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Insight into the molecular pathophysiology of myelodysplastic syndromes: targets for novel therapy

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Abstract

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by abnormal cellular differentiation and maturation with variable progression to acute leukemia. Over the last decade, scientific discoveries have unraveled specific pathways involved in the complex pathophysiology of MDS. Prominent examples include aberrations in cytokines and their signaling pathways (such as tumor necrosis factor-alpha, interferon-gamma, SMAD proteins), mutations in genes encoding the RNA splicing machinery (SF3B1, SRSF2, ZRSR2, and U2AF1 genes), mutations in genes disrupting the epigenetic machinery (TET2, DNMT3A, DNMT3B, EZH2, ASXL1). In addition, abnormalities in regulatory T-cell dynamics and atypical interactions between the bone marrow microenvironment, stroma and progenitor cells, and abnormal maintenance of telomeres are also notable contributors to the complex pathogenesis of MDS. These pathways represent potential targets for novel therapies. Specific therapies include drugs targeting aberrant DNA methylation and chromatin remodeling, modulating/activating the immune system to enhance tumor-specific cellular immune responses and reduce anomalous cytokine signaling, and blocking abnormal interaction between hematopoietic progenitors and stromal cells.

Key words myelodysplastic syndromes; pathogenesis; cytokines; bone marrow microenvironment; immune dysregulation; cellular senescence; telomeric erosion; epigenetic regulation; targeted therapies

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Introduction

Myelodysplastic syndromes (MDS) are clonal, heterogeneous, hematopoietic stem cell disorders characterized by ineffective hematopoiesis, peripheral blood cytopenias, dysplasia in one or more myeloid cell lines, and an inherent risk of transformation to acute myeloid leukemia (AML). De novo MDS is usually a disorder of the elderly, commonly presenting in the 7th decade of life (1). Several molecular pathways have been implicated in the pathogenesis of MDS, with somatic mutations evident in over 80% of patients (2). Besides acquired somatic mutations, cytokine aberrations, immune dysregulation, alterations in the bone marrow microenvironment, abnormal RNA splicing, changes in telomeres and epigenetic dysregulation also play an integral role in the pathogenesis.

Cytokine aberrations

Transforming growth factor-beta (TGF-β) signaling and the SMAD proteins

Aberrations in the production and signaling of cytokines, such as tumor necrosis factor-alpha (TNF-α), TGF-β, interferon-gamma (IFN-γ), and interleukins (IL) have been described in MDS. TGF-β exhibits myelosuppressive properties and stimulates autocrine production of other myelosuppressive cytokines (such as IL-6 and TNF-α). Ineffective
hematopoiesis in MDS arises as a result of increased apoptotic susceptibility and diminished sensitivity of bone marrow stem cells to growth factors (3, 4). These effects are believed to be the result of the excess production of inflammatory cytokines and T-cell dysregulation along with abnormal signaling, a finding most pronounced in lower-risk patients with MDS (5, 6). TGF-β signal transduction is carried out by phosphorylation of SMAD proteins (principally SMAD2 and SMAD3), a class of intracellular proteins that regulate gene expression and cellular events. SMAD2 has been reported to be over-expressed in MDS progenitor cells. In a report by Zhou et al. (7) in 2008, immunohistochemical staining of progenitor cells from patients with MDS showed an increased expression of activated/phosphorylated SMAD2 protein, with increased number of staining cells and increased intensity of nuclear staining, in comparison with progenitor cells from non-MDS controls. Not only this, but gene expression profiling revealed a significant upregulation of the SMAD2 gene in MDS progenitors, a finding most likely arising as a result of sustained TGF-β signaling. As TGF-β ligand interaction with its receptor and the coupled TGF-β receptor 1 kinase (TBR1-kinase) is responsible for the activation/phosphorylation of SMAD2 for signal transduction, manipulation of this interaction represents a potential therapeutic option for patients with MDS (4). Zhou et al. (7) delivered short hairpin RNA (shRNA), an artificial RNA molecule used for silencing gene expression, targeting TBR1-kinase to MDS progenitors via lentiviral vectors. Expression of shRNA resulted in down-regulation of TBR1-kinase, leading to functional inhibition of the TBR1-kinase-SMAD2 signal transduction pathway. MDS progenitor cells expressing the anti-TBR1-shRNA showed resistance to TGF-β-mediated anti-proliferative signals and formed larger colonies of erythroid and myeloid cell lineages in vitro.

SMAD7, another SMAD protein, interacts with activated TBR1 and blocks association and activation/phosphorylation of SMAD2, thereby inhibiting TGF-β signaling. In another study published by Zhou et al. (8) in 2011, gene expression profiling showed markedly reduced SMAD7 in progenitor cells isolated from MDS patients in comparison with non-MDS controls, indicating SMAD7 downregulation to be another mechanism of hematopoietic suppression via unchecked TBR1-kinase-SMAD2 signal transduction. Chromosomal deletions involving the SMAD7 gene, located on the q-arm of chromosome 18, may be responsible for its downregulation. In fact, -18/del(18q) were reported to be commonly observed chromosomal abnormalities (up to 8%) in multiple studies involving patients with MDS (9–11). As discussed later in this review, abnormal DNA methylation and epigenetic silencing are dominant pathological alterations in MDS and it is likely that the SMAD7 gene may be affected by this process in a subset of patients (12).

In the aforementioned studies, the effects of novel TBR1-kinase inhibitors SD-208 and LY2157299 (galunisertib) on MDS progenitors were also studied in vitro. Progenitors from patients with low-risk MDS treated with various cytokines in the presence of SD-208 showed a significant increase in erythroid and myeloid colony numbers, highlighting the therapeutic potential of TBR1-kinase inhibition, especially in patients with low-risk MDS (7). The same findings were evident in similar in vitro experiments using galunisertib (8). These results indicate that inhibition of TBR1-kinase abrogates the myelosuppressive effects of sustained TGF-β signaling and SMAD2 activation by mimicking the effects of SMAD7. A recent study by Rodón et al. (13) showed galunisertib to be well-tolerated and effective in patients with malignant gliomas. Studies involving patients with MDS are warranted to determine whether the in vitro effects of TBR1-kinase inhibition on MDS progenitors are reproducible in vivo.

Additionally, other agents targeting the TGF-β/SMAD pathway are currently being developed. ACE-001 (sotatcept) is a chimeric fusion protein that acts as an activin-receptor type 2 ligand trap, antagonizing activin, and other TGF-β ligands (14). ACE-536 (luspatercept) is another fusion protein that is also an activin-receptor type 2 ligand trap that preferentially targets growth differentiation factor (GDF)-11 and GDF-8 (15). Both agents interfere with downstream signaling cascades of their respective targets, having pronounced inhibitory effects, especially on the SMAD pathway (14). Sotatcept and luspatercept promote late stage, erythropoietin-independent erythropoiesis and red cell maturation, thereby alleviating red cell transfusion dependence in low- and intermediate-risk MDS. Preliminary results from a clinical trial investigating luspatercept (ClinicalTrials.gov: NCT01749514) demonstrated significant efficacy in reducing red cell transfusion requirements in low- and intermediate-risk patients with MDS (16). Similar results were also reported for sotatcept (ClinicalTrials.gov: NCT01736683).

**TNF-α, IFN-γ, and B7-H1**

As MDS progress, the progenitor cells become less susceptible to apoptosis and become phenotypically more immature in comparison with non-clonal bone marrow progenitor cells. B7-H1, an immune-inhibitory molecule induced by the prolonged presence of cytokines such as TNF-α and IFN-γ, has been implicated in this. B7-H1+ MDS progenitors are shown to have greater proliferative capacity in comparison with those that do not express B7-H1. Furthermore, B7-H1 suppresses T-cell expansion and blocks T-cell-mediated apoptosis of MDS blasts, a process noted to keep the proliferation of the dysplastic clone in check early within the disease process. Blasts from patients with high-risk MDS are also noted to have increased expression of B7-H1 than those from low-risk MDS (17). Nuclear factor-κB (NF-κB) transduces the signal from TNF-α and IFN-γ, leading to the production and
expression of B7-H1 in MDS blasts. The NF-κB inhibitor pyrrolidine dithiocarbamate has been shown to block this signal transduction in vitro (17). Extensive in vitro and in vivo studies may lead to development of pyrrolidine dithiocarbamate as a targeted therapy for patients expressing high levels of B7-H1, particularly those with high-risk disease and impending evolution toward AML.

**Immune dysregulation**

There is evidence for immune dysregulation and abnormal dynamics between T-cell subtypes and their responses in MDS. Regulatory T cells (CD4+CD25+, FOXP3+) modulate immunity by preventing over-exuberant immune activation that may lead to autoimmunity. However, these cells also exhibit detrimental effects on immune surveillance and anti-tumor responses (18). A number of studies have shown polyclonal expansion of CD4+ T cells with oligoclonal/monoclonal expansion of CD8+ T cells and markedly decreased numbers of regulatory T cells (CD4+, CD25+, FOXP3+) in low-risk patients with MDS, leading to autoimmune cytotoxicity against progenitor cells, myelosuppression, and ineffective hematopoiesis (3). Conversely, the number of regulatory T cells in late stage and high-risk MDS is markedly increased, compromising the anti-tumor mechanisms of the immune system and leading to immune escape and unchecked expansion of the abnormal progenitor clone and eventual progression to AML (18). Based on these observations, the role of immunosuppressive therapy (usually with anti-thymocyte globulin and/or cyclosporine) and immunomodulatory agents (such as thalidomide and lenalidomide) has been defined in the treatment of patients, particularly showing efficacy in those with low-risk and early-stage MDS (3, 19). Specific characteristics, such as elevated serum thrombopoietin levels (20), positivity for HLA-DR15 (21), presence of MDS progenitors deficient in glycosyl-phosphatidylinositol-anchored proteins (22), and loss of the HLA-A allele due to uniparental disomy of the q-arm of chromosome 6 (23) potentially predict a positive response to immunosuppressive therapy in a subset of patients.

Activation of T lymphocytes results in the expression of the immune-inhibitory molecule CTLA-4 on the cell surface. CTLA-4 demonstrates greater avidity for B7 than the stimulatory molecule CD28, thus providing a checkpoint that prevents uncontrolled T lymphocyte proliferation. Blocking CTLA-4 with monoclonal antibodies and interfering with this checkpoint has shown great enhancement of tumor-specific T lymphocyte expansion and killing in studies involving solid tumors (24). Although autoimmune phenomena (especially involving the gastrointestinal tract) arise as complications of this strategy, they are easily manageable with additional therapies (24, 25). In the case of MDS, anti-CTLA-4 therapy may remove the prohibiting checkpoints on pre-existing T lymphocyte immunity against the abnormal progenitor clone.

The highly promising results of ipilimumab in patients with metastatic melanoma have provided a model for potential use of anti-CTLA-4 therapy in a wide variety of tumors. Results of early phase 2 and phase 3 trials in a wide variety of cancers have demonstrated feasibility, safety, and activity of these agents, thus suggesting a potential therapeutic role of anti-CTLA-4 therapy to be further investigated in MDS (26, 27). There are currently two ongoing clinical trials (ClinicalTrials.gov: NCT01757639, ClinicalTrials.gov: NCT02530463) investigating the activity of ipilimumab (an anti-CTLA-4 monoclonal antibody) against advanced stage MDS.

Programmed cell death protein 1 (PD-1) is another inhibitory molecule expressed by T lymphocytes. PD-1 also functions as an immune checkpoint by inducing the apoptosis of antigen specific T lymphocytes while inhibiting the apoptosis of regulatory T lymphocytes (28). This provides the basis of using another class of drugs, PD-1 inhibitors, to enhance immune system activation and increase anti-tumor activity of T lymphocytes. Anti-PD-1 agents, such as the monoclonal antibody nivolumab, have also shown remarkable response rates and activity against several solid tumors (27, 29). Like anti-CTLA-4 agents, anti-PD-1 inhibitors may also prove to be beneficial in MDS, improving responses and prolonging survival. Currently, the efficacy of nivolumab against MDS is being investigated in an ongoing clinical trial (ClinicalTrials.gov: NCT02530463). Another anti-PD-1 monoclonal antibody, pembrolizumab, is being studied in a phase 1 clinical trial involving patients with MDS and other advanced hematologic malignancies (ClinicalTrials.gov: NCT01953692).

**Bone marrow microenvironment and stromal cells**

Abnormal bone marrow microenvironment and altered functions of bone marrow stromal cells and mesenchymal stromal cells play an important role in the pathogenesis of MDS. Mesenchymal stromal cells in bone marrow samples from patients with MDS have shown pronounced chromosomal abnormalities and dysfunction (30). Similarly, bone marrow stromal cells derived from patients with MDS have been shown to function abnormally, producing high levels of inflammatory cytokines such as TNF-α and IL-6 (31). These abnormalities in the microenvironment result in disruption of the integrity of normal hematopoiesis, leading to an increased apoptotic index, aberrant cellular biology, and dysplasia of bone marrow progenitor cells. An abnormal microenvironment may also function as a milieu for selective expansion of the MDS clone and lead to disease progression (18). Based on these findings, therapeutic strategies that target the interaction of abnormal cells with their microenvironment may delay/halt disease progression and increase the sensitivity of abnormal cells to other therapeutic
agents. An example of such a strategy is blocking the interaction between the chemokine ligand CXCL12 and its receptor CXCR4, which is a major mechanism of interaction between cells and their microenvironment (32). However, the effectiveness of this strategy has yet to be studied in more detail.

**Aberrant RNA splicing**

Post-transcriptional premessenger RNA (pre-mRNA) processing involves splicing to generate mature mRNA transcripts for translation into functional proteins. The spliceosome is composed of small nuclear ribonucleoproteins (snRNPs) and other accessory proteins essential for spliceosome assembly, alternative splicing, and recognition of spliceosome donor and acceptor sites (33). Somatic mutations in genes encoding different spliceosome components have been described in ~45–80% of patients with MDS (33, 34).

*SF3B1* mutations are most frequent and exhibit a strong correlation with the presence of bone marrow ring sideroblasts (35). Other mutations include *SRSF2*, *ZRSR2*, and *U2AF1* (33, 36). Majority of the spliceosome mutations lack frameshift and nonsense changes, indicating either neomorphic (altered function) or gain-of-function alterations in splicing proteins (3, 37). Hence, therapeutic inhibition of the altered spliceosome may be an attractive choice for targeted therapy.

**Cellular senescence and telomeres**

Cellular senescence is a process by which normal cells lose their ability to divide after a specific number of cell divisions. Senescence in the context of MDS is related to shortening of telomeres, which are repeat sequences of DNA added to chromosomes by telomerase. MDS progenitor cells have been shown to have abnormal shortening of telomeric repeat sequences. Studies using southern blot, quantitative polymerase chain reaction (PCR), multiplex-quantitative reverse transcriptase-PCR, and flow-FISH have shown large reductions in telomere length in MDS blasts relative to cells from healthy controls, with no correlation with gender or age (38, 39). These studies have also shown large variations in telomere length among MDS patients, with shorter telomere length correlating with complex karyotypes, higher IPSS scores, marked transfusion dependence, greater percentage of bone marrow blasts, and higher risk of developing AML. Telomerase mutations/polymorphisms can occur sporadically in MDS. Notable mutations in this context affect the TERC (telomerase RNA component), TERT (telomerase reverse transcriptase component), *RTEL1* and *TINF2* genes, which have been shown to affect the telomerase complex activity and are correlated with shortened telomeres in patients with MDS carrying the mutations (40–42), and may also be one of the factors responsible for de novo MDS in younger patients (43).

Data from a recent study involving mouse models of MDS show that telomere dysfunction-induced DNA damage brings about cellular events that affect several cellular processes, including homogenous downregulation of genes encoding the mRNA-spliceosome machinery, particularly the *SRSF2* gene (40). *SRSF2* is an extensively studied splicing factor playing an integral role in mRNA splicing (36). Treating the mouse models with VE-821, an ATR-kinase inhibitor, leads to significant improvement in mRNA splicing in bone marrow progenitor cells, indicating an ATR-kinase-mediated pathway is responsible for altering the expression of splicing genes in cells with telomere dysfunction. Interestingly, the same study showed that *SRSF2* haploinsufficiency resulted in increased number damaged DNA foci related to telomere dysfunction, signifying that *SRSF2* mutations/delotions lead to abnormal splicing of mRNA transcribed by genes responsible for telomere maintenance (40). These findings advocate a close relation between mRNA splicing and telomere maintenance biology.

Telomere dysfunction-induced DNA damage was also shown to selectively down-regulate the expression of genes encoding the cohesin complex (*RAD21, STAG1, SMC2*, and *SMC5*), which plays a key role in the detection and repair of DNA postreplication and are commonly found to be mutated in MDS and AML (40, 44).

**DNA methylation and epigenetic silencing**

Molecular events affecting the epigenetic regulation of genes are also notable processes in the pathophysiology of MDS. Hypermethylation of CpG islands within the promotor regions of several genes, such as DNA repair genes, cell-cycle regulators, and apoptotic genes, leads to epigenetic silencing and is one of the most common molecular abnormalities in the pathogenesis and clonal evolution of MDS (12, 45). In this context, *TET2* is the most commonly mutated epigenetic regulator gene in MDS (approximately 25% of patients) (46). *TET2* encodes a protein involved in demethylation of DNA by hydroxylating the modified DNA-based methyl-cytosine to hydroxymethyl-cytosine and plays an important role in normal hematopoiesis and stem cell differentiation (47). To date, the prognostic relevance of *TET2* mutations in MDS remains unclear (48). Recurrent mutations involving the *DNMT3A* and *DNMT3B* genes (encoding DNA-methyltransferases 3A and 3B, respectively, which modulate epigenetic regulation via methylation of CpG islands on DNA) are found in up to 8% of patients with MDS and also correlate with worse overall survival and accelerated transformation to AML (49). Mutations affecting the isocitrate dehydrogenase (an enzyme responsible for the oxidative decarboxylation of isocitrate to alpha-ketoglutarate) genes, *IDH1* and *IDH2*, are observed in 4–12% of patients
with MDS. These mutations result in the accumulation of (D)-2-hydroxyglutarate which inhibits the function of enzymes that are dependent on alpha-ketoglutarate, leading to hypermethylation of DNA and histones which results in aberrant gene expression that can activate oncogenes and inactivate tumor-suppressor genes (50). They are associated with adverse prognosis in patients with MDS harboring these mutations, especially in the **IDH1** gene (49). Enhancer of zeste homolog 2 (EZH2) is a histone-lysine N-methyltransferase that catalyzes the methylation of histone H3 at lysine 27 (51). **EZH2** mutations occur in approximately 6% of patients with MDS and are frequently observed in early-stage, low-risk MDS while being exceptionally rare in AML, which is an interesting finding considering the fact that **EZH2** mutations predict adverse outcomes and reduced overall survival (52). The **ASXL1** gene (a regulator of epigenetic markers and gene expression by interacting with polycomb-complex proteins and various transcription activators and repressors) (53) is another frequently mutated gene in MDS (in 10–20% of patients) which shows a positive correlation with shorter time to evolution into AML and shorter overall survival (54).

Hypomethylating agents, such as 5-azacitidine and decitabine, inhibit DNA-methyltransferases 3A and 3B and are currently US FDA approved for the management of MDS (12, 45). IDH inhibitors have demonstrated good clinical activity in patients with myeloid malignancies. Currently, two IDH inhibitors, AG-120 targeting **IDH1** and the AG-221 targeting **IDH2**, are being investigated in clinical trials (ClinicalTrials.gov NCT02074839 and ClinicalTrials.gov NCT01915498, respectively). Preliminary results from these two studies have shown clinical activity in AML patients. AG-120 has shown complete response (CR) rates of 15% and an overall response rate (ORR) of an encouraging 31%, with responses lasting up to 11 months (55). Similarly, AG221 has shown CR rates of 17% and ORR as high as 40%, with the duration of responses up to 15.7 months (56). Both agents have proven to be well-tolerated with limited side effect profiles in both ongoing clinical trials.

There are other agents targeting epigenetic dysregulation. These include inhibitors of DOT1L (a H3K79 histone methyltransferase), for example, EPZ004777 (57), and lysine-specific demethylase 1 (LSD-1), for example, GSK2879552 (58), which have shown activity in AML,
indicating that MDS may also be amenable to treatment with these agents. Several inhibitors of bromodomain and extraterminal (BET) proteins, such as I-BET151, I-BET762, and JQ1, have proven to be potential targeted therapies for hematological malignancies (59). Currently, CPI-0610, another BET inhibitor, is being investigated in a phase 1 study involving patients with AML, MDS, and myeloproliferative neoplasms (ClinicalTrials.gov NCT02158858).

Other mechanisms

Several other mechanisms have been associated with the pathogenesis of MDS. For example, iron dysregulation with abnormal mitochondrial ferritin has been associated with MDS, especially in the refractory anemia with ringed sideroblasts subtype (60, 61). Another mechanism is abnormal control of apoptosis. Early-stage MDS shows an increase in the expression of pro-apoptotic proteins, such as Bax and Bad, which leads to widespread progenitor cell death and may account for hypercellular marrow often seen in early-stage disease. As MDS progresses, the abnormal clone demonstrates overexpression of Bcl-2, an anti-apoptotic protein which is found to be high in late stage and high-risk MDS (2, 60), accounting for unchecked proliferation of MDS blasts and development of AML.

An understanding of these multiple mechanisms involved in the pathogenesis in MDS will ultimately lead to opportunities to develop targeted therapies. Targeted therapies specific for these defects may possibly hold minimal side effect profiles with impressive response rates, thereby improving therapeutic outcomes and quality of life for patients afflicted with MDS. Some of the pathways discussed in this review are illustrated in Fig. 1.

Conflicts of interest

None.

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