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Molecular Biology of Myelodysplastic syndromes

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Abstract

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic disorders marked by ineffective hematopoiesis, peripheral cytopenias, and an increased risk of transformation to acute myeloid leukemia. Multiple processes govern hematopoietic progenitor proliferation and natural differentiation into mature myeloid elements. Molecular events that disrupt any of these processes have the potential to lead to ineffective hematopoiesis and an MDS phenotype. Recent advances in genomic analysis have identified a number of new genes that may be involved. The molecular description of MDS will lead to better understanding, classification, and treatment of this disease.

Introduction

The myelodysplastic syndromes (MDS) are a group of myeloid neoplasms characterized by abnormal differentiation, morphology, and maturation of myeloid cells. Clinically, patients present with cytopenias of one or more lineages and have an increased risk of progressing to acute myeloid leukemia (AML). In contrast to the peripheral cytopenias characteristic of MDS, the bone marrow is typically hypercellular. Molecular characterization of MDS has contributed to our understanding of this disease and has been incorporated into standard prognostic measures. In the International Prognostic Scoring System (IPSS) cytogenetics help determine risk of developing aggressive disease with complex cytogenetics (>3 abnormalities) and chr 7 abnormalities giving higher risk and normal cytogenetics, isolated del(5q), isolated del (20q), and –Y giving lower risk scores. In particular, the isolated del(5q) syndrome is associated with favorable outcomes with treatment responses to lenalidomide. Mapping within the 5q region has identified ribosomal protein *RPS14* and micro-RNAs miR-145 and miR-146 to be the key genes involved. However, other genes in the region such as *SPARC* may also affect the disease course and response. The 5q-syndrome is reviewed in detail separately in this issue. Over 50% of patients, however, have normal karyotypes.¹ Currently, these patients are classified as having good prognosis although their outcomes are varied. The advent of newer methods in genomic analysis including next generation sequencing and higher density SNP arrays has uncovered molecular changes beyond chromosomal abnormalities. Mutations in single genes have been implicated and can now be used to characterize patients with normal karyotype. This genetic information has increased our understanding of the molecular events that are involved in MDS. With more defined genetic changes, perhaps other subgroups besides del(5q) will be identified with characteristic clinical course or responses to treatment.

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Transcription Factors

The differentiation of hematopoietic stem cells to mature myeloid cells requires activation of specific genetic programs. The disruption of this program may lead to a partial block in differentiation and increased survival of progenitor cells. Likely, this inability to fully develop mature cells results in the cytopenias typical of MDS. In this fashion, mutations in transcription factors are a common mechanism towards the development of MDS.

RUNX1

The runt-related transcription factor 1 gene (RUNX1, also known as AML1, CBFA2) is a subunit of core-binding factor (CBF) transcription factor complex. It belongs in a family of 3 related proteins (RUNX1–3). Through the N-terminal runt domain it dimerizes with CBF β and binds DNA where it acts as a transcriptional activator through its C-terminal domain. RUNX1 regulates expression of various genes involved in hematopoiesis, including IL3, CSF2, and CD4. Loss of Runx1 is embryonic lethal in mice while conditional deletion in the adult hematopoietic compartment results in reduction of lymphoid progenitors, increase in myeloid progenitors, and defective platelet maturation.² Mouse models expressing RUNX1 mutations found in MDS/AML patients in a bone marrow transplant model induce an MDS phenotype.³ Mutations of RUNX1 occur in approximately 10–25% of AML cases and 10–20% of MDS cases.⁴ RUNX1 can also be disrupted through translocations such as t(8;21), though this fusion gene is much more commonly seen in AML. These mutations disrupt the transactivation activity of RUNX1 and can lead to dominant negative effects. RUNX1 mutations in MDS patients have been reported to be associated with poorer prognosis.⁵ Germline mutations in RUNX1 are associated with Familial platelet disorder with propensity to myeloid malignancy (FPD/AML) syndrome.⁶ Carriers of this mutation have an increased incidence of AML and MDS.

MDS1-EVI1

Ecotropic viral integration site 1 (EVI1) was initially identified as a common integration site of retroviral induced murine myeloid malignancies. There are 2 reported isoforms, EVI1 and a fused MDS1/EVI1 gene where the myelodysplasia syndrome gene1 is spliced in frame to the second exon of EVI1. Both transcripts are over-expressed in selected MDS samples compared to normal hematopoietic progenitors.⁷ The gene can also be over-expressed through a rare translocation with RUNX1, t(3;21)(q26;q22). Evidence of this translocation can be used in the diagnosis of MDS. EVI1 expression is associated with poor prognosis, deletions of chromosome 7, and epo-unresponsive anemias.⁸ EVI1 acts as a transcription factor that can inhibit erythroid differentiation through direct interaction with GATA1, a transcription factor critical in erythropoiesis.⁹ Mice over-expressing Evi1 develop a MDS phenotype and have defective erythropoiesis and thrombopoiesis.¹⁰ This is associated with decreased expression of EpoR and c-Mpl, receptors regulating proliferation of myeloid lineages. Evi1 also blocks transactivation of PU.1 through direct interaction and, thus disrupts the expression of genes involved in myeloid differentiation.¹¹ Evi1 has been shown to regulate GATA2 expression.^{12,13} GATA2 and potentially other target genes regulated by Evi1 play a role in maintaining stem cell proliferative capacity of hematopoietic progenitors. In a functional assay, Evi1 $-/-$ HSCs were unable to reconstitute the hematopoietic system of lethally irradiated mice.¹³ By contrast, continued and over-expression of Evi expression may contribute to increased progenitor cell number.

TP53

The protein p53 is a pleiotopic tumor suppressor involved in cellular senescence, cell cycle check point, and apoptosis. The genetic data demonstrating TP53 is an important tumor suppressor has been observed in a variety of tumor types through somatic mutations and

chromosome 17p deletions. TP53 acts as a tetrameric transcription factor for cell cycle and apoptosis regulating genes including p21 and BAX. Loss of p53 renders cells more resistant to apoptosis and cell death signals. Abnormal cell cycle and apoptosis controls render hematopoietic stem cells more prone to transformation. Mutations in TP53 are associated with higher-risk MDS with poor prognosis and poor response to therapy.¹⁴ Mutations are found in approximately 10% of patients with MDS, with an increase in frequency in those with secondary MDS.¹⁵

Signal Transduction

In spite of the peripheral cytopenias, patients with MDS typically have hypercellular bone marrows. An increase in the proliferation of blasts also marks potential transformation to AML. Acquisition of mutations in proliferative signals, then, may give rise to dominant clones that make up MDS.

NRas

The RAS family of oncogenes are small GTPase that activate signaling pathways and lead to increased proliferation. Activating RAS mutations have been identified in a variety of cancer types. In hematopoietic cells, RAS pathway signaling is often activated through receptor signaling including stimulation by FLT3 and KIT, ligands that support the growth of hematopoietic progenitors. Mutations in RAS give rise to ligand-independent proliferation or prolonged growth signals. RAS mutations are found in approximately 15% of MDS.¹⁶ NRAS is the most commonly affected RAS allele in myeloid malignancies and mutations are associated with poorer prognosis.

JAK2

Janus tyrosine kinases (JAK1–3) are proteins involved in cytokine signaling and cell proliferation. They signal by tyrosine phosphorylation of STATs, transcription factors that depend on this phosphorylation for dimerization, nuclear translocation, and transcription of target genes. JAK proteins are activated by cytokine receptors, and in the hematopoietic system, these including signaling through the GM-CSF receptor family, EpoR, and the thrombopoietin receptor. The JAK2V617F mutation has been most strongly associated with myeloproliferative neoplasms (MPNs) with a valine to phenylalanine substitution at amino acid 617. JAK2V617F mutation being the most common. This mutation is seen in 97% of patients with polycythemia vera, 57% with essential thrombocythemia, and 50% with idiopathic myelofibrosis. Mutations in JAK2 are rare in MDS and are found in only 5% of cases.¹⁷ It is, however, found commonly in a subgroup of MDS patients having ring sideroblasts and thrombocytosis (RARS-T). A majority of these patients have mutated JAK2 and have a clinical syndrome of overlapping MDS and MPN.¹⁸

CBL

The C-cbl E3 ubiquitin ligase gene (CBL) is an enzyme involved in the degradation of receptor tyrosine kinases. Inactivation of this gene leads to accumulation of receptor signaling complexes that may provide increase growth signals and lead to enhanced proliferation. Mutations in CBL have been shown to cause loss of E3 ligase activity. Expression of C-cbl mutants in hematologic cells shows prolonged signaling through JAK2, KIT receptor, and FLT3 receptor and increased sensitivity to cytokines including SCF, IL3, TPO, and FLT3 ligand.¹⁹ In the same set of experiments, mutants were shown to have a dominant negative effect on activity of the wild type protein. Cbl knockout mice have an increased hematopoietic stem cell population, enlarged spleens, and abnormal hematopoietic development.²⁰ CBL mutations were identified in various myeloid malignancies through increased frequency of uniparental disomy (UPD) on chromosome 11q. CBL missense

mutations were found in 10 of 110 (9%) patients with secondary AML, 2 of 38 (5%) of CMML, and 1 of 115 (<1%) of MDS and was associated with a poorer prognosis.²¹

Epigenetic modifications

DNA Methylation

Besides mutations in individual genes, epigenetic modifications are likely to be involved in the pathogenesis of MDS. The two most common modes of epigenetic regulation involve DNA methylation and histone modifications. DNA methylation occurs with modification of cytosine in cytosine-phospho-guanine (CpG) regions, and generation of 5-methylcytosine. Maintenance methylases DNA methyltransferase (DNMT) carry out this methylation and maintain this modification through mitosis. CpG methylation is associated with silencing of transcription. In part, this silencing is carried out through methylated DNA-binding proteins that recruit other repressive protein complexes. DNA from MDS patient samples have been found to have increased methylation and increased promoter methylation is associated with poorer prognosis.²² Multiple genes have been identified as targets for silencing by methylation in MDS including cell cycle regulators such as *p15INK4B*.²³ The most convincing evidence for the central role of methylation in MDS come from clinical trials demonstrating the efficacy of hypomethylating agents in treating this disease. Decitabine and 5-azacitidine have both been shown to have benefit in treatment MDS in terms of improvement of cytopenias and decreasing the rate of progression to AML.^{24,25}

TET2—*TET2* is a member of a recently characterized family of proteins first identified in translocations associated with MLL, ten-eleven translocation (TET). TET1 has been shown to have enzymatic activity in 5-hydroxy modification of methylcytosine residues.²⁶ The enzyme requires 2-oxoglutarate (2OG) and Fe(II) as co-factors. Formation of 5-hydroxymethylcytosine (5-hmC) is hypothesized to regulate demethylation. Knockdown of *Tet1* expression in mouse ES cells demonstrated a requirement for *Tet1* function in ES cell maintenance.²⁷ Mechanistically this may be through regulation of the *Nanog* promoter, a key embryonic stem cell factor. Knockdown of *Tet1* resulted in reduced *Nanog* expression, which was correlated with increased DNMT dependent methylation of the *Nanog* promoter. TET2 has been shown to have similar enzymatic activity as TET1. Loss of TET2 may lead to increased survival and expansion of hematopoietic progenitor cells.²⁸ Transplantation of CD34+ cells from patients of MPD into mice resulted in expansion of the *TET2*-mutated clones compared to *TET2* wild type clones within the same sample. TET2 may also be involved in differentiation as *TET2* mutant cells were also more skewed towards myeloid versus lymphoid differentiation in the same experiments. Although TET1 has a DNA binding domain, TET2 does not and likely requires other proteins to interact with DNA. There are also isoforms of TET2 that lack the C-terminal region; the function of these variants is unknown.

TET2 was identified as a gene commonly mutated in MDS, in up to 26% (27/102) of samples examined.²⁹ Mutations were scattered throughout the protein sequence, although missense mutations were frequently identified in conserved domains. Subfractionation of patient bone marrow cells showed the mutation was present CD34+ cells, suggesting loss in an early stage clone. In one study of 96 patients, mutation of *TET2* in MDS was associated with a favorable prognosis and better 5 year survival.³⁰ However in a larger cohort of 320 patients with 12% (39/320) mutation frequency, *TET2* mutation was not correlated with overall prognosis.³¹ *TET2* mutations have also been identified in a wide variety of myeloid malignancies, including AML, MPD, and CMML.^{28,32} In isolated studies *TET2* was not prognostic in AML but was associated with worse prognosis in CMML.^{30,33} *TET2* has been associated with various other mutations in myeloid malignancies and can be acquired at an early stage of initiation or later upon disease progression.^{28,34} Mutations can be seen in

heterozygous states as well being associated with UPD or LOH. Given the diverse context within which mutations are found, further work is required to define its potential tumor suppressor function.

Histone modification

Another means of epigenetic regulation is histone modification. Eight histones form a core complex upon which DNA is packaged forming a nucleosome subunit. Histones H3 and H4 in particular are involved in regulating transcription. The tails of these subunits extend from the nucleosome and are subject to a variety of modifications including methylation, acetylation, phosphorylation, ubiquitination, and sumoylation. Acetylation of histone tails is thought to open the chromatin complex and allow for active gene transcription. Methylation can either be an activating or repressive signal depending on context. Histone 3 lysine 4 (H3K4) methylation is associated with activation while H3K9 and H3K27 methylation are associated with silencing. Activating marks associated with methylation of H3K4 are carried out by the Trithorax group of proteins. In contrast repressive methylation marks are carried out by the polycomb group (PcG) of proteins, specifically the polycomb repressive complex 2 (PRC2). Dysregulation of histone modifying complexes are known to contribute to myeloid malignancies. *MLL*, a H3K4 methylase, is a frequently rearranged gene in acute leukemias. *CBP*, a histone acetyltransferase, has also been found to be rearranged in some acute leukemias. Furthermore, CBP acts as a transcriptional co-activator that is recruited to RUNX1 complexes to initiate transcription.

ASXL-1

The Additional Sex-comb Like-1 (*ASXL1*) is a member of the Enhancer of Trithorax and Polycomb genes first identified in *Drosophila* as genes involved in Hox gene regulation and cell fate determination. *ASXL1* contains a PHD (plant homeodomain) finger in the C-terminus that is known to bind chromatin. It functions in maintenance of both activation and silencing of genes, depending on the interacting complex. In *Drosophila* where the gene was first identified, *Asx* mutation was shown to cooperate with both Trithorax (transcriptional activators) and Polycomb (transcriptional repressors) mutations. In mammalian systems, *ASXL1* also acts as an co-activator with retinoic acid (RA) receptor in RA responsive cells.³⁵ *Drosophila* *Asx* interacts with a protein Calypso that has Histone H2A deubiquitinase activity, and a homologous complex was identified in human cells.³⁶ H2A ubiquitination has been associated with transcriptional silencing and DNA repair. *ASXL-1* has also been shown to interact with HP1, a heterochromatin associated protein, and LSD1, a H3 demethylase.³⁷ Thus, loss of *ASXL1* may lead to inappropriate repression or activation of target genes depending on context. In mouse models, loss of *Asxl1* was shown to disrupt expression of Hox genes, specifically *Hoxa4*, *Hoxa7*, and *Hoxc8*, causing homeotic transformations.³⁸ Using the same model to analyze hematopoiesis, *Asxl-1* loss causes a defect in differentiation of lymphoid and myeloid progenitors and also results in mild splenomegaly.³⁹ No defects were observed in more primitive progenitors from the fetal liver or bone marrow, and no phenotype was seen in the peripheral blood in terms of cytopenias, MPD, or leukemia. This may be due in part, to compensation by *Asxl2* and *Asxl3* homologs. Mutations in *ASXL1* were found in 11% (4/35) patients and 18.5% (12/65) with MDS in two separate studies.^{40,41} *ASXL1* mutations were seen more frequently in advanced MDS RAEB-2 in one of the studies, 47% (9/19).⁴¹ Like *TET2*, *ASXL-1* mutations have also been identified in various other myeloid malignancies including MPN and AML.^{42,43} Mutations were most often identified in exon 12 before the C-terminal domain, causing frameshift or non-sense mutations. At this time there is no clear association of *ASXL1* mutations with prognosis or response to treatment.

EZH2

Enhancer of Zeste Homolog 2 (EZH2) is a polycomb group protein that has activity in methylating histone-3 and inducing transcriptional repression. It is a member of the polycomb group of proteins and part of the PRC2 complex along with Suz12 and Eed proteins. Recent studies have identified somatic EZH2 mutations in 6% (8/126) of MDS patients.⁴⁴ This gene maps to chromosome 7q36 and may be one of the genes implicated in chromosome 7 deletion phenotypes in poor risk MDS. Mutations in EZH2 have also been identified in MPN and CMML as well as lymphomas and solid tumors,^{45,46} however unlike other malignancies the mutations in MDS patients are most commonly frameshift/nonsense suggesting that loss of EZH2 function contributes to MDS pathogenesis.

The stem cell niche

Normal hematopoietic development depends on interactions between hematopoietic progenitors and bone marrow cells creating a particular niche for stem cells. Cytokines and growth factors regulate a delicate balance of stem cell renewal and differentiation. TNF- α has been studied in MDS patients as a regulator of apoptosis and elevated levels were seen to inhibit normal hematopoiesis.⁴⁷ IL-32 has also been identified as an unregulated cytokine in MDS and may cooperate with TNF- α in disrupting normal hematopoiesis.⁴⁸ The importance of interactions with the microenvironment has also been observed in mouse models. Disruption of Dicer in normal osteoprogenitors in mice disrupts normal stem cell interactions and induces myelodysplasia and secondary AML.⁴⁹ The relevance of an immune and cytokine regulatory effect on MDS is seen by clinical responses in select patients treated with immune suppression.⁵⁰ Antithymocyte globulin and cyclosporine were seen to induce response in terms of improved peripheral blood counts and transfusion independence. Patients with Trisomy 8 and HLA-DR15 have been reported to be the most likely responders to immunotherapy.

Therapeutic Implications

With the recent identification of molecular pathways which contribute to MDS pathogenesis, we can envision targeted therapies based on our improved fundamental understanding of the disease. It is already apparent that MDS is a molecularly heterogeneous disease with distinct outcomes. This is typified by the 5q- syndrome, which is associated with dramatic sensitivity to lenalidomide. Hypomethylating agents are another potential class of targeted therapies; it is possible that mutations in TET2 and methylation studies in MDS patient samples may provide insight into their therapeutic role in MDS. It will be interesting to see if patient response to decitabine or 5-azacitidine can eventually be correlated to specific mutations or methylation profiles. Although we do not yet have agents directed at specific transcription factors affected in MDS, therapies affecting transcription are being actively pursued in clinical trials. Histones integrate multiple epigenetic signals to regulate transcription, and one class of agents being intently studied is those affecting histone modification. As a class, the histone deacetylase inhibitors have been widely explored in phase I and II clinical trials.⁵¹ There is some early evidence that HDAC inhibitors alone or in combination with hypomethylating agents may have potential therapeutic benefit in MDS.^{52,53} Retinoic acid has also been tested as a potential therapy which can activate differentiation programs. Early reports suggest that it may have a benefit in the treatment of MDS related anemia in patients with low epo levels.⁵⁴ Disrupted signaling pathways in MDS are also targets for therapy. Clinical trials with receptor kinase inhibitors, specifically FLT-3 inhibitors are actively being tested in patients with MDS and AML with RTK mutations and have shown some preliminary efficacy.⁵⁵ Although Ras family members have been difficult to target, its downstream pathways may be amenable to manipulation. Inhibitors of downstream signaling components -- RAF, MEK, AKT, mTOR,

etc. all serve as potential targets of therapy. In the subset of MDS and MDS/MPN patients with **JAK2** mutations, JAK2 kinase inhibitors may be of benefit.⁵⁶ Many of these agents are newly in development and we await results from ongoing clinical trials to determine the potential benefits from these targeted therapies.

Conclusion

As more powerful genetic techniques are applied to studying MDS, we are gaining greater insights into the molecular events that contribute to MDS pathogenesis. Multiple processes required in hematopoietic development including transcriptional programs in differentiation, protein translation by ribosomes, proliferation, signaling, epigenetic regulation, and maintenance of the stem cell niche can go awry and lead to an MDS phenotype (see Figure). By better understanding the specific events involved we can hope to further subclassify patients and to develop better- directed treatments.

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Figure. Molecular mechanisms leading to MDS. Disruption of signaling transduction (A), transcription factors (B), epigenetic regulation (C), translation (D), and stromal interactions (E) all can contribute to the myelodysplastic phenotype.

Table

Molecular abnormalities in MDS.

Gene	Frequency	Comment
<u>RUNX1</u>	20%	associated with poor prognosis
<u>MDS-EV1</u>	n/a	~5% cases over-expressed Also dysregulated through translocations such as t(3;21)
<u>TP53</u>	10%	associated with poor prognosis, more common in therapy related MDS
<u>RAS</u>	15%	associated with poor prognosis, <u>NRAS</u> most common potential treatment targets in downstream pathway members
<u>JAK2</u>	5%	most often seen in RARS-T potential treatment with JAK2 inhibitors
<u>CBL</u>	<1%	seen in various myeloid malignancies
<u>TET2</u>	12–26%	seen in various myeloid malignancies potential link to DNA methylation and treatment with hypomethylating agents
<u>ASXL-1</u>	11–18%	seen in various myeloid malignancies associated with RA receptor, potential treatment with ATRA potential treatment targets in histone modifying enzyme
<u>EZH2</u>	6%	seen in various myeloid and solid tumor malignancies potential treatment targets in histone modifying enzyme