant clones indicating that these abnormalities belonged to the malignant clone.

DC's are equipped with Toll-like receptors and C-type lectin receptors which help in recognition of pathogens. DC's are usually in a nascent state and upon contact with potential pathogens they mature. During maturation there are morphological changes due to which the function of DC's changes from pathogen capturing to cytokine secretion and antigen presentation. The processed antigens on MHC's are presented by DC's to naïve T cells which then elicit an immune response.

Various researchers have reported aberrant immune response by DC's in different cancer types [62–66]. This defect could be in the frequency of DC's, maturation, cytokine secretion profile, and ability to induce T cell proliferation [7]. Abnormalities and defects in number of dendritic cells lead to escape of tumor cell from immune recognition, hence, resulting in higher rate of relapse after allogeneic HSCT [23]. The number and frequency of DC's in the peripheral blood of MDS cases is much reduced as compared to healthy individuals [60, 61, 67]. Further their capability to stimulate T cells is also reduced due to lower surface expression of

molecules such as CD54, CD80 and CD86 [60]. Ma et al. [68] have reported lower IL12 secretion and higher IL10 secretion in DC's from MDS patients. Saft et al. [23] reported lower myeloid and plasmacytoid precursor DC's in high-risk MDS as compared to low-risk MDS patients.

4.5 Natural killer cells

Natural Killer cells (NK) play an important role in innate immunity. NK cells secrete cytokines and play an important role in host defense [69]. Their main role is to kill non-self cells, i.e., cells that lack self-major histocompatibility complex (MHC). NK cell cytotoxicity can either be activated or inhibited based on the sum of the activating and inhibitory receptors present on their surface. The inhibitory receptors could either be Killer immunoglobulin like receptors (KIR) or CD94/NKG2A dimer which protect self-cells from NK cell lysis by binding to their cognate MHC Class I ligands [70], whereas, the activating receptors are the natural cytotoxic receptors (NCRs), namely, NKp46, NKp44, NKp30 and NKG2D [69, 71].

The role of NK cells in immune dysregulation in MDS results from reduction in total number or functional NK cells leading to defects in immune surveillance and hence disease progression. Since NK cells eliminate the aberrant cells from the system, deficiency of NK cell functionality has been associated with poor prognosis in MDS, hence, in older patients, where in the NK cell activity is already low due to aging, the NK cell activity decreases further.

Various reports have measured NK cells in MDS patients [72–74]. Epling-Burnette et al., reported 2 types of NK cells based in their cytotoxicity: low function NK cells which have been associated with high-risk MDS, and normal function NK cells. Furthermore, they also reported defects in number of activating NK receptors such as NKG2D leading to progression of disease. They observed reduced NK cell cytolytic activity in MDS patients as compared to healthy donors. The activating receptors NKp30 and NKG2D was downregulated in these patients [74].

Fujii et al. [24], reported deficiency of IFN-gamma producing NK T cells in blood and marrow of MDS patients. This led to decreased cytolytic function leading to tumor load. Zhang et al. [25], report lower expression of $CD3^{-}CD56^{+}CD16^{+}$ NK cells in MDS patients as compared to healthy individuals. $CD16^{+}$ NK cells play an important role in tumor surveillance, and hence decreased count of these cells correlate with the tumor cell load in MDS patients [75, 76].

$CD56^{+}$ is a marker for NK cells that produce high levels of cytokines. Circulating NK cells usually exhibit $CD56^{dim}$ which produce lower amounts of cytokines as compared to $CD56^{bright}$ NK cells. Hence, $CD56^{bright}$ are known to have a more immunomodulatory role as compared to $CD56^{dim}$ which are mostly cytotoxic in nature [77]. In patients with MDS, there is a higher prevalence of $CD56^{dim}CD16^{+}$ cells as compared to healthy controls which results in dysregulation of immune surveillance and ineffective hematopoiesis [25]. Hejazi et al. [72] conducted a study on 75 MDS patients, wherein it was observed that majority of the patients in the high risk group had a NK Cell deficiency, moreover, in another group, it was observed that although NK cells were present, they were non-functional. $CD56^{dim}$ NK cells were predominantly present which result in reduction of cytotoxicity.

4.6 Regulatory T cells

Tregs are T helper cells that are involved in immune tolerance and modulation of immune reactions. Regulatory T cells (Tregs) cells are $CD4^{+}CD25^{high}FOXP3^{+}$ or $CD4^{+}CD25^{high}CD127^{low}$. Tregs carry out immune tolerance as well as modulation of immune response and are the important for immune surveillance [27] by secreting IL10

and TGF-Beta which are immunosuppressive cytokines [78]. Any impairment in Tregs results in defective immune activation and decreased immune surveillance against the tumor cells [26]. Lower numbers of functional Tregs result in autoimmunity.

The exact role of Tregs in MDS progression is not very clear. However, variation in number of Tregs has been reported to be associated with disease progression, risk of transformation to AML and overall survival in MDS patients [29, 31, 49, 79]. The very first study for the involvement of Tregs in MDS was demonstrated by Kordasti et al. [29], wherein, an association between increased CD_4^+ Tregs and disease progression was shown. Low number of Tregs in bone marrow have been linked to increased CD_8^+ T cells and the recruitment of Th17 proinflammatory cells [44, 80]. The number of Tregs differs based on the disease stage. In low-risk MDS, lower numbers of Tregs or impaired Tregs have been reported in association with T cell cytotoxicity leading to increased apoptosis, whereas, higher number of Tregs are seen in high-risk MDS patients and are associated with impaired antineoplastic immunity and immune suppression [15, 28, 29].

Costantini et al. [81] conducted a study to investigate the effect of 5-azacytidine on Tregs functionality in intermediate/high risk MDS patients. Following treatment, lower numbers and Tregs with lower suppressive function were observed in these patients. Alfinito et al. [80] reported an inverse relationship between levels of Tregs and the degree of dyserythropoiesis. Hamdi et al. [49], reported an inverse ratio between Treg and CD_8^+ frequencies in MDS patients. Low risk MDS patients have higher number of Th17 cells which is inversely correlated with Tregs numbers. Hence in these patients the Th17/Tregs ratio is found to be increased [44]. Shao et al. [30] have shown increased number of Th22 (IL22 producing T cells) in high-risk MDS patients. This increased number of Th22 is correlated with release of pro-inflammatory cytokines leading to increased apoptosis.

5. Immunosuppressive therapy

The most curative treatment for MDS is allogeneic hematopoietic stem cell therapy; however, since MDS develops mostly later in life, most patients are rendered ineligible for transplantation and supportive care is their only option for treatment. As previously discussed, many researchers have shown the involvement of immune cells in the pathogenesis of MDS and hence, immunosuppression involving targeting these cells has evolved as one of the treatment modalities.

Molldrem et al. and Jonasova et al. [82, 83] were the first to report the use of immunosuppressive therapy (IST) in MDS with Antithymoglobulin (ATG) in 1997 and Cyclosporin (CsA) in 1998 respectively. Jonasova et al. [83] reported positive response in MDS-RA patients treated with CsA. On the contrary, another study showed no advantage to CsA and also reported CsA related renal toxicity [84]. CsA acts by blocking IL-2 production which leads to inhibition of expansion of CTLs and suppression of cytokines like TNF-α which are involved in apoptosis [85]. ATG has a role in depleting T cells and restoring normal hematopoiesis [86]. It acts by interfering with the normal function of DC's and hence inhibiting the interaction between the T cell and the antigen presenting cell. Moreover, ATG restores normal hematopoiesis by diminishing cytokine release by activated T cells [87].

Since reduced number of Tregs are associated with disease progression in low-risk MDS, introduction of ex-vivo expanded Tregs is a potential targeted therapy in such patients. Not only the number but also the function of Tregs is important, hence, it is absolutely necessary to be sure that the ex-vivo expanded Tregs population do not have non-regulatory effector cells. Functional Tregs can be also be induced by vaccination using tolerizing DC's [88].

6. Conclusions

It is now well known that genetic, epigenetic and aberrant immune factors play a major role in the pathogenesis of MDS, making it one of the most challenging disorders to design therapies for. Leaving aside the MDS patients in whom the major factor is genetic mutations, there is a group of patients in whom immune cells play a major role in the disease progression. In these patients, immunosuppressive therapies (IST) form part of the major treatment strategy. It is known that there is improved marrow function and increased survival in patients undergoing IST. However, identifying patients that will respond favorably is a challenge. Some targeted therapies involve introducing tolerogenic DC's and expansion of Tregs. The therapeutic approach becomes more difficult because of the difference in the immune environment that exists between low-risk and high-risk MDS cases. For this the exact immune mechanisms involved and the specific immune cells responsible for the pathogenesis in the particular group of patients need to be identified in order to establish targeted therapies in the future.

Conflict of interest

The authors declare that there is no conflict of interest.

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Chapter 5

Myelodysplastic Syndromes: An Update on Pathophysiology and Management

Wanxing Chai-Ho and Gary J. Schiller

Abstract

Myelodysplastic syndromes (MDS) comprise a set of clonal hematopoietic stem cell (HSC) disorders characterized by ineffective hematopoiesis that manifest as cytopenia of variable severity. The result often is an increased risk of infection, transfusion dependence, and a potential to transform to acute myeloid leukemia (AML). For the past decade, hypomethylating agents remain the only FDA-approved therapy. Given that MDS is more prevalent in the elderly who often have comorbid conditions, supportive care remains the mainstay of therapy. Curative treatments are restricted to younger, healthy individuals with histocompatible-matched donors for allogeneic transplant able to tolerate more intensive chemotherapeutic treatment. Understanding of the pathophysiology of MDS advanced over the past decade, which leads to an increasing array of new agents under clinical investigation. This review focuses on our recent enhanced understanding of MDS molecular biology, and promising novel agents that go beyond the hypomethylating agent.

Keywords: myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), bone marrow transplant, hypomethylating agent, somatic mutation

1. Introduction

The myelodysplastic syndromes (MDS) comprise an heterogeneous group of malignant hematopoietic stem cell disorders characterized by dysplastic and ineffective blood cell production and a variable risk of transformation to acute leukemia. Based on the United States Surveillance, Epidemiology, and End Results (SEER) Program, the incidence of MDS is about 4.1–4.6 cases per 100,000 population per year, with approximately 86% of patients aged ≥60 years at time of diagnosis (median age 76 years). The incidence rate is higher in men than women. [1] The prevalence is slightly lower in Europe with reported 1.24–3.7 cases per 100,000 population per year, also with observed male predominance.[2, 3] With an aging population and improved awareness of disease, it is likely that the number of new patients diagnosed with MDS each year will increase in the future.

Pathogenesis of MDS is incompletely understood. Studies have revealed age, male gender, alcohol, cigarette smoking, ionizing radiation, chemotherapy such as alkylating agents and topoisomerase II inhibitor, immunosuppressive therapy, viral infection, benzene and other environmental/occupational exposures as possible implicating factors. [4–8] However, disease caused by these risk factors are estimated

to account for only 20–30% of cases, which are described as secondary MDS, with remainders as primary MDS [4]. The major subsets of secondary MDS are therapy-related MDS (t-MDS) and MDS with predisposition to familial myeloid neoplasm.

The risk for MDS and AML is increased in certain familial predisposition syndromes, such as inherited bone marrow failure disorders like Diamond-Blackfan syndrome, Fanconi anemia, dyskeratosis congenital, Shwachman-Diamond syndrome, and Down syndrome, Noonan syndrome/Noonan syndrome-like disorders and neurofibromatosis [9]. Accurate diagnosis and recognition of these syndromic disorders allows opportunities to improve clinical care. Genetic counseling should be offered to family members of affected individual. One should avoid using heterozygous sibling as bone marrow transplant donor. Recently, a growing number of germline mutations including CEBPA, DDX41, ANKRD26, ETV6, GATA2, RUNX1 were identified to associate with familial thrombocytopenia and development of MDS and acute leukemia in up to 40% of patients [10, 11]. Special attention should be noted that many patients with familial MDS and acute leukemia predisposition syndromes develop disease in adulthood rather than childhood. To increase awareness of this entity of disease, myeloid neoplasm with above mentioned germline predisposition was incorporated into the updated WHO 2016 classification [12].

2. Diagnosis

2.1 Clinical presentation

MDS usually presents as cytopenia in one or more lineage. Fatigue, dyspnea on exertion, infection, easy bruising or bleeding are the most common symptoms. Lymphadenopathy and hepatosplenomegaly are infrequent and should raise suspicion for chronic myelomonocytic leukemia (CMML) [13, 14]. It has been estimated that various autoimmune features such as subacute vasculitis, fever, arthritis, peripheral edema, and pulmonary infiltrates, may be present in up to 10% of patients [15–18]. Certain autoimmune syndromes have correlated with distinct cytogenetic abnormalities; including Behcet's disease with trisomy 8, Sweet's syndrome and pyoderma gangrenosum with del(5q) [19]. Acquired hemoglobin H disease has been documented in approximately 8% of cases of MDS [20–22]. An acquired somatic mutation of ATRX, an X-linked gene encoding a chromatin-associated protein, has been linked to this entity, [21] as have acquired deletions of the alpha globin loci.

2.2 Pathology evaluation and WHO criteria

Bone marrow aspiration and biopsy are critical to the diagnosis of MDS. In general, the marrow is normo- or hypercellular due to ineffective hematopoiesis. However, up to 20% of MDS patients have hypocellular marrow, making it difficult to distinguish from aplastic anemia or paroxysmal nocturnal hematuria [23, 24]. Dysplastic neutrophils are commonly found in the peripheral blood smear. These cells may demonstrate reduced segmentation, increased size, the so-called pseudo-Pelger-Huet cell [25], often accompanied by reduced or absent granulation [26], and are associated with del(17)p [27]. Hypersegmentation with greater than 5 nuclear lobes is another feature of neutrophil dysplasia [28]. Red cells are usually normocytic or macrocytic, although ring sideroblasts, ovalomacrocytosis, teardrops, stomatocytes or acanthocytes may be seen [28]. Platelet morphology is usually normal, but micromegakaryocytes, mononuclear megakaryocytes, dumbbell-shaped nuclei, multinuclearity with multiple isolated nuclei ("Pawn ball" changes) may be seen [29].

Classification of MDS has been a challenge. In 1982, the French-American-British (FAB) Cooperative Group published the first seminal classification system that distinguished five subcategories of MDS based on marrow morphological criteria and myeloblasts proportions: refractory anemia, refractory anemia with ring sideroblasts (RARS), refractory anemia with excess of blasts (RAEB), RAEB "in transformation" (RAEB-T), and chronic myelomonocytic leukemia (CMML) [30]. Presence of more than 30% blasts in the bone marrow was defined as AML.

In 2001, World Health Organization (WHO) published new classification system on myeloid malignancy with modifications to the FAB system: The diagnosis of AML requires 20% myeloblasts. RAEB-T is classified as AML, and CMML is categorized as a new entity of myeloid neoplasms with both MDS and myeloproliferative features. In addition, MDS with isolated del(5q) is acknowledged as distinctive features in forms of disease with a low blast count, severe anemia and thrombocytosis (5q- syndrome) [31]. The revised 2008 WHO criteria maintained these modifications [32]. In the absence of definitive morphologic features of MDS, MDS-defining cytogenetic abnormalities were included in the diagnostic criteria (**Table 1**). The presence of chromosome 7, Y, or del(20q) does not meet criteria as an MDS-defining abnormality.

The 2016 revision of WHO (**Table 2**) incorporated rapidly accumulating molecular genetic information into the classification [12]. The same cytogenetic abnormalities listed in the 2008 WHO classification remain MDS-defining in a cytopenic patient. Given recent data showing 1 chromosomal abnormality in addition to the del(5q) poses no adverse effect [33–35], the entity "5q- syndrome" may be diagnosed if there is 1 additional cytogenetic abnormality besides the del(5q), unless that abnormality is monosomy 7 or del(7q). Mutations like SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1, U2AF1, TP53, and EZH2 can be found in 80–90% MDS patients [36, 37]. Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of healthy older individuals without MDS, so-called "clonal hematopoiesis of indeterminate potential" (CHIP), or patients with mild cytopenia but without dysplastic changes or specific cytogenetic and/or genetic abnormalities considered as presumptive evidence of MDS (idiopathic cytopenia of undetermined significance, ICUS) [38, 39]. Although some CHIP and ICUS subsequently develop MDS, there have not been sufficient data to support using the presence of aforementioned mutations as surrogate diagnostic marker of MDS. Based on the link between ring sideroblasts and an SF3B1 mutation, MDS

Unbalanced abnormalities	Balanced abnormalities
−7 or del(7q)	t(11;16)(q23;p13.3)
−5 or del(5q)	t(3;21)(q26.2;q22.1)
i(17q) or t(17p)	t(1;3)(p36.3;q21.1)
−13 or del(13q)	t(2;11)(p21;q23)
del(11q)	inv(3)(q21q26.2)
del(12p) or t(12p)	t(6;9)(p23;q34)
del(9q)	
idic(X)(q13)	
Complex karyotype (3 or more chromosomal abnormalities) involving one or more of the above abnormalities.	

Table 1.
Recurring chromosomal abnormalities considered as presumptive evidence of MDS in the setting of persistent cytopenia or undetermined origin in the absence of definitive morphologic features of MDS, according to World Health Organization 2008 and 2016 criteria.

Myeloproliferative neoplasms
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB, or FGFR1, or with PCM1-JAK2
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) Chronic myelomonocytic leukemia(CMML) Atypical chronic myeloid leukemia (aCML), BCR-ABL1 Juvenile myelomonocytic leukemia (JMML) MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) MDS/MPN, unclassifiable
Myelodysplastic syndrome MDS with single lineage dysplasia MDS with ring sideroblasts (MDS-RS) MDS-RS and single lineage dysplasia MDS-RS and multilineage dysplasia MDS with multilineage dysplasia MDS with excess blasts MDS with isolated del(5q) MDS, unclassifiable *Provisional entity: Refractory cytopenia of childhood*
Myeloid neoplasms with germline predisposition
Acute myeloid leukemia (AML) and related neoplasms Includes AML with myelodysplasia-related changes and therapy-related myeloid neoplasms
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
B-lymphoblastic leukemia/lymphoma
T-lymphoblastic leukemia/lymphoma

Table 2.
Classification of myeloid neoplasms and acute leukemia, according to World Health Organization 2016 criteria.

with ring sideroblasts and multilineage dysplasia, marked thrombocytosis, lacking excess blasts or an isolated del(5q) abnormality is included into the category of MDS with ring sideroblasts, and correlates with a favorable prognosis [40–43].

2.3 Differential diagnosis

MDS must be distinguished from other marrow dysplasia secondary to reversible causes, such as folate and vitamin B12 deficiency, viral infections (e.g. HIV), antibiotics, benzene, ethanol, or lead poisoning. Other primary bone marrow disorders presenting as pancytopenia, such as aplastic anemia, paroxysmal nocturnal hematuria, hairy cell leukemia, large granular lymphocytic leukemia can be distinguished by marrow morphology, flow cytometry features and gene mutation profile [8].

2.4 Risk stratification

The natural history of MDS in patients varies. The heterogeneity reflects both known and unknown differences in the pathophysiology of specific disease subtypes and patient related characteristics. Several prognostic scoring systems were developed and validated for MDS patients. In 1996, the International Prognostic Scoring System (IPSS) was developed by the International MDS Risk Analysis Workshop based on FAB classification [44]. Based on percent bone marrow blasts, specific cytogenetic abnormalities, and the number of cell lines involved with dysplasia and cytopenia, individual patient are placed into 4 groups: low,

intermediate-1, intermediate-2, and high. The median survival in these four risk categories is 5.7 years for low risk, 3.5 years for intermediate-1 risk, 1.2 years for intermediate-2 risk, and 0.4 year for high risk.

In 2012, a revised IPSS (IPSS-R) was developed based upon data from 7012 patients with primary MDS diagnosed using the FAB or WHO classifications [45]. It incorporated new cytogenetic categories [35], and differentially weighed the degree of cytopenias in newly diagnosed patients. Patient age is an optional variable that can be incorporated to predict overall survival, but not evolution to AML. Individual patient was categorized into five risk groups: very low, low, intermediate, high and very high risk, that translates into median survival of 8.8, 5.3, 3.0, 1.6 and 0.8 years respectively. IPSS-R is simple to use, and is perhaps the most commonly used prognostication system today. However, there are several potential limitations to the IPSS-R. Both IPSS and IPSS-R were developed using data from patients who were observed without treatment. While outcomes might be different now that a variety of interventions are available, an analysis of a separate population suggested that the predictive value of the IPSS-R also applies to those treated with lenalidomide and azacitidine. [46] The prognosticating system only considered patients with *de novo* MDS. It is well recognized that patients with secondary MDS are more likely to have shorter survival. Much of this reflects the association between secondary MDS and "unfavorable cytogenetics". In addition, the IPSS-R seems to be most reliable at predicting outcomes at initial disease diagnosis, as the hazards in mortality and leukemia transformation diminishes over time in higher-risk but remains stable in lower-risk patients [47]. With increasing knowledge of MDS clonal genetics, the future risk stratification system might incorporate the prognostic value of mutation profile, which will be discussed in the next section.

WHO prognostic scoring system (WPSS) was designed to include information on red blood cell (RBC) transfusion need and cytogenetic information [48]. A subset of patients in the study cohort had data from multiple time points for a time-dependent analysis, therefore had the advantage over the IPSS of being able to be used at any time during the disease course.

The MD Anderson Cancer Center (MADCC) MDS model was developed based on a retrospective analysis of 856 patients with *de novo* or therapy-related MDS [49]. Age, cytogenetics, degree of anemia and thrombocytopenia, bone marrow blast percentage were identified as prognostic markers. Subsequently it was prospectively validated in 1915 patients, accounted for the duration of MDS and prior therapy [49]. One should take note that the MDACC model should only be applied to the population of patients with lower-risk (low or intermediate-1 IPSS) MDS, and patients who received various of MDS treatment, from which it was derived [50].

3. Pathogenesis

The pathogenesis of MDS is considered as a multistep process involving sequential acquisition of oncogenic mutations [51, 52]. The interplay between genetically altered HSCs and an abnormal bone marrow microenvironment may allow for selection of a predominant dysplastic clone [51–56].

3.1 Clonal heterogeneity and evolution

MDS is driven by a multistep process characterized by recurrent mutations affecting basic cellular pathways, including RNA splicing, epigenome regulation, myeloid transcription coordination, DNA damage response and growth factor signaling. It has been long recognized that HSC with certain pathogenic alterations

has a competitive advantage and drives clonal expansion at the stem cell level. Clonal cytogenetic abnormalities are detected in up to 50% of *de novo* MDS cases and 80% of therapy-related cases [57, 58]. Over the past decade, a number of large, MDS-focused sequencing studies further demonstrated that MDS is a genetically complex and heterogeneous disease [36, 37, 42, 59–61].

However, clonality alone is not sufficient to cause or diagnose disease, because increased clonal hematopoiesis can remain functionally intact [38, 39]. Recently published data on a large cohort of cytopenia (ICUS) patients delineated the natural history of patients with clonal vs. nonclonal cytopenia [62]. Patients with clonal ICUS had a much higher rate of progression than patient with nonclonal ICUS. Spliceosome gene mutations such as SF3B1 SRSF2 and U2AF1 and co-mutation patterns involving TET2, DNMT3A or ASXL1 had clinical characteristics resemble low-risk MDS patients, and higher progression to myeloid neoplasm when comparing with patient with somatic TET2, DNMT3A and ASXL1 mutation alone [39].

The diversity of clinical MDS phenotypes associated with specific mutations may be related to differential coregulation of the HSC self-renewal and lineage-specific differentiation capacity. Accurate prediction of the natural history of individual patient is certainly of high clinic interest. Our growing knowledge suggests that individual mutations occur in highly stereotyped order and strong patterns of co-mutation association and exclusivity (**Figure 1**) [36, 60, 63]. Mutations affecting epigenetic modifier genes (DNMT3A, TET2, ASXL1, EZH2, etc.) or RNA spliceosome components (SF3B1, SRSF2, and U2AF1) tend to arise in the initiation and early progression phase of MDS and rarely occur at the time of transformation. By contrast, mutations in growth factor signaling pathways (NRAS, KRAS, PTPN11, FLT3, etc.) are rarely found in early phase of disease, and instead, they are frequently acquired and expanded in subclones at time of progression to high-grade MDS or secondary AML [63–66]. A recent study [67] suggested that at the time of

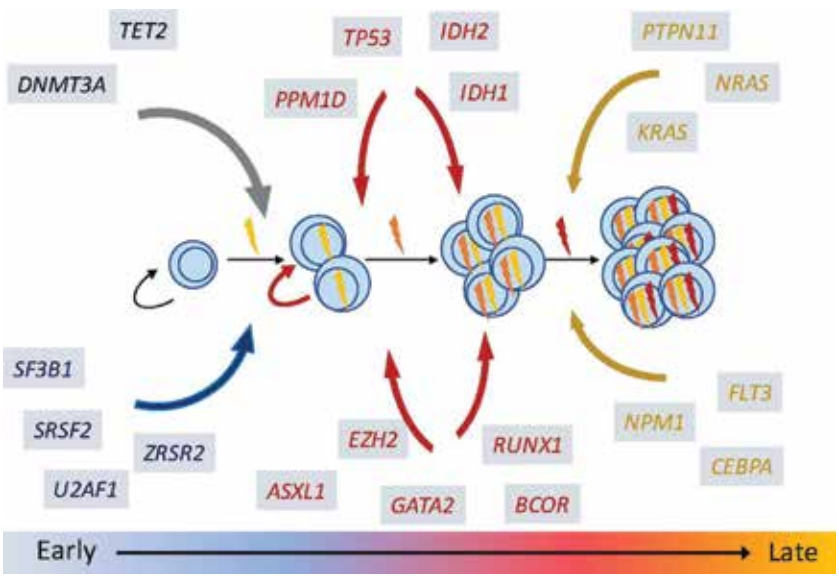

Figure 1.
Gene mutations have stereotyped positions in the MDS clonal hierarchy. Recent knowledge suggests that individual mutations occurs in highly stereotyped order and strong patterns of co-mutation association and exclusivity (Mutations affecting epigenetic modifier genes (DNMT3A, TET2, ASXL1, EZH2, etc.) or RNA spliceosome components (SF3B1, SRSF2, and U2AF1) tend to arise in the initiation and early progression phase of MDS and rarely occur at the time of transformation. Mutations in growth factor signaling pathways (NRAS, KRAS, PTPN11, FLT3, etc.) are frequently acquired and expanded in subclones at time of progression to high-grade MDS or secondary AML.

secondary AML transformation, the founding clone persisted at high variant allele fraction, but there was selective emergence and dominance of at least one genetically distinct subclone. In t-MDS, mutations in PPM1D or TP53 were present in 46% of patients, and they were the only gene mutations that were significantly associated with t-MDS [63, 68–71].

Various studies have also assessed the value of risk stratification based on MDS mutation profile [36, 42, 72]. TP53, ETV6, ASXL1, EZH2 and RUNX1 mutation confers adverse outcomes that are independent of IPSS risk assessment. SF3B1, which is frequently mutated in patients with ring sideroblasts, is associated with distinct and favorable clinical features [37, 38, 40].

3.2 Bone marrow microenvironment

HSC and genetically altered HSC all reside in a highly complex and dynamic cellular microenvironment in the bone marrow, that is composed of endothelial cells, multipotent mesenchymal stem cells, and sympathetic nerve fibers. There have been many *in vivo* studies demonstrated the concept of niche-induced disease initiation of MDS [73, 74] or AML [75, 76]. Evidence to support this in humans is mainly based on the not-so-rare occurrence of donor-derived leukemia in bone marrow transplant recipients, where changes in the preexisting niche in the host is thought to be leukemogenic [77]. In the review by Pleyer et al., [78] a variety of functional and molecular alterations were observed in *ex vivo* expanded mesenchymal stromal cells from MDS and AML patients, including their differentiation potential and HSC supportive activities, as well as chromosomal aberrations, transcriptional, and epigenetic changes. *In vivo* evidence also suggested that endothelial cells-specific gene alterations causes myeloproliferative disorders [79, 80].

3.3 Dysregulated immune pathways

Regulators of inflammation and innate immunity have always been thought to play an important role in pathogenesis of malignancies, but only until recent have the specific immune effectors and their cell-intrinsic functional roles in MDS stem cell biology been elucidated [81, 82]. Therapeutic targeting of over-activated innate immune components such as Toll-like receptors [83], IL-1 receptor–associated kinase/tumor necrosis factor receptor–associated factor-6 [84], IL8/CXCR2 [85], and IL1RAP [86] signaling pathways in MDS HSCs is being attempted pre-clinically.

4. Treatment

Treatment for MDS is guided by clinical symptoms, disease risk classification, patient age, comorbidities and performance status. Supportive care with transfusion and timely treatment of infection with antibiotics are important adjuncts for all MDS patients. Incorporating iron chelation therapy for patients requiring chronic transfusion and all candidates for allogeneic stem cell transplant is being increasingly emphasized to prevent cardiac and liver damage from iron overload [87, 88]. Pharmacologic treatment is usually reserved for symptomatic patients. Treatment goal for lower-risk MDS patients is to minimize symptoms, improve quality of life, and avoid toxicity from therapy. Erythropoiesis stimulating agent (ESA) can be used for symptomatic anemia and a low serum erythropoietin [89–92]. Together with low-dose G-CSF, hemoglobin improvement can be seen in up to 40% of lower-risk patients [93, 94]. Immunosuppressive therapy with antithymocyte globulin and cyclosporine A can produce response in a selected subset of

patients. Those most likely to benefit are younger than 60 years, with less than 5% bone marrow blasts, hypoplastic MDS, presence of a paroxysmal nocturnal hemoglobinuria clone, trisomy 8, human leukocyte antigen DR15 positive, and with short duration of transfusion dependence [95]. Low-dose lenalidomide at 10 mg daily is FDA-approved for lower-risk MDS characterized as the 5q- syndrome. Transfusion independence was achieved in 67% of patients in the phase 2 trial [96], and 56% in the phase 3 trial [97]. For ESA refractory lower-risk MDS patients without 5q- syndrome, lenalidomide in combination with ESA also demonstrated efficacy at reducing transfusion need [98–100]. So far, the only FDA approved therapies for higher-risk MDS are the HMAs azacitidine and decitabine. The use of these agents results in complete (CR) and partial response (PR) each in 10–20% patients, with median duration of response about 10 months [101–104]. An additional 20–30% patients achieve hematologic improvement without an objective response. Despite survival benefit demonstrated with azacitidine in high-risk patients [101], HMAs are not curative. For young and fit patients, allogeneic stem cell transplant is the only curative treatment option. Therefore, there remains a significant unmet therapeutic need beyond HMAs. Novel agents under clinical investigation and the use of allogeneic stem cell transplant will be discussed here.

4.1 Next-generation hypomethylating agents

HMAs are S-phase specific. Conventional HMAs all have a very short half-lives (less than 30 min) due to rapid clearance of azanucleoside by cytidine deaminase. The focus of newer generation HMA development has been to meet the need of prolonged drug exposure, allowing greater drug incorporation into DNA.

Oral film-coated azacitidine (CC-486) was first studied in an open-label pilot trial. It demonstrated 17% mean bioavailability after a single dose at 80 mg [105]. In a subsequent phase 1 dose finding study in MDS, CMML and AML patients, overall response rate was 73% in previously untreated patients, and 35% in previously treated patients [106]. Extended dosing schedule of CC-486 for 14 or 21 days is being evaluated in a phase 3 trial (NCT01566695) in lower-risk MDS [107]. CC-486 is also being studied in combination with immune check point inhibitor in the second line setting (NCT02281084).

ASTX727 is a novel formulation of oral decitabine paired with an oral cytidine deaminase inhibitor E7727 to overcome the rapid clearance from cytidine deaminase in gut and liver. In the early phase studies with intermediate- or high-risk MDS, ASTX727 (35 mg decitabine, 100 mg E7227) successfully emulated the pharmacokinetic profile of intravenous decitabine [108, 109]. In the phase 2 trial, clinical benefit was observed in 62% patients, with 16% CR, 28% marrow complete response (mCR), and 18% hematologic improvement [109].

Another strategy to circumvent the rapid degradation of azanucleotide is to develop a novel formulation that is relatively resistant to cytidine deamination. Guadecitabine (SGI-110) is a novel dinucleotide of decitabine and deoxyguanosine, linked by a phosphodiester bond, that leads to a slower release of the active decitabine moiety, prolonging cellular exposure to the drug [110]. In the phase 2 study with guadecitabine in intermediate and high risk MDS and CMML patients, CR was observed in 7/49 treatment naïve patients (14%) while CR + mCR were observed in 11/53 previously treated patients (21%) [111].

4.2 Histone deacetylase inhibition

Both DNA-promoter hypomethylation as well as post-translational modification of histone tails (e.g., deacetylation) lead to transcriptional silencing of tumor-suppressor

genes and genes involved in differentiation and apoptosis [112, 113]. Histone deacetylase inhibitors (HDACi) have limited single-agent efficacy in both high risk MDS and AML [114–116]. Preclinical evidence supported synergy between HMAs and HDACi [117]. However, a few phase 2 randomized clinical trials failed to demonstrate improvement in response rates or survival when azacitidine was combined with HDACi entinostat, vorinostat, valproic acid, or pracinostat [118–122]. Currently, a few clinical trials in MDS are ongoing using HDACi in combination with other novel agents such as immune checkpoint inhibitors (NCT 02936752) or pracinostat in combination with azacitidine using different dosing scheme (NCT 03151304). At this moment, how to best incorporate HDACi in MDS treatment remains uncertain.

4.3 Other epigenetic modification agents

Beyond targeting DNA methylation and HDAC recruitment, there has also been an increasing effort to develop epigenetic modification agents targeting posttranslation or posttranscription pathways, to mitigate malignant myeloid transformation in MDS.

Bromodomain and extraterminal (BET) proteins are epigenetic readers that recognize acetylated lysine tails of histones, and thus areas of open chromatin structure. It has been suggested that AML relies on BET protein BRD4 [123, 124], therefore led to great interest in utilizing BET inhibitors in myeloid malignancy. Various clinical trials are investigating the use of JQ1, the first selective BET inhibitor, in myeloid malignancy including MDS (NCT 02158858, NCT 02308761).

Overexpression of the mono and dimethyl lysine demethylase, LSD1 has been implicated in myeloid malignancies [125]. Clinical trials are ongoing evaluating LSD1 inhibitors in combination with ATRA or HMA in previously treated AML and MDS patients (NCT02273102, NCT02717884, NCT02929498).

4.4 Immune checkpoint inhibition

Upregulation of immune checkpoint molecules like PD-1/PDL-1 and CTLA4 is commonly observed in many malignancies, including AML and MDS [126, 127] to evade immune surveillance. However, preliminary experience suggested limited activity of immune checkpoint inhibitor use as single agent after HMA failure in MDS patients [128]. Several clinical trials are ongoing evaluating the efficacy of immune checkpoint inhibitors plus HMAs or HDACis (NCT02530463, NCT03092674, NCT02775903, NCT03094637, NCT02599649).

4.5 Other targeted therapies: extrapolating experience from AML

Based on the mutation profile, FLT3 inhibitor and IDH1/2 inhibitors are now FDA approved for AML. However, these mutations are less common in MDS [129]. The early phase ½ studies of IDH1 and IDH2 inhibitors included MDS patients, with reported response [130, 131]. Especially given their tolerability profile and single agent activity, these agents deserves further investigation in MDS.

Spliceosome mutations, such as SF3B1, SRSF2 and U2AF1 are the most common mutations in MDS [37]. Based on the encouraging activity in preclinical study [132], there is now a phase 1 study in myeloid malignancies including MDS, with splicing modulator H3B-8800, an oral modulator of the SF3B complex (NCT02841540).

Venetoclax, a selective BCL-2 inhibitor was granted breakthrough designation by FDA in combination with decitabine in 2017 for treatment–naive AML patients age greater than 65 years. This decision was based on result from two ongoing phase ½ clinical trials [133]. This combination is now being evaluated in higher-risk MDS in both frontline and HMA failure settings (NCT02966782, NCT02942290).

4.6 Management of anemia in lower-risk MDS

Luspatercept and sotatercept are modified activin receptor type II (ActRII) chimeric fusion proteins that consist of the modified extracellular domain of ActRIIB and ActRIIA respectively, trap TGF-β superfamily ligands to promote late-stage erythropoiesis [134, 135]. In the phase 2 trial of luspatercept for patients with lower-risk MDS who were ineligible for or refractory to ESAs, RBC transfusion independence was seen in 38% patients, and 63% hematologic improvement [136]. Similar efficacy was seen in the phase 2 trial for sotatercept, with 47% hematologic response in patients with high transfusion burden, and 58% with low transfusion burden [137]. Ongoing phase 3 clinical trial is evaluating the efficacy of ActRII antagonist in lower-risk MDS and MDS with ring sideroblasts who require regular RBC transfusions (NCT 02631070).

Rigosertib is a PI3K and polo-like kinase pathways small-molecule inhibitor. In the recent phase 2 study for transfusion-dependent lower-risk MDS patients, 20 of 62 (32%) patients achieved transfusion independence lasting for more than 8 weeks [138]. Validation of these results in future clinical trials is anticipated.

Roxadustat is a drug which acts as a HIF prolyl-hydroxylase inhibitor and thereby increases endogenous production of erythropoietin, which stimulates production of hemoglobin and RBCs. Roxadustat is shown to be safe and effective as anemia treatment for patient with underlying chronic kidney disease, not on dialysis [139]. A phase 3 trial is ongoing to evaluate the efficacy of roxadustat in low-risk MDS patients with low transfusion burden (NCT03263091).

4.7 Allogeneic stem cell transplant

Allogeneic stem cell transplant is the only curative therapy for MDS, but restricted to younger and fit patients. Disease free survival rates are approximately 30–50%. Treatment failure is attributed by transplant-related mortality in low-risk patients, and relapse in higher-risk patients [140]. In general, bone marrow transplant is offered to intermediate-2 and high-risk MDS patients. Over the past decade, reduced-intensity conditioning transplant made more older patients eligible for transplant [141].
An ongoing clinical trial is comparing the efficacy of reduced intensity allogeneic stem cell transplant to HMA in patients aged 50–75 with higher-risk disease [142]. In the study by Della Porta et al. [143], IPSS-R was prognostic for outcomes of patients in the high and very high-risk groups, but not in the low- and intermediate-risk groups.

There have been emerging data on the prognostic value of mutation profile and minimal residual disease pre- and post-transplantation. It was shown that only a minority of patients with MDS was in deep hematologic remission by flow cytometry minimal residual disease (MRD) and cytogenetic analysis before transplant [144]. For myeloablative conditioning, MRD positive and MRD negative patients had similar post-transplant outcome. However, relapse rate was higher for MRD positive patient who received non-myeloablative conditioning. Multiple studies have shown that TP53 mutation is an independent marker for short survival post-transplant [59, 61, 145]. EZH2, ETV6, RUNX1, ASXL1, JAK2, and mutations in the RAS signaling pathway have all been implicated to associate with short relapse-free interval post-transplant [59, 61, 145, 146].

5. Conclusion

Over the past decade, knowledge was gained in understanding the pathogenesis of MDS. However, many gaps remain to change the natural history of MDS. With

increasing number of novel treatments under investigation, it is likely that we are getting closer to more therapeutics options for MDS in the near future.

Conflict of interest

Wanxing Chai-Ho reports no conflict-of-interest.

Gary Schiller receives research funding from Forma, Janssen, Onconova, Pfizer, Incyte and BioMed Valley Discoveries, and receives consulting fees from Agios, Amgen, Astellas, Bioline RX, Celgene, Gamida, Fanssen, Karyopharm, Leukemia Lymphoma Society, Medallion, Medeor, Merck, Pharmacyclics, Sanofi, Seattle Genetics, Spectrum, Stanford University Health, Sunesis, and Takeda.

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Chapter 6

Noncoding RNAs in Myelodysplastic Syndromes

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Abstract

The discovery of short regulatory RNAs has recently directed the attention of scientists to parts of the genome that previously had been regarded as "junk" DNA because they did not encode protein products. The revelation that even protein-noncoding sequences had biological functions began the era of discovering the world of noncoding RNAs (ncRNAs). Of these ncRNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) are the most numerous and best-known ncRNA groups. miRNAs and lncRNAs are important regulators of hematopoiesis, and their abnormal function has serious implications for phenotypes. Deregulation of these ncRNAs is found in hematopoietic disorders, and they also contribute to the development and progression of myelodysplastic syndromes (MDS). Properties of ncRNAs such as stability and tissue specificity make these molecules highly promising diagnostic and prognostic markers as well as interesting therapeutic targets. This chapter summarizes our knowledge on the contribution of ncRNAs to the pathogenesis of MDS and discusses their potential applicability in disease diagnostics and prognosis.

Keywords: myelodysplastic syndromes, noncoding RNAs, microRNAs, long noncoding RNAs, pathogenesis

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell (HSC) disorders, characterized by dysplastic and ineffective blood cell production, with a tendency for transformation to acute myeloid leukemia (AML). The diagnosis is generally suspected based on the presence of an abnormal complete blood count and is confirmed by performing bone marrow (BM) aspiration and biopsy, demonstrating morphological evidence of dysplasia. A number of additional tests, including cytogenetics, flow cytometry, and molecular genetics, are needed to complete the laboratory evaluation of patients with MDS [1].

Because of the large heterogeneity of MDS, the development of additional molecular tools able to refine the prognostic scoring system, to predict outcome, and to monitor the response to treatment is required. Recently, application of new high-throughput methods such as next-generation sequencing (NGS) has identified recurrent somatic mutations in MDS cells. In particular, point mutations in the TP53, EZH2, ETV6, RUNX1, and ASXL1 genes have been shown to be associated

with specific clinical features and poor overall survival, independent of established risk factors [2]. Even though approximately 78% of MDS patients carry at least one oncogenic mutation [3], there is a long list of mutations in more than 50 genes with often unclear etiology, complicating the use of somatic mutations as simple and universal markers of MDS prognosis.

Concerning MDS pathogenesis, substantial progress has been made in recent years. A vast literature has become available regarding the spectrum of cytogenetic abnormalities, gene mutations, epigenetic modifications, gene expression patterns, and deregulated signaling pathways (e.g., apoptosis, proliferation, immune response, chromatin remodeling, RNA-splicing machinery, oxidative damage/DNA repair, microenvironment interactions, and others) associated with the disease. In this review, we discuss the contributions of noncoding RNAs (ncRNAs) to the pathogenesis of MDS as well as their potential applications as novel molecular markers for clinical purposes.

2. Noncoding RNAs

At the end of the last millennium, the importance of noncoding RNAs was completely unknown. Up to that point, the scientific community focused on genes that coded for proteins. The classic dogma of molecular biology postulated that DNA was transcribed into RNA, which was then translated into protein, ignoring all non-protein-coding sequences. Only in 1993 did the importance of miRNAs begin to be revealed. The discovery of the first miRNA, lin-4, from *Caenorhabditis elegans* [4, 5] initiated a new scientific era that definitively overcame the absolute sanctity of the central dogma. Interest in this field was further stimulated by the finding that almost all of the mammalian genome was transcribed at some level [6], raising speculation that much of this pervasive transcription was likely functional. This idea was epitomized by the ENCODE (Encyclopedia of DNA Elements, www.encodeproject.org) consortium that claimed to have assigned "biochemical functions for 80% of the genome" [7, 8]. From the beginning of this era, researchers identified thousands of previously unknown types of noncoding RNAs and indeed started to reveal their multiple functions affecting various features of cells.

2.1 Types of noncoding RNAs

Functional ncRNAs can be divided into two main types: infrastructural and regulatory ncRNAs. Infrastructural ncRNAs appear to have housekeeping roles in translation and splicing; they include species such as ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and small nuclear RNAs (snRNAs) that are involved in splicing events. Regulatory ncRNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs) are involved in the modification and interactions with other RNAs. However, ncRNAs can also be categorized on the basis of length (small, 18–31 nt; medium, 31–200 nt; and long, >200 nt), structure (circular RNAs (circRNAs)), or subcellular localization (small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs)). Other infrequent ncRNA types such as trans-spliced transcripts and macroRNAs that comprise enormous genomic distances, or multigene transcripts containing several genes or even the whole chromosome, further complicate efforts at systematic classification [9]. Moreover, clear categorization of ncRNA species is difficult, as many ncRNA transcripts often share properties of several categories. The major classes of ncRNAs are summarized in **Table 1**.

ncRNA class	Description	Description	Length
rRNA	Ribosomal RNA	RNA that is directly incorporated into the ribosome	Large subunit (5S–121 nt, 5.8S–156 nt, 28S–5070 nt) Small subunit (18S–1869 nt)
tRNA	Transfer RNA	Transfer amino acids to the ribosome for protein construction	76–90 nt
snRNA	Small nuclear RNA	Small RNA located in the nucleus, involved in spliceosomes (e.g., U1, U2, U5, U4, and U6), RNA modification, and other functions. Also commonly referred to as U-RNAs	~150 nt
snoRNA	Small nucleolar RNA	RNA located in the nucleolus, mostly involved in modification of other RNAs, such as rRNA (C/D-box and H/ACA box snoRNAs) or spliceosomal RNA (scaRNA)	~60–250 nt
miRNA	microRNA	A short single-stranded RNA that usually suppresses the translation of target mRNA by binding to 3′ UTR through RNA interference pathways	21–25 nt
siRNA	Small interfering RNA	Double-stranded RNA that guides sequence-specific degradation of target mRNA through RNA interference pathway	10–25 bp
piRNA	Piwi-interacting RNA	A large class of small ncRNAs involved in retrotransposon silencing through interactions with piwi proteins	26–31 nt
Y-RNA	Y RNA	Components of the Ro ribonucleoprotein complex, repressing its activity. It is also required for DNA replication	69–112 nt
circRNA	Circular RNA	RNA derived from precursor mRNAs forming covalently closed continuous loop. It is more resistant to exonuclease-mediated degradation	1–5 exons
lncRNA	Long noncoding RNA	A non–protein-coding transcript with >200 nt	>200 nt

Table 1.
The major categories of ncRNAs.

Of the various classes of ncRNAs, miRNAs and lncRNAs are the most numerous and are probably the best-known ncRNA groups (numbers of publications concerning miRNAs and lncRNAs are shown in **Figure 1**). Having different regulatory functions, these types of ncRNAs are important players in the majority of cellular processes, including hematopoiesis, and their abnormal function has serious implications for phenotypes. Deregulation of miRNAs and lncRNAs is frequently found in hematopoietic disorders, contributing substantially to the disease development and progression. Therefore, the following sections of this review will primarily focus on these two categories of ncRNAs and their functions in MDS.

3. MicroRNAs

miRNAs are short single-stranded noncoding RNA molecules of approximately 21–25 nt in length. Their sequences are highly conserved in both plants and animals and are thought to be an evolutionarily ancient component of gene regulation. miRNAs posttranscriptionally regulate gene expression through the RNA

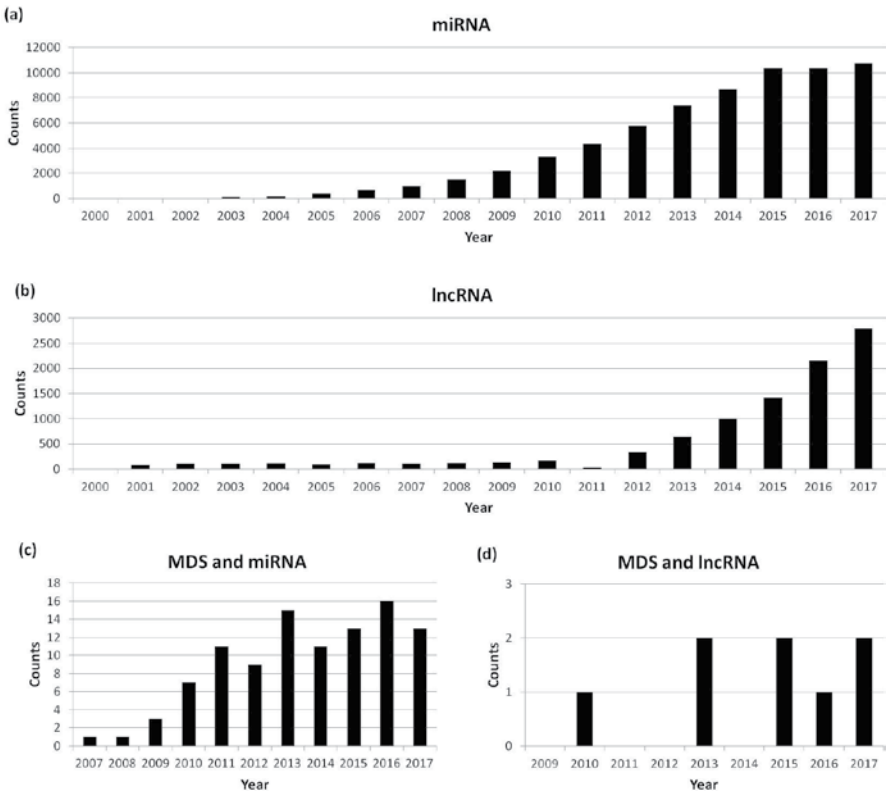

Figure 1.
Numbers of publications found on Pubmed using search terms: (a) miRNA (b) lncRNA, (c) MDS and miRNA, and (d) MDS and lncRNA (since the year 2000).

interference pathway. During the last decade, it has repeatedly been proven that miRNAs play crucial roles in a wide variety of biological processes such as development, differentiation, proliferation, and apoptosis. Because miRNAs influence the expression of genes involved in fundamental signaling pathways, their deregulation often triggers various pathological processes, including cardiovascular diseases, neurological diseases, and cancer.

The first miRNA, lin-4 from *Caenorhabditis elegans*, was discovered in the early 1990s [4, 5]. However, miRNAs were not recognized as a distinct class of biological regulators until the early 2000s. To date, thousands of miRNAs have been identified in humans and other species, and miRNA online sequence repositories, such as the miRBase database (www.mirbase.org), are available. According to the current version of the miRBase database (release 22), there are 1982 precursor miRNAs and 2693 mature miRNAs known in humans.

The biogenesis of miRNAs is a multistep process. miRNA genes are transcribed from genomic DNA by RNA polymerase II, resulting in primary miRNA (pri-miRNA) transcripts that usually encode sequences for several miRNAs. In the nucleus, these pri-miRNAs are cleaved by endonuclease Drosha, releasing approximately 70 nucleotide-long hairpin precursor miRNAs (pre-miRNAs). Pre-miRNAs are transported into the cytoplasm, where they are cleaved by Dicer into dsRNA duplexes containing both mature miRNA strand (miRNA) and its complementary strand (miRNA*). In general, the mature miRNA strands are preferentially loaded into the miRNA-induced silencing complex (miRISC), whereas the complementary strands are excluded and degraded. Once processed from the hairpin and loaded

into the silencing complex, the miRNA pairs with messenger RNA (mRNA) to direct posttranscriptional repression. At sites with extensive pairing complementarity, the miRNA directs argonaute-catalyzed mRNA cleavage. More commonly, however, the miRNAs direct translational repression, mRNA destabilization, or a combination of the two [10].

It has been shown that an individual miRNA is able to control the expression of more than one target mRNA and that each mRNA may be regulated by several miRNAs. Generally, it is believed that miRNAs regulate more than 30% of protein-coding genes in the human genome [11]. The ability of miRNAs to interact with thousands of mRNAs has raised intensive interest in their role in physiological and pathological conditions. Like mRNAs, the majority of miRNAs are expressed in tissue-specific manners. For example, miR-122 is preferentially expressed in liver [12], miR-124 in neurological tissues [13], miR-133 in muscles [14], and miR-208a in heart [15]. Moreover, it has been demonstrated that changes in the spectrum of tissue miRNAs correlate with various pathophysiological conditions [16].

3.1 Extracellular miRNAs

In recent years, cell-free circulating miRNAs have been found in various body fluids such as blood, cerebrospinal fluid, saliva, and urine [17]. The first extracellular small RNAs were observed in blood in 2004 [18]. Unlike the comprehensively described function of cellular miRNAs, the function of miRNAs present in the extracellular environment remains somewhat speculative. However, a growing body of evidence has suggested that these molecules are not mere leftovers of cellular degradation without any specific functions, but that active exchange of miRNAs between cells can play an important role in long-distance cell-to-cell communication.

In 2008, Mitchell et al. [19] reported that extracellular miRNAs were stable in human plasma/serum. This high stability of circulating miRNAs despite high levels of RNase activity in blood indicates that circulating miRNAs must somehow be protected from degradation. To date, a number of miRNA carriers have been described: membrane-derived vesicles (shedding vesicles, exosomes), lipoproteins, and ribonucleoprotein complexes (with argonaute-2 (AGO2) or nucleophosmin 1 (NPM1) proteins) have been found to transport extracellular miRNAs. It has been reported that the sorting of miRNAs into various types of vesicles can be selective. Diehl et al. [20] compared the content of miRNAs in microvesicles and their maternal cells. These authors demonstrated a significantly varied spectrum of miRNAs in both samples, suggesting a selective packaging of miRNAs into microvesicles.

3.2 miRNAs in normal hematopoiesis

The differentiation and homeostasis of the hematopoietic system requires complex and interconnected molecular networks that need careful regulation. During the last decade, the role of miRNAs in the hematopoietic system has been extensively studied, and many miRNAs serving as critical regulators of both normal immune functions and diseases have been discovered (**Figure 2**).

The first study of a role of miRNAs in the differentiation of the immune system showed that forced expression of miR-181 in hematopoietic stem cells (HSCs) markedly increased the number of B lymphocytes, with a concomitant reduction of T lymphocytes [21]. Since then, other miRNAs specific for the maintenance of the "stemness" of HSCs and for the development of individual blood cell lines have been determined. For example, miR-125a was found to be sufficient as a single miRNA to

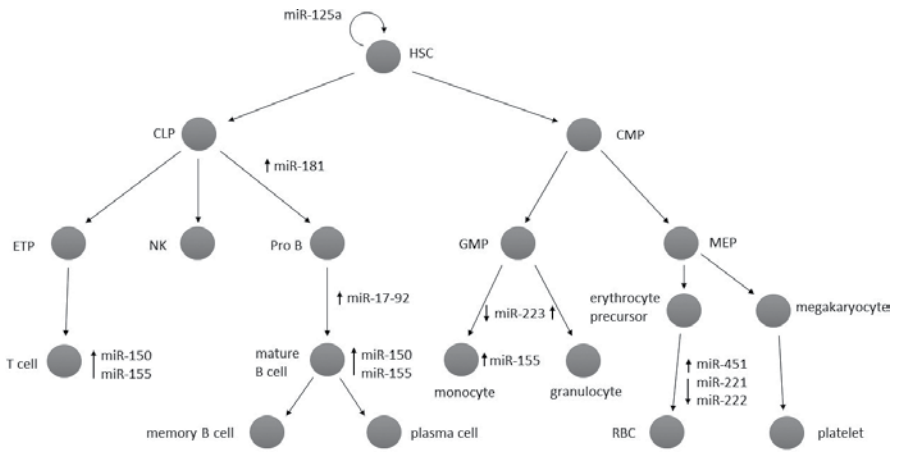

Figure 2.
Schema of lineage differentiation in hematopoiesis and miRNAs involved in the process. CLP—common lymphoid progenitor, CMP—common myeloid progenitor, ETP—early thymic progenitor, GMP—granulocyte macrophage progenitor, HSC—hematopoietic stem cell, MEP—megakaryocyte erythroid progenitor, NK—natural killer cell, RBC—red blood cell.

modulate HSC self-renewal and numbers and to protect lineage-negative progenitor cells from apoptosis [22]. A key miRNA that regulates granulocytic differentiation and function is miR-223. This miRNA shows a highly lineage-specific pattern of expression with low levels in HSCs and common myeloid progenitors. The expression of this miRNA is steadily upregulated during differentiation to granulocytes and is repressed during differentiation to the alternative monocytic fate [23]. miR-451 is expressed predominantly in erythroid cells, and its expression is significantly increased during maturation of erythrocytes. In contrast, miR-221 and miR-222 are downregulated during erythroid differentiation. This downregulation enables the expression of their target gene KIT (KIT proto-oncogene receptor tyrosine kinase, CD117), whose activation triggers erythroblast expansion [24]. Another miRNA, miR-150, is highly expressed in mature lymphocytes, whereas it is not active in HSCs. The target gene of miR-150 is transcription factor MYB (myeloblastosis) that regulates lymphocyte development [25]. miR-155, with its high levels in activated B cells, T cells, and monocytes, also participates in lymphoid differentiation. The development of B cells is positively regulated by miRNAs encoded by cluster miR-17-92. This cluster of miRNAs inhibits the expression of the apoptotic gene BIM (proapoptotic BH3-only Bcl-2 family member) and thus plays a key role in pro-B cells to pre-B cells transition [26]. In our laboratory, we analyzed miRNA expression in individual cell types from the peripheral blood of healthy individuals and determined a panel of 13 miRNAs whose expression profile enables differentiation of individual blood cell lines and determination of the cellular origin of in vitro cultured lines [27].

3.3 miRNAs in malignant hematopoiesis

In oncogenesis, miRNAs act both as oncogenes and as tumor suppressors. Mechanisms of deregulation are similar to those of protein-coding genes (chromosome aberrations, mutations, and epigenetic modifications). In 2002, the loss of two miRNAs (miR-15a and miR-16-1) due to a deletion in the 13q14 region in patients with chronic lymphocytic leukemia was described for the first time as directly associated with malignant disease [28]. In subsequent years, several miRNAs with key roles in the pathogenesis and prognosis of hematological malignancies were detected.

Several publications involved investigations of the role of miRNAs in AML. miRNA expression profiling revealed marked differences in miRNA expression between common cytogenetic subtypes of AML. Jongen-Lavrencic et al. [29] identified upregulation of several miRNAs (miR-382, miR-134, miR-376a, miR-127, miR-299-5p, and miR-323) in AML patients with t(15;17) and significant downregulation of miRNAs from let-7 family in AML with t(8;21) as well as in AML with inv(16). Moreover, miRNA signatures have been reported to be associated with recurrent molecular abnormalities in cytogenetically normal AML [30]. For example, upregulation of miR-10, let-7, and miR-29 family members and downregulation of miR-204 and miR-128a were found in AML with *NPM1* mutations [31] and high expression of miR-181a and miR-181b was associated with *CEBP*A (CCAAT/enhancer binding protein alpha) mutations [32].

3.4 miRNA deregulation in MDS

Several preliminary reports focused on identification of miRNA expression profiles that were either common in MDS or specific for individual MDS subcategories. For example, Pons et al. [33] measured levels of expression of 25 mature miRNAs in mononuclear cells (MNCs) of MDS patients. The authors reported overexpression of miRNA cluster miR-17-92 in MDS and differential expression of miR-15a and miR-16 between low- and high-risk subgroups of patients. Hussein et al. [34] showed that the miRNA profiles in BM cells discriminated MDS with chromosomal alterations from patients with normal karyotypes. Sokol et al. [35] examined miRNA signature in BM MNCs and found deregulation of several miRNAs (increase of miR-222 and miR-10a; decrease of miR-146a, miR-150, and let-7e) in MDS. In our study, we analyzed miRNA expression on a genome-wide level in $CD34^+$ BM cells. We observed significant differences in miRNA expression between early and advanced MDS; an apparent changeover was found between MDS with excess blast 1 (MDS-EB1) and MDS-EB2 subtypes. In particular, we identified strong upregulation of proapoptotic miR-34a in early subtypes of MDS [36].

Although many studies were conducted regarding miRNA profiling in MDS, there have been very few overlaps among them. This inconsistency may mirror the heterogeneity of the disease but also may possibly be explained by variations between the protocols and platforms used for miRNA detection.

There are several lines of evidence that many miRNAs are deregulated in MDS; however, the functions of miRNAs in MDS pathogenesis remain rather unknown. Identification of target genes of miRNAs in MDS and their functional proofs in both in vitro cell cultures and in vivo animal models are necessary to realizing this goal.

3.4.1 miRNAs in MDS with del(5q)

One of the best-characterized MDS subtypes is MDS with isolated del(5q), formerly referred to as 5q– syndrome. Haploinsufficiency of specific genes within common deleted region (CDR) localized in 5q31.3-5q33 locus is essential for the specific phenotype of MDS with del(5q). In addition to protein-coding genes, 13 genes encoding miRNAs are located in CDR. Most importantly, Starczynowski et al. [37] correlated del(5q) haploinsufficiency with loss of two miRNAs that are abundant in hematopoietic stem/progenitor cells (HSPCs), miR-145 and miR-146a. Knockdown of miR-145 and miR-146a together in mouse HSPCs resulted in thrombocytosis, mild neutropenia, and megakaryocytic dysplasia [37].

Kumar et al. [38] showed that miR-145 loss in MDS with del(5q) affects megakaryocyte and erythroid differentiation. These authors found that miR-145 functions through the repression of FLI1 (Friend Leukemia Integration 1 Transcription

Factor), a megakaryocyte and erythroid regulatory transcription factor. Inhibition of miR-145 increases the production of megakaryocytic cells relative to that of erythroid cells. Moreover, the authors proved that combined loss of miR-145 and RPS14 (a ribosomal gene that is required for the maturation of 40S ribosomal subunits and that maps to the CDR) cooperates to alter erythroid-megakaryocytic differentiation in a manner similar to that of the 5q– syndrome [38].

In our studies, we detected high expression of miR-34a in MDS del(5q) patients [36, 39]. The expression of miR-34a is induced by p53, activating apoptosis through inhibition of BCL2 gene (B-Cell CLL/Lymphoma 2, Apoptosis Regulator). This result is consistent with the increased apoptosis of progenitor cells seen in MDS del(5q).

3.4.2 miRNAs related to prognosis of MDS

Several publications have focused on miRNA expression in MDS with regard to prognostic potential. The earliest study in this area associated miRNA profiles with the International Prognostic Scoring System (IPSS) score [35]. A unique signature consisting of 10 miRNAs was closely associated with IPSS risk category permitting discrimination between lower- and higher-risk disease. Selective overexpression of miR-181 family members was detected in higher risk MDS, indicating pathogenetic overlap with AML. Survival analysis revealed shorter survival in patients with high expression of miR-181 family than in patients with low miR-181 expression [35].

Another miRNA that has been identified as having prognostic value in MDS is miR-22 [40]. This miRNA was upregulated in MDS and its level correlated with poor survival. Transgenic mice expressing miR-22 in the hematopoietic cells displayed reduced levels of global 5-hydroxymethylcytosine and increased HSC self-renewal accompanied by defective differentiation. Over time, these mice developed MDS. Interestingly, TET2 gene (Ten-Eleven Translocation 2, Tet Methylcytosine Dioxygenase 2) was identified as a key target of miR-22 in this context [40]. TET2 is a major regulator of DNA demethylation by conversion of methylated cytosine into 5-hydroxymethylcytosine.

3.4.3 miRNAs in the treatment of MDS

Expression profiles of miRNAs also appear to be applicable predictors of treatment responses. Lenalidomide is an immunomodulatory and antiangiogenic agent used for the treatment of MDS with del(5q). In recent years, several studies have analyzed miRNA expression levels before and after lenalidomide treatment in these patients [41–44]. Oliva et al. [41] investigated expression of selected genes/miRNAs at baseline and after 3 and 6 months of lenalidomide treatment. These authors showed that the expression levels of miR-145, miR-146, and miR-155 gradually increased during the course of the treatment. The significant role of miR-143 and miR-145 in response to lenalidomide was confirmed by Venner et al. [42], who showed that lenalidomide selectively abrogated progenitor activity in cells depleted of miR-143 and miR-145, supporting their key role in the sensitivity to lenalidomide in MDS with del(5q). In our studies, the most significant changes in expression levels (decreases) after lenalidomide treatment showed miR-34a and several miRNAs clustered within the 14q32 locus [43, 44]. However, a question remains as to whether the changes in expression levels were due to direct response to lenalidomide or whether they were caused by a reduction of the pathological clone.

Therapy with hypomethylating agents (HMAs) such as azacitidine and decitabine is currently considered to be the standard therapy for higher-risk MDS

and AML with myelodysplasia-related changes. Several studies investigated miRNA expression with respect to HMA treatment in AML [45–47]. Blum et al. [45] proposed miR-29b as a predictive factor for the stratification of older AML patients treated with decitabine; however, this was not confirmed by other studies [46]. Butrym et al. [47] showed that low expression of miR-181 at diagnosis was a predictor of complete remission and prolonged survival in a subset of older AML patients treated with azacitidine.

In relation to HMA therapy in MDS, it was found that the level of extracellular miR-21 was associated with overall response rate and progression-free survival [48]. Furthermore, reduced expression of miR-124 (caused by abnormal methylation) was found in MDS/AML patients responding to decitabine. These patients exhibited significantly lower expression levels of the CDK6 gene (cyclin-dependent kinase 6) that is the target of miR-124 [49]. Moreover, hypermethylation of miR-124-3p gene appeared to be a good prognostic marker of overall survival [50]. In our report, we found that the overall response rate to azacitidine treatment was significantly higher in MDS/AML patients with upregulated miR-17-3p and downregulated miR-100-5p and miR-133b and that the high level of miR-100-5p was associated with shorter overall survival [51].

3.4.4 Extracellular miRNAs in MDS

To date, only a few studies have investigated circulating miRNAs in MDS [48, 52–54]. Two papers [48, 52] focused on specific circulating miRNAs (miR-21, let-7a, and miR-16) that were preselected based on information regarding their deregulation in blood cells and their importance in similar diseases. Researchers monitored the plasma/serum levels of these miRNAs and showed that their levels could serve as prognostic markers for MDS. Kim et al. [48] showed that serum level of miR-21 was significantly associated with overall response rate and progression-free survival in MDS patients treated with HMAs. The publication that studied let-7a and miR-16 demonstrated that high plasma levels of these miRNAs can serve as semi-invasive markers of poor outcome for MDS patients [52].

Zuo et al. [53] measured expression of 800 human miRNAs in MDS plasma. These authors identified a 7-miRNA signature (let-7a, miR-144, miR-16, miR-25, miR-451, miR-651, and miR-655) as an independent predictor of survival in MDS patients with normal karyotypes.

In our study, we investigated the spectrum (2006 human miRNAs) of circulating miRNAs in the plasma of MDS patients [54]. With regard to prognosis, the levels of miR-27a-3p, miR-150-5p, miR-199a-5p, miR-223-3p, and miR-451a were lower in higher risk MDS. Moreover, miR-451a was an independent predictor of progression-free survival, and there was a significant association of miR-223-3p with overall survival [54].

These pioneer studies suggested that plasma levels of specific miRNAs were associated with MDS patient outcome and may add information beyond the currently used scoring systems. Despite these early promising results, there remain insufficient data regarding the full spectrum of extracellular RNAs in MDS. To date, the possible presence of various forms of small noncoding RNAs (apart from mature miRNAs), pathways for their protection, and identification of their cells of origin have not been explored in MDS. These missing information would expand the knowledge regarding extracellular RNAs in this disease, and beyond that, it would definitely contribute to better interpretation of alterations of individual miRNAs with the potential to become specific prognostic markers in MDS.

4. Long noncoding RNAs

lncRNAs form perhaps the most numerous group of ncRNAs. These RNAs are defined as protein-noncoding transcripts longer than 200 nucleotides. This length was proposed to distinguish lncRNAs from small noncoding RNAs. In contrast with protein-coding RNAs, lncRNAs contain only short open reading frames or completely lack them. This group of ncRNAs is characterized by high levels of structural and functional diversity, low levels of GC nucleotides, and lower expression levels, in contrast with protein-coding transcripts. lncRNAs are transcribed by RNA polymerase II or III and subsequently can be spliced and polyadenylated at the 3′ end or may contain a 5′ cap, depending on their biogenesis. Their expression is developmental and tissue-specific.

Some lncRNAs regulate (negatively or positively) the expression of genes in *trans* or *cis* by affecting RNA polymerase II recruitment or by inducing chromatin remodeling. In addition, antisense transcripts can pair with their specific sense RNA, facilitating alternative splicing. When lncRNAs interact with proteins, they can influence protein activity or localization or even help to form cellular substructures or ribonucleoprotein complexes. lncRNAs can be processed to yield small, single- or double-stranded RNAs that can act as endogenous small interfering RNAs (siRNAs) or miRNAs. Moreover, they can also act as "miRNA sponges" that affect the competitive endogenous RNA (ceRNA) network. However, additional functions and detailed signaling pathways of lncRNAs remain to be clarified [55].

According to their position relative to protein-coding mRNAs, lncRNAs are further subcategorized into several groups. Long intergenic noncoding RNAs (lincRNAs) are lncRNAs that are located between annotated protein-coding genes and that are at least 1 kb away from the nearest protein-coding genes. Intronic lncRNAs are coded within introns of protein-coding genes. Sense and antisense lncRNAs are transcribed from the sense or antisense strands of protein-coding genes and often contain exons of this gene with mutual overlap. Bidirectional lncRNAs are oriented head-to-head with protein-coding genes within 1 kb. Bidirectional lncRNA transcripts often exhibit similar expression patterns to those of their protein-coding counterpart, suggesting that they may be subject to shared regulatory pressures. Another group of lncRNA is TERRA (Telomeric Repeat-Containing RNA), transcribed from constitutive heterochromatin-rich regions, or T-UCR (Transcribed Ultraconserved Regions), transcribed from highly conserved regions of the genome.

Although more than 100,000 lncRNAs have been identified to date, only a small number of them have been characterized in detail. To integrate the data describing various lncRNAs, their expression profiles, molecular features, and functions in a variety of cell systems, several databases containing a comprehensive list of lncRNAs have been developed and are continually being updated. Among the most comprehensive databases are LNCipedia (compendium of human lncRNAs, lncipedia.org) [56], lncRNAdb (reference database for functional lncRNAs, lncRNAdb.org) [57], and NRED (database of lncRNA expression, nred.matticklab.com) [58].

4.1 lncRNAs in normal hematopoiesis

Recently, lncRNAs have emerged as important regulators of cell fate. These RNAs play a variety of roles in controlling various steps in hematopoietic differentiation, including maintenance of HSCs and differentiation of myeloid, erythroid, and lymphoid lineages. To date, there have been several descriptions of lncRNAs

crucial for correct function of the hematopoietic system (**Table 2**). The first hematopoiesis-associated lncRNA, EGOT (Eosinophil Granule Ontogeny Transcript), was described in 2007. EGOT, a conserved transcript localized antisense to ITPR1 (Inositol 1,4,5-Trisphosphate Receptor Type 1) that modulates the development of eosinophils, is normally expressed in human $CD34^+$ HSCs, and its expression level increases during eosinophil development, helping to regulate production of eosinophil granule proteins [59].

The relatively well-known lncRNA, H19, maintains quiescence of adult HSCs. The H19 transcript was in fact the first lncRNA to be identified, enriched in the embryonic fetal liver but downregulated after birth [60]. H19 is active in long-term HSCs and becomes gradually downregulated in short-term HSCs and multipotent progenitors. Deletion of H19 from the maternal allele resulted in increased HSC activation and proliferation as well as impaired repopulating ability. This effect is mediated by derepression of maternal IGF2 (Insulin-Like Growth Factor 2) expression and by increased IGF1R (IGF1 Receptor) translation, resulting in increased signaling through the IGF1R [61].

Zhang et al. [62] identified HOTAIRM1 (Homeobox Antisense Intergenic RNA Myeloid 1), which is encoded within the human HOXA (Homeobox A) gene cluster and plays a role in the differentiation of myeloid cells. The expression of this transcript is upregulated during granulocyte differentiation. Knockdown of HOTAIRM1 reduced transcription of HOXA1 and HOXA4 in an acute promyelocytic leukemia cell line, resulting in a decreased expression of genes associated with granulocyte activation, defense response, and maturation [62].

LncRNA	Localization	Function	Reference
EGOT (Eosinophil Granule Ontogeny Transcript)	3p26.1, antisense to ITPR1 gene	Modulates development of eosinophils	[59]
H19	11p15.5, antisense to IGF2	Maintains quiescence of adult HSCs	[60]
HOTAIRM (Hox Antisense Intergenic RNA Myeloid 1)	7p15.2, encoded within the HOXA gene cluster	Induces differentiation of myeloid cells	[62]
lincRNA-EPS	Mouse 4qC7	Promotes red blood cell maturation	[63]
T-ALL-R-LncR1	6q24.3	Induces apoptosis in T-ALL	[66]
LUNAR1 (Leukemia-Induced Noncoding Activator RNA)	15q26.3	Positive regulator of cell division	[67]
HOTAIR (Hox Transcript Antisense RNA)	12q13.13, encoded within the HOXC gene cluster	Oncogene, promotes chromatin relocalization	[68]
MALAT-1 (Metastasis Associated Lung Adenocarcinoma Transcript)	11q13.1	Regulates transcription and cell cycle	[69]
MEG3 (Maternally Expressed Gene 3)	14q32.2	Tumor suppressor	[70]
XIST (X-Inactive Specific Transcript)	Xq13.2	Regulates X chromosome inactivation during embryogenesis	[73]

Table 2.
Examples of lncRNAs involved in normal and malignant hematopoiesis.

Hu et al. [63] studied the lncRNA transcriptome of the erythroid lineage and uncovered numerous erythroid-specific lncRNAs that become induced during terminal differentiation of mouse fetal liver red blood cells in vivo. These authors showed that lincRNA-EPS (erythroid prosurvival) acts to promote red blood cell maturation by downregulating proapoptotic pathways. Knockdown of lincRNA-EPS severely compromised terminal differentiation of erythroid progenitors and resulted in elevated apoptosis. Conversely, its ectopic expression protected erythroid progenitors from apoptosis triggered by erythropoietin starvation. Functional studies indicated that lincRNA-EPS acts by repressing a number of proapoptotic proteins, most prominently the caspase-activating adaptor protein Pycard [63].

Recent studies have also provided evidence for the importance of several lncRNAs in immune cell function. For example, lncRNA NeST (Nettoie Salmonella pas Theiler's, cleanup Salmonella not Theiler's), also named Tmevpg1, modulates the ability of mice to respond to viral and bacterial infections. NeST is specifically expressed by the T_H1 subset of helper T cells. The expression of NeST regulates the degree of inflammation induced by infecting pathogens, such as Thelier's virus or Salmonella [64]. lncRNA-Cox2 (cyclooxygenase 2) acts during inflammatory signaling by modulating the expression of several immune response genes via interactions with regulatory complexes [65].

4.2 lncRNAs in malignant hematopoiesis

lncRNAs not only participate in normal hematopoiesis but also contribute to the pathogenesis of hematologic malignancies, representing a new class of potential biomarkers and therapeutic targets. These RNAs have significantly different expression levels in primary tumors and metastases, functioning both as oncogenes or as tumor suppressors. Some cancer-related lncRNAs could affect the development and progression of tumor by means of p53, polycomb repressive complex 2 (PRC2), and other signaling pathways. Others are not observed in normal tissue but are detected in cancer. For example, T-ALL-R-lncR1 appears to induce (together with protease-activated receptor 4 (PAR-4)) cellular apoptosis in T cell acute lymphoblastic leukemia cells (T-ALL) [66]. LUNAR1 (leukemia-induced noncoding activator RNA) is highly expressed in T-ALL cells, and its expression is dependent on signaling through the oncogenic NOTCH1 receptor [67]. lncRNAs HOTAIR (homeobox transcript antisense RNA) and MALAT-1 (metastasis-associated lung adenocarcinoma transcript) are associated with metastasis and recurrence [68, 69].

4.2.1 lncRNA deregulation in MDS

The first study describing lncRNA in the context of MDS was published in 2010 by Benetatos et al. [70] who studied lncRNA MEG3 (maternally expressed gene 3). Abnormal methylation of its promoter was observed in a third of MDS patients and in half of AML patients [70]. MEG3 was the first lncRNA described to have a tumor suppressor function. MEG3 is expressed in many human normal tissues, and numerous studies have demonstrated that its expression level is lost in various cancers. Low expression of MEG3 is associated with an increased risk of metastasis and poor prognosis in cancer patients [71, 72].

In 2013, Yildirim et al. [73] conditionally deleted lncRNA XIST (X-inactive specific transcript) in mice hematopoietic cells. XIST is perhaps the most well-understood lncRNA to date. This lncRNA is located on the X chromosome and is required for X chromosome inactivation during embryogenesis. Yildirim et al. [73]

demonstrated that mutant females developed a highly aggressive myeloproliferative neoplasm and MDS (mixed MPN/MDS) with 100% penetrance.

The first study examining the deregulation of lncRNAs on a genome-wide level in MDS was published in 2017 [74]. The authors combined NGS and microarray data in $CD34^+$ BM cells and identified several lncRNAs (linc-ARFIP1-4, linc-TAAR9-1, lincC2orf85, linc-RNFT2-1, and linc-RPIA) deregulated in MDS-EB2. In the same year, Yao et al. [75] profiled lncRNA expressions in 176 adult patients with primary MDS and identified four lncRNAs (TC07000551.hg.1, TC08000489.hg.1, TC02004770.hg.1, and TC03000701) with expression levels significantly associated with overall survival. Subsequently, the authors constructed a risk-scoring system with the weighted sum of these four lncRNAs. Higher lncRNA scores were associated with higher marrow blast percentages, higher-risk subtypes of MDS, complex cytogenetic changes, and mutations in RUNX1, ASXL1, TP53, SRSF2, and ZRSR2, whereas they were inversely correlated with the SF3B1 mutation. Patients with higher lncRNA scores had significantly shorter overall survival and higher 5-year leukemic transformation rate than did those with lower scores [75].

Although increasing numbers of deregulated lncRNAs are currently being described in MDS, only a few have been functionally characterized so far. Transcriptomic data may be used to construct network modules consisting of lncRNAs and protein-coding genes to enable functional analysis of lncRNAs with unknown functions. These networks are subsequently linked with annotated signaling pathways and gene ontologies. The resulting outputs provide a degree of functional annotation for differentially expressed lncRNAs in the disease and their potential roles in pathophysiology [74].

5. Other types of noncoding RNAs in MDS

Only very limited information regarding other groups of ncRNAs have been published for MDS to date. However, we can anticipate that introduction of next-generation sequencing of RNAs (so-called RNA-seq) will bring to MDS research many novel insights regarding various ncRNAs in the near future. This technology enables sensitive global detection of various RNAs across an unparalleled dynamic range. Particularly, small RNA-seq is predominantly used for detection of miRNAs. However, during library preparation, small RNAs are selected by electrophoresis with sizes typically ranging from 20 to 50 nt. This range of size selection allows for the capture of many other species of small RNAs in addition to miRNAs.

5.1 Piwi-interacting RNAs

In 2011, Beck et al. [76] conducted one of the early studies to apply small RNA-seq in MDS. These authors compared expression of small RNAs between low-grade (refractory anemia, RA) and high-grade (MDS-EB2) MDS patients and demonstrated the first evidence of piwi (P-element-Induced Wimpy Testis)-interacting RNAs (piRNAs) in MDS BM cells and their particular enrichment in low-grade MDS. PiRNAs are a relatively newly defined class of small ncRNAs with lengths from 26 to 32 nt. These RNAs lack sequence conservation and are more complex than miRNAs. PiRNAs have been linked to both epigenetic and posttranscriptional gene silencing of retrotransposons and other genetic elements in germline cells, particularly those involved in spermatogenesis.

Transcription of particular piwi proteins (*piwil1* and *piwil2*) that are required for the accumulation of piRNAs was also significantly upregulated in RA [76]. Recent studies indicated that the piwi-piRNA complex may have a role in

posttranscriptional silencing of damaged DNA fragments and that interrupting piwi-piRNA formation can lead to DNA double-strand breaks [77]. In summary, the study from Beck et al. [76] suggested that the enrichment of piRNAs in low-grade MDS may potentially protect DNA from the accumulation of mutations, a mechanism not observed in high-grade MDS. Moreover, they proposed that piRNAs might be used as diagnostic markers for low-grade MDS; however, further studies of piRNA roles in MDS pathogenesis are warranted.

5.2 Transfer RNAs

The abovementioned pioneer study from Beck et al. [76] also provided an early insight into the deregulation of tRNAs in MDS. The authors showed that ratios of tRNA to rRNA were significantly higher in MDS-EB2 compared to those of RA and controls. Because tRNAs are building blocks for protein synthesis and are required during translation, this may indicate an increased regulation of translation at this disease stage. Interestingly, a significant increase of tRNAs in tumor samples was reported by Pavon-Eternod et al. [78] In addition, tRNAs have been shown to inhibit cytochrome c-activated apoptosis [79]. Taken together, Beck et al. [76] hypothesized that high tRNA content seen in EB2 may contribute to the two well-known characteristics of high-grade MDS, decreased apoptosis, and high rate of leukemic transformation.

Guo et al. [80] performed small RNA-seq in paired pre- and posttreatment samples from MDS patients receiving therapy with HMAs. In the sequencing data, the number of reads aligned to tRNA-derived small RNAs (tDRs) (78.81%) vastly outnumbered those aligning to miRNAs (4.43% of reads). The tRNA fragments that were captured by miRNA-seq might be a result of either active cleavage or artifacts of the miRNA-seq library construction. The authors identified six tDR fragments that were differentially expressed between MDS and normal samples. Three tDRs demonstrated increased expression in MDS (chrM.tRNA10.TC, chr12.tRNA8.AlaTGC, and chr16.tRNA4.ProAGG), while three were decreased (chr1.tRNA58-LeuCAA, chr19.tRNA8-SeC(e)TCA (SeC(e)TCA), and chr19.tRNA4-ThrAGT). Moreover, they identified a panel of four tRNA fragments (chr6.tRNA157.ValCAC, chr11.tRNA17.ValTAC, chrM.tRNA12.TS1, and chrX.tRNA4.ValTAC), whose combined expression in the pretreatment samples together was predictive of the likelihood of response. Deeper focus on mitochondrial tRNAs revealed that MT-TS1 (mitochondrially encoded tRNA serine 1) was the only mitochondrial tRNA to have a significant association with treatment response [80].

6. Conclusions

The discovery of ncRNAs has initiated a new era in molecular biology, completely changing our view of “junk” DNA that it is no longer considered unnecessary ballast. Mouse models have clearly demonstrated key functions of ncRNAs in regulatory networks and their ability to significantly influence biological processes. In the hematopoietic system, ncRNAs represent important regulators of HSC “stemness” and differentiation; therefore, it is not surprising that deregulation of ncRNAs also occurs in MDS.

Currently, we possess comprehensive information regarding the impact of miRNA deregulation on the pathogenesis of MDS. The efforts of current research activities aim to apply these findings to clinical practice, testing the potential diagnostic/prognostic value of selected miRNAs for MDS. However, miRNAs also represent promising therapeutic agents or targets. miRNA-based drugs are designed to

reduce the expression of oncogenic miRNAs or, conversely, to increase levels of miRNAs with tumor suppressor functions. Unlike targeted inhibition or activation of a single protein-coding gene, the administration of miRNA antagonists or their mimics may potentially improve the desired effects, as these molecules can regulate several genes, often in specific signaling pathways implicated in tumorigenesis. Several pharmaceutical companies already have miRNA therapeutics in their developmental pipelines [81]. In 2012, the first cancer-targeted miRNA drug, MRX34 (a liposome-based miR-34 mimic), entered phase I clinical trials in patients with advanced hepatocellular carcinoma, and this mimic has attracted considerable attention from both academic researchers and pharmaceutical companies [82]. MRG-106, a synthetic antagonist of miRNA-155, is currently being tested by MIRagen Therapeutics in patients with cutaneous T-cell lymphoma [83]. However, testing miRNAs as potential therapeutic agents or targets in MDS therapy still requires initial exploration in in vitro models before evolving to future clinical trials.

Information regarding the contribution of other categories of ncRNAs, including lncRNAs, to the pathogenesis of MDS remains scarce. However, given the large number of ncRNAs encoded in the human genome and the complexity of their interactions, it can be expected that, in the near future, we will reveal a number of ncRNAs involved in MDS. It can also be anticipated that we will identify new predictive markers of progression and responses to therapy among these molecules.

To conclude, the diagnostic and therapeutic possibilities of ncRNAs undoubtedly have profound potential in MDS. However, although the effects of some miRNAs have already been demonstrated, it is certain that the importance of ncRNAs in MDS will be fully understood only in the future and that many years of research and clinical trials remain before the eventual application of ncRNAs in clinical practice to classify, monitor, and treat this disease.

Acknowledgements

This work was supported by the research grants no. 16-33617A and 17-31398A and the project for conceptual development of research organization no. 00023736 (all from the Ministry of Health of the Czech Republic).

Conflict of interests

The authors declare that they have no competing interests.

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Edited by Ota Fuchs

This book deals with the rapid progress in the area of myelodysplastic syndromes (MDS). MDS are a group of age-associated heterogeneous malignant bone marrow stem cell disorders. MDS are characterized by ineffective hematopoiesis, which leads to refractory cytopenias and to clonal instability. Patients with MDS have myeloid dysplasia, intramedullary apoptosis and an increased risk of transformation to acute myeloid leukemia (AML). The use of next generation sequencing has allowed for the identification of molecular mutations in several genes in about 90% of MDS patients. Several mutations will likely be incorporated into future prognostic scoring systems for MDS. About 50% of MDS cases are characterized by the presence of cytogenetic abnormalities. The correct morphological and cytogenetic analysis interpretation plays an important role in diagnosis and prognosis of these disorders. Cell death and an inflammatory gene signature are associated with MDS. Better understanding of the genetic and molecular mechanisms of MDS pathogenesis provides an opportunity for new treatment strategies to be developed. Promising novel therapies targeting pathophysiological mechanisms of MDS are being studied but the drugs currently used in MDS therapy remain limited. The only curative therapy for MDS is allogeneic hematopoietic stem cell transplantation. Recent advances in strategies to minimize transplant-related toxicity make this treatment possible for more MDS patients who are sufficiently fit.

Published in London, UK

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ISBN 978-1-83962-040-9