Molecular pathology of myelodysplastic syndromes: Biology of medullary stromal and hematopoietic cells (Review)

MASANOBU KITAGAWA, MORITO KURATA, KOUHEI YAMAMOTO, SHINYA ABE, SHIHO SUZUKI and SHIGEAKI UMEDA

Department of Comprehensive Pathology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

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Abstract. Myelodysplastic syndromes (MDS) have been defined as a disease entity based on clinical features and morphological findings. Despite similarities in clinical manifestations, genetic abnormalities occurring in hematopoietic cells are heterogeneous among the syndromes. However, recent investigations have revealed that there are common biological events in the bone marrow of MDS cases. Most notably, excessive apoptosis of hematopoietic cells was observed to be induced by the bone marrow microenvironment. The apoptosis was mediated by paracrine as well as autocrine factors, suggesting that medullary stromal and hematopoietic cells play a role in the pathology of disease. Pro-inflammatory cytokines, such as TNFα, in the bone marrow microenvironment are predominantly paracrine mediators of apoptosis. Regarding autocrine stimulation mechanisms, it has recently been shown that the deregulation of ribosomal protein is capable of initiating a stress response in the hematopoietic cell through a p53-mediated signaling pathway. Thus, both the stromal cells of the bone marrow microenvironment and hematopoietic cells themselves possess a common and characteristic biology in this heterogeneous disease entity.

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Correspondence to: Dr Masanobu Kitagawa, Department of Comprehensive Pathology, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
E-mail: masa.pth2@tmd.ac.jp

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1. Introduction

Myelodysplastic syndromes (MDS) are characterized by the clinical manifestation of a variable cytopenia, despite normo- or hypercellular bone marrow, with a subset of cases progressing to overt leukemia (OL). MDS occur predominantly in the elderly and thus, the prevalence of patients with MDS is on the increase due to an aging population and increased awareness of these syndromes. In MDS bone marrow, it has been postulated that hematopoietic stem cells are initially exposed to genetic hits that eventually cause abnormality, and then develop a growth advantage over their normal counterparts, leading to clonal expansion and monoclonal hematopoiesis (1,2) (Fig. 1). Cell cycle kinetic studies demonstrated that MDS bone marrow is actively proliferative and cycling more rapidly than even normal bone marrow cells (3). However, despite such active proliferation of bone marrow cells, the presence of cytopenias (ineffective hematopoiesis) remains to be elucidated (Fig. 1). This review summarizes the mechanism of the underlying ineffective hematopoiesis in MDS, which appears to be caused by the apoptotic death of hematopoietic cells. In early MDS, stromal and hematopoietic cells produce high levels of pro-apoptotic cytokines, whereas in advanced MDS or OL, these cells produce anti-apoptotic molecules (Fig. 1). Therefore, apoptosis was the key finding inducing common and significant biologic characteristics in different sub-types of MDS patients. Regarding MDS bone marrow, a number of additional common abnormalities have recently been reported and are evaluated in this review.

2. Excessive apoptosis in the MDS bone marrow: cytokines and the bone marrow microenvironment

Cytokines and their receptors. The paradox of cytopenia, despite cellular marrow in MDS patients, was resolved by findings regarding programmed cell death in the 1990s. The excessive proliferation of bone marrow cells in MDS patients appeared to correspond to an equally excessive intramedul- lary apoptosis of hematopoietic cells (4-7). This apoptosis was observed in all FAB categories of MDS patients, although the frequency was higher in the early stage of disease and proportionately lower in patients with an increasing percentage of blasts. Furthermore, it was shown that excessive apoptosis was mediated by a number of pro-apoptotic factors, including pro-
inflammatory cytokines that were overexpressed in the MDS bone marrow. These factors include tumor necrosis factor α (TNFα), interferon γ (IFNγ), transforming growth factor β (TGFβ), interleukin 1 β, FAS-ligand (FAS-L) and inducible nitric oxide synthase (5-11). Pro-apoptotic factors, such as FAS-L were mainly produced by stromal cells, including macrophages, or partly by hematopoietic cells, whereas receptors, such as FAS and TNF receptor (TNFR), were expressed in hematopoietic cells (6,12).

As indicated by Raza et al (13), a second crucial paradox at this point was the presence of clonal expansion and monoclonality in cells that showed a tendency for apoptotic death. A model was proposed to explain this second paradox in which an unknown, poorly understood, initial lesion in a pluripotential hematopoietic stem cell leads to this cell developing a growth advantage (8). The cells are stimulated further to proliferate through the effects of pro-inflammatory cytokine TNFα. This proliferation eventually leads to a monoclonal hematopoiesis. Notably, as the daughter cells mature, TNFα driving the proliferation of the progenitors is capable of exerting a dual action, in that apoptosis is induced in the maturing progeny. This phenomenon was supported by demonstrating a shift in the expression of the cytokine receptors of hematopoietic cells in MDS. Pro-apoptotic receptor TNFRI and pro/anti-apoptotic receptor TNFRII were expressed differentially in response to the disease stages (12). The tendency to undergo apoptosis is not inherited uniformly by all the cells of the subsequent generations; instead, there is a spectrum of sensitivity to pro-inflammatory cytokines. The cells most sensitive to apoptosis are those undergoing apoptotic death in the bone marrow, whereas apoptosis-resistant cells actually enter the bloodstream. This phenomenon has been supported by the finding that granulocytes in MDS patients, although clonal in nature, are more resistant to apoptosis than granulocytes obtained from normal, healthy donors (14).

Early hematopoietic progenitor appears to develop a growth advantage and clonal expansion, leading to monoclonality. The subsequent generations of this transformed clone have an unequal tendency towards apoptotic death in the presence of increased amounts of a cascade of pro-inflammatory cytokines. One of the key systems for this switching appears to be TNFα and its receptors. Therefore, MDS should be considered as a disease of not only hematopoietic cells, but also of the bone marrow microenvironment, a concept that becomes critical when developing therapeutic strategies. Thus, cell proliferation followed by marked apoptosis was the most significant biological characteristic present in all subtypes of MDS.

Angiogenic factors. The bone marrow microenvironment of MDS is also affected by the production of angiogenic factors and matrix metalloproteinases (MMPs), which may regulate marrow microvascular density (MVD). Bellamy et al (15) have reported that in hematopoietic cells of MDS, the production of vascular endothelial growth factor (VEGF) and its receptor may promote leukemia progenitor self-renewal and cytokine elaboration and may also provide a biological condition for the generation of foci of abnormal localized immature precursors in advanced disease. Verstovsek et al (16) reported that VEGF expression is the determining factor of the biological behavior of MDS and AML. Findings of a recent study revealed a significant increase in marrow MVD in de novo AML patients compared to controls (17). In MDS marrow, MVD was higher than that of controls, but lower than that of de novo AML patients. MDS patients exhibited a significantly lower MVD following transformation to OL, as demonstrated by a pairwise comparison of samples collected from the same patient on diagnosis and at leukemic evolution. Furthermore, AML secondary to MDS revealed a MVD lower than that of de novo AML patients, similar to that of healthy controls. Genes encoding for pro-angiogenic factors exhibited a similar expression pattern. Of note, TGFβ, an anti-angiogenic mediator, exhibited an opposite expression pattern, since its expression was higher in AML secondary to MDS than in MDS and de novo AML.

Matrix metalloproteinases. Concerning the role of MMP in MDS bone marrow, Iwata et al (18) demonstrated that stromal cells induced the expression of MMP-9 in monocytes. MMP-9 expression was inversely correlated with bone marrow cellularity and percentage of cytogenetically abnormal cells. In their study, Travaglino et al (19) showed that MMP-2 and MMP-9 expression levels were higher in MDS patients than in healthy controls. The two MMPs localized in the cytoplasm of maturing myeloid cells. A positive correlation was observed between MMP-2 erythroblast expression and erythroid dysplasia, and an inverse correlation was observed between MMP-2/MMP-9 myeloid expression and marrow blast cell percentages. Particularly, in early MDS, the abnormal MMP expression profile correlated with an increased apoptotic rate, and high MMP levels were associated with longer overall and evolution-free survival.

Niche-induced myelodysplasia: bone progenitor dysfunction. The majority of normal and malignant tissues appear to form ‘stroma’ with specific mesenchymal cell involvement in the regulatory niches of stem cells. By examining the manner in which mesenchymal osteolineage cells of the bone marrow modulate hematopoiesis, Raaijmakers et al (20) showed that deletion of Dicer1 disrupts the integrity of hematopoiesis in mouse osteoprogenitors, but not in mature osteoblasts. These mice exhibited myelodysplasia followed by the development of AML that had acquired several genetic abnormalities, whereas Dicer1 remained intact. As a result of Dicer1 deletion, the expression of Sbds was reduced. Sbds is the gene mutated in Schwachman-Bodian-Diamond syndrome, a human bone marrow failure and leukemia pre-disposition condition. As expected, deletion of Sbds in mouse osteoprogenitors induced bone marrow dysfunction with myelodysplasia. Therefore, perturbation of specific mesenchymal subsets of stromal cells disorders differentiation, proliferation and apoptosis of heterologous cells, and disrupts tissue homeostasis. This study clearly indicated the essential role of niche as well as the microenvironment in the pathogenesis of MDS.

3. Recent findings for hematopoietic cell defects of MDS

The majority of MDS and AML patients harbor cytogenetic and molecular defects that identify entities with specific
biological/clinical features and distinct therapeutic responses (21-26). The incidence of chromosomal abnormalities is 40-60% in de novo MDS, 50-60% in AML and 70-90% in secondary MDS/AML (sMDS/sAML). A significant point that indicates a distinct pathogenesis for MDS and AML is that the genetic defects observed in de novo MDS are different from those of de novo AML. In de novo MDS, the incidences of chromosomal lesions with deletions (50%) and numerical defects (10%) dominate over the incidence of chromosomal translocations (<5%), although the most common cytogenetic abnormalities of AML are the balanced translocations (40%) (22). Thus, the hematopoietic cell (or myeloid blast) defects of MDS appear to be distinct from those of de novo AML.

Genetic bases of 5q syndrome. A special type of MDS involving an isolated del(5q) is called 5q syndrome (27). The syndrome is characterized by macrocytic anemia, normal or slightly elevated platelet counts, normal blast count, hypolobated megakaryocytes and a long-term risk of OL evolution of approximately 10% (28).

Bone marrow in 5q syndrome is essentially normo- or hypercellular with >90% of the hematopoietic stem cells being clonal (29). Despite the expansion of immature hematopoietic cells, the common manifestation is anemia, designated as ineffective hematopoiesis. By knocking down all 40 genes located within the common deleted region (CDR) at 5q32-33, Ebert et al (30) demonstrated that a decreased expression of RPS14 results in poor erythroid development and increased erythroid apoptosis. Forced expression of RPS14 in del(5q) marrow progenitors rescued the disease phenotypes. RPS14 is a ribosomal gene, coding for a component of the ribosomal subunit 40S. Other ribosomal genes, in particular RPS19, have been shown to cause Diamond-Blackfan anemia (31), also characterized by a macrocytic anemia. In addition, animal models have demonstrated that defects in ribosomal genes may increase the risk of cancer (32).

Using large parallel sequencing of small RNA libraries, Starczynowski and Karsan (33) identified two down-regulated micro-RNAs, miR-145 and miR-146a, located close to the 5’ end and with an abundant expression in marrow progenitors. Knock-down of miR-145 and miR-146a leads to a phenotype with key features of the 5q syndrome; i.e., hypolobated megakaryocytes and peripheral thrombocytosis. Moreover, predicted targets for miR-145 and miR-146a were associated with the innate immune response pathway. Two genes in the Toll-like receptor signaling pathway were verified as true targets: TIRAP for miR-145 and TRAF6 for miR-146a. TIRAP interacts with TRAF6 and subsequently results in the activation of NF-xB. A proportion of mice transplanted with TRAF6 overexpressing marrow developed bone marrow failure or AML in due course.

A mouse model of 5q syndrome has been generated (34). Since the CDR of 5q syndromes is split into two regions in mice, on chromosomes 11 and 18, chromosomal engineering was used to delete small segments within these regions. The only deletion that exhibited a clear phenotype was the Rps14 deficiency, while hypolobated megakaryocytes and peripheral thrombocytosis. Moreover, predicted targets for miR-145 and miR-146a were associated with the innate immune response pathway. Two genes in the Toll-like receptor signaling pathway were verified as true targets: TIRAP for miR-145 and TRAF6 for miR-146a. TIRAP interacts with TRAF6 and subsequently results in the activation of NF-xB. A proportion of mice transplanted with TRAF6 overexpressing marrow developed bone marrow failure or AML in due course.

As summarized by Jädersten and Eva Hellström-Lindberg (35), various genetic alterations cooperate to induce the characteristic phenotype of 5q syndrome. The anemia is likely to be caused by RPS14 deficiency, while hypolobated megakaryocytes and thrombocytosis may be the result of a deficiency of miR-145 and miR-146a. However, the CDR of 5q syndromes contains a number of other genes that may be of importance for clonal expansion, such as the tumor suppressor gene, SPRC (36). SPRC is anti-adhesive and modulates the cell matrix, induces apoptosis and may inhibit angiogenesis (37). Haploinsufficiency of SPRC may increase the adhesion of the hematopoietic stem cells to the supporting stromal cells and provide a clonal advantage for proliferation.
Novel gene mutations identified in MDS. Although the frequency is not usually high, various novel mutations have been identified in patients with MDS. JAK2 mutations (the most common one being V617F) were found in a significant proportion of patients with myeloproliferative neoplasms (MPN) (38), leading to the constitutive activation of proliferative and survival signaling in hematopoietic cells. In addition, an activating mutation in the thrombopoietin receptor (MPL) has been found in a small proportion of essential thrombocythemia and primary myelofibrosis (39). Multipotent hematopoietic stem cells with these mutations generate a myeloid clone that expands to replace hematopoietic cells without the mutation (40). These types of gene mutations have also been reported in MDS and MDS/MPN. JAK2 mutations occur in approximately 50% of patients with RARS with marked thrombocytosis (RARS-T), although the mutation is extremely rare in RARS with normal platelet count (41,42).

In 2009, mutations in the Ten-Eleven Translocation-2 (TET2) gene located on 4q were found in MDS and MPN (43-47). Among 320 patients with myeloid malignancies, TET2 mutations were found in 12% of patients with MPN, 19% of MDS and 22% of patients with CMML (44). Moreover, hematopoietic stem cells (HSC) harboring the mutation had a growth advantage compared to normal HSC. Another study revealed that 26% of 102 MDS patients carried TET2 mutations (46). These TET2 mutations were found to be more frequent in IPSS low (41%) and intermediate-1 risk (27%) MDS patients than in those with intermediate-2 (13%) and high-risk (14%) MDS. However, the prognostic significance of TET2 remains controversial (48). Although the cellular function of TET2 is unknown, TET1 was revealed to mediate the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, and was involved in the epigenetic control of the gene expression (49). Therefore, TET2 appears to be involved in DNA methylation.

Recently, mutation of the C-cbl E3 ubiquitin ligase gene (CBL) that is located on chromosome 11q was found in MDS and MDS/MPN. This ligase is involved in the degradation of receptor tyrosine kinases, and its inactive mutation may cause a growth advantage in the hematopoietic cells. The CBL mutation was identified in 2 of 38 (5%) patients with CMML and 10 of 110 (9%) with secondary AML (50). However, only 1 of 115 (1%) of the patients with MDS carried mutant CBL. Mutations were revealed to associate with poor survival on multivariate analysis.

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Another recently identified gene mutation in MDS is the polycomb-associated gene ASXL1, which is involved in the regulation of chromatin remodeling. ASXL1 mutations were found in 11% of 35 MDS patients and 43% of the 39 CMML cases (51). Boulwood et al studied 300 patients with MDS and AML and found that 62 (21%) patients carried an ASXL1 mutation (52). The mutation was observed in 5 of 79 (6%) RA patients, 17 of 55 (31%) RAEB-1 or RAEB-2 patients and 17 of 67 (25%) AML patients. Thus, the mutation was more frequent in high-risk MDS and AML patients compared to lower-risk MDS patients.

Uniparental disomy. Small genetic lesions, such as uniparental disomy (UPD), have been identified by high-density single nucleotide polymorphism (SNP) arrays. In MDS patients, 10-15% of those with normal karyotypes had regions of DNA that were derived from only one parent. This phenomenon has been termed UPD (53-55). In addition, small genetic lesions such as amplifications and deletions, which had remained undetected on conventional cytogenetics or FISH analyses, were also identified. UPDs were constitutional and not limited to the clonal cells, whereas the amplifications and deletions proved to be acquired. These data suggest that individuals who are born with constitutional UPDs are predisposed toward genetic instability and are at an increased risk of developing MDS. For example, the TET2 gene mutation is known to be frequently affected by UPD (47).

Toll-like receptor. Toll-like receptors (TLRs) play a crucial role in host defense against invading microorganisms by identifying pathogen-associated molecular patterns (56,57). A number of endogenous molecules have been reported to be ligands of TLRs (58). Some of these molecules are known to be expressed in cancer tissue and to activate intracellular signal pathways via TLRs during cancer progression. At the time of initial diagnosis, MDS bone marrow hematopoietic cells tended to express higher levels of TLR2, TLR4 and TLR9 than control bone marrow cells (59). Among these TLRs, TLR9 exhibited a significant decrease of expression at the time of transformation to OL. The expression of TLR9 and TNFα showed a significant correlation in bone marrow cells from patients with MDS and OL. Bone marrow cells in MDS exhibit frequent apoptosis, whereas OL cells are prone to immortality. Thus, TLR9 may be associated with the regulation of apoptotic/proliferative signals via TNFα in the MDS bone marrow.

4. Animal models of MDS

Although MDS is characterized by numerous complex phenotypes, such as ineffective hematopoiesis, peripheral blood cytopenias, morphological dysplasia of hematopoietic cells and susceptibility to OL evolution, attempts have been made to generate effective animal models mimicking MDS pathology. These models include Evi-1 overexpression in a mouse bone marrow transplantation (BMT) system, Npm1 hetero mice, Nup98/HoxD3 transgenic mice, mutated Am1l-bearing BMT system and Dicer1 deletion mouse system. In mouse hematopoietic organs, a forced expression of Evi-1 initially determines an excessive proliferation of bone marrow cells with the down-regulation of genes involved in erythroid differentiation and platelet formation followed by a profound and fatal peripheral cytopenia resembling human MDS (60). However, the condition does not progress to leukemia unexpectedly. Npm1+ mice exhibited features resembling MDS, although Npm1− mice showed embryonic lethality (61). In contrast to the Evi-1 model, these mice developed hematological malignancies of myeloid as well as lymphoid origin after a 2-year follow-up.

Similarly, Nup98/HoxD13 transgenic mice develop a disease that faithfully exemplifies all the key features of MDS, including peripheral blood cytopenias, bone marrow dysplasia, apoptosis and transformation to OL (62). These mice exhibit a uniformly fatal MDS, dying of either severe anemia/leucopenia or leukemic evolution. However, mice with
BMT of Nup98/HoxD13 cells did not develop OL. A recent study demonstrated that the BMT of Aml1 mutants exhibited MDS-like features and evolution to OL (63). In this model, different mutations of Aml1 resulted in different types of MDS. Finally, results from the DicerI deletion mouse system were highly significant (20). As described above in the section on microenvironment-niche-induced myelodysplasia, these results clearly revealed the significance of niche-like bone progenitor cells in the pathogenesis of MDS.

5. Conclusions

As shown in Fig. 1, a heterogeneous disease entity, MDS, is a complex regulation of cell dynamics, resulting in cellular proliferation and/or apoptosis. Under the various phenotypic manifestations, common biology may exist as regulatory mechanisms. One of the most characteristic of these mechanisms, distinct from AML and MPN, is the induction of apoptosis in hematopoietic cells. Fig. 2 summarizes the genetic/molecular modifications recently identified in the bone marrow of MDS. Interaction between hematopoietic cells and the bone marrow microenvironment is likely to define the balance between apoptosis/cell proliferation of MDS bone marrow cells.

Apoptosis is induced in hematopoietic cells only when the subsequent generations of the initial MDS progenitor cell mature and begin to express the appropriate cytokine receptors. These insights led to the novel translational approach of using anti-cytokine therapy to improve the cytopenias by protecting the maturing cells from dying. This strategy was attempted without a serious concern for causing leukemic transformation as the therapeutic procedure did not affect earlier progenitors. Since the rate of apoptosis is inversely related to the risk of transformation, this strategy was ideal for patients with lower-risk MDS where anti-TNF agents such as thalidomide, lenalidomide, infliximab and etanercept have been found to be effective in improving cytopenias in such as thalidomide, lenalidomide, infliximab and etanercept for patients with lower-risk MDS where anti-TNF agents related to the risk of transformation, this strategy was ideal for patients with lower-risk MDS where anti-TNF agents related to the risk of transformation, this strategy was ideal for patients with lower-risk MDS where anti-TNF agents related to the risk of transformation, this strategy was ideal for patients with lower-risk MDS where anti-TNF agents.

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References


