

# Biology of the Myelodysplastic Syndromes

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## Introduction

The myelodysplastic syndromes (MDS) cover a range of clonal stem cell disorders that result in disorderly and ineffective hematopoiesis. Since the French-American-British (FAB) classification of MDS<sup>[1]</sup>, a great deal has been learned about their natural history and prognosis. Elderly populations are predominantly affected, although MDS are increasingly recognized in younger individuals, including children. Patients usually present with chronic cytopenias that gradually worsen due to progressive bone marrow failure or transformation into acute myeloid leukemia (AML). It has become abundantly clear that blast cell percentage, karyotypic status, and the severity of peripheral blood cytopenias are major determinants of survival as well as transformation to AML. The International Prognostic Scoring System (IPSS)<sup>[2]</sup> has incorporated all the major prognostic determinants and identified four subgroups with significantly different prognosis in both survival and transformation to AML.

Precise pathogenesis of MDS is unknown, but is generally assumed that MDS arise from a hematopoietic stem cell harboring irreversible DNA damages. A step wise accumulation of genomic lesions within the affected hematopoietic stem cells may lead to qualitative and quantitative abnormalities in hematopoietic cells. Morphological dysplasia, impaired differentiation, defective cellular functions as well as the genomic instability are the fundamental abnormalities shared by the abnormal MDS clone.

## Clonal nature of MDS

Quantitative and qualitative abnormalities of blood cells in many cell lineages are indicative of the clonal origin of MDS. The clonal nature of hematopoiesis in MDS has been established by a variety of different techniques, including karyotyping, fluorescent in situ hybridization (FISH) analysis, detection of gene mutations, and X-chromosome inactivation studies<sup>[3]</sup>. In fact, a biologically essential feature in MDS is that once the abnormal hematopoietic clone is established, the clone predominates with time. No matter what techniques are employed in clonal analysis, we have to be aware of certain drawbacks inherent to each method. Karyotypes can only be assessed on mitotic cells. FISH has an advantage in that it can be applied to non-dividing mature cells as well, but is applicable only in cases with known cytogenetic abnormalities. Point mutation in the *ras* and *fms* oncogenes may arise at any stages of disease progression and do not necessarily track expansion of the "pre-malignant" clone. X-chromosome inactivation is based on restriction fragment length polymorphism (RFLP) methylation analysis, in which active and inactive X chromosome can be distinguished by differences in methylation pattern. Skewed distribution of

alleles in normal females and alteration in methylation status of "malignant" cells need to be taken into consideration. The polymorphism of a short tandem repeat in the X-linked human androgen-receptor (HUMARA) gene has recently proven its usefulness<sup>[4]</sup>. This HUMARA assay benefits from a high heterozygosity frequency (~90 %) in the 5' located CAG repeat and stable methylation patterns. Although the results of these different assays are in favor of a clonal mutation in the myeloid, erythroid, and megakaryocytic lineages, it still remains controversial whether the lymphoid cell population is also clonally involved. As a consequence, it is still a matter of debate whether the primary clonal mutation in MDS arises in the most primitive common hematopoietic stem cell or at a more committed stage. Perhaps some types of MDS arise from pluripotential cells, but it is in only rare instances or under rare conditions that these progenitors are able to give rise to lymphocytes and, possibly, lymphoid malignancies.

Thus, except for lymphocytes, all blood cells are low in number and abnormal in function and morphology. Dysplastic morphological changes, in turn, represent abnormality in the process of differentiation. Peripheral cytopenia, cellular dysplastic morphology, dysfunction, and impaired differentiation are different facets of the same abnormal MDS clone. In addition, genetic instability as suggested by cytogenetic studies is another feature shared by the MDS clone. These biological abnormalities, in particular clonal origin affecting the three hematopoietic cell lineages, may be projected to the biological difference between AML developing from MDS and AML without preceding MDS phase (de novo AML).

## Cell culture and cell biology

### 1. Abnormality in cell proliferation and differentiation

The ability of all of the hematopoietic progenitor cells committed to granulocyte-macrophage (CFU-GM), erythroid (BFU-E and CFU-E), megakaryocyte (CFU-Meg) and to multiple lineages (CFU-GEMM) to form colonies is either reduced or absent in MDS. Failure of cultures may be due partly to the abnormal function of accessory cells. The bone marrow cells from patients with MDS are unable to produce a healthy adherent cell layer in long-term marrow culture. However, the marrow cells from patients cultured over a normal adherent layer do not grow well either. Thus far, MDS marrow stroma has no consistent functional defect when studied for its activity to sustain the growth of normal hematopoietic progenitors<sup>[5,6]</sup>. Impaired or aberrant responsiveness to hematopoietic growth factors has been reported, but the weight of evidence suggests that MDS CFU-GM

have normal sensitivity to growth factors<sup>[7]</sup>. High endogenous levels of erythropoietin (Epo), granulocyte colony-stimulating factor (C-CSF), and CSF-1 have also been reported. Serum levels of interleukin-6 (IL-6) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) are often elevated in MDS. A high expression of TNF- $\alpha$  and transforming growth factor  $\beta$  (TGF- $\beta$ ) has been suggested in a histochemical study of MDS marrow biopsy samples<sup>[8]</sup>.

Both liquid and clonal culture studies show that the marrow cells from MDS patients have delayed maturation. Phenotypic analysis has suggested that NMS marrow CD 34+ cells were predominantly committed to nonerythroid lineages with impaired differentiation<sup>[9]</sup>. Available data suggest that a proportion of MDS cells can mature in vitro, although a considerable overlap in growth and differentiation pattern was seen between MDS and AML. While cell maturation is less than normal in MDS, maturation is clearly greater than that seen in AML. Delayed cell maturation may account for the increased number of early myeloid cells seen in the bone marrow of patients with MDS.

### **2. Suppression of normal hematopoiesis**

Once the abnormal hematopoietic clone is established, the clone predominates with time. Like other human malignancies, MDS is viewed as a disease that develops as a consequence of multiple genetic changes. During the course of multiple genetic insults to the hematopoietic stem cells, the MDS clone will progressively replace the residual normal stem cells. Macrophage populations from MDS marrow have been suggested to suppress normal hematopoiesis<sup>[3]</sup>. Two populations of enriched marrow macrophages suppressed the growth of CFU-GM from normal marrow in coculture experiments. The extent of suppression appears to correlate with the progression of the MDS clones<sup>[3]</sup>. The precise relationship of the suppression of normal CFU-GM by MDS marrow macrophages to other candidate inhibitors of hematopoiesis remains unanswered. Of interest was the finding that CFU-GM from MDS marrow was resistant to the inhibitory effects<sup>[3]</sup>. This would indicate participation of macrophage population from MDS marrow in the progressive expansion of abnormal MDS progenitors at the expense of normal granulocytopoiesis.

### **3. Ineffective hematopoiesis as studied in vitro**

Clonal CFU-GM assays appear to recapitulate the in vivo hematopoietic defect in that cells may grow initially but fail to proceed to further proliferation and maturation. Daily in situ observation of individually proliferating cells mapped in methylcellulose culture dishes demonstrated that a significantly greater proportion of cells in MDS proliferated initially, but failed to do so thereafter and disintegrated in culture. Cells with these abortive growth characteristics apparently contributed to ineffective hematopoiesis<sup>[3]</sup>. This would explain why CFU-GM cultures from most MDS patients end up with poor colony formation and increased cluster formation. Although there was no clear evidence for apoptosis in these experiments even after extensive search,

dying cells might have been phagocytized by adjacent marrow cells. From these observations, we postulated that the abortive growth is inherent to MDS progenitors themselves and that ineffective hematopoiesis is due to premature intramedullary cell death via apoptosis<sup>[10]</sup>.

### **Apoptosis as the cell biological abnormality in MDS**

Ineffective hematopoiesis is a condition in which cellular bone marrow is unable to produce and deliver adequate numbers of mature cells to the peripheral blood. As such, it is a poorly-defined condition, but it is likely to involve an excessive intramedullary cell death in the form of apoptosis. Premature apoptotic cell death within the bone marrow may well explain the apparent paradox in MDS of persistent cytopenia in spite of cellular bone marrow. Recent findings suggestive of excessive apoptosis in MDS marrow include 1) light and electron microscopic observation of bone marrow particles, 2) histochemical in situ end-labeling (ISEL) study of marrow biopsy specimens, 3) a high sensitivity to apoptosis induction of MDS-derived P39 myelomonocytoid leukemia cell line, 4) increased incidence of apoptosis following short-term culture of MDS marrow cells, 5) decrease of marrow apoptosis in patients following successful response to the combination treatment with G-CSF and Epo, 6) a high proportion of CD34+ cells with sub-G1 as stained with propidium iodide, 7) altered expression of proapoptotic Myc vs antiapoptotic Bcl protein, or altered ratio of proapoptotic Bad & Bax versus antiapoptotic Bcl-2 & Bcl-X in marrow CD34+ cells, and 8) a high proportion of apoptotic CD34+ as assessed by annexin V-FITC binding<sup>[11,12]</sup>.

Most of these studies support the view that apoptosis is more prominent in early stages of MDS than in advanced stages<sup>[13]</sup>. In accord with this notion is a recent histochemical study of Bcl-2 expression in immature myeloid blast cells from patients with MDS, which showed cell survival activity was higher in advanced MDS and AML than in early stages of MDS<sup>[14]</sup>. The early myeloid precursors, predominantly myeloblasts, were examined in marrow biopsy sections following immunostaining, and when sequential biopsies were studied, Bcl-2 expression was found to correlate with stages of the disease. The cell survival activity as assessed by Bcl-2 expression was lower in early MDS than in late stages of the disease, and an increased level of activity was associated with disease progression over time to AML. These data collectively support the hypothesis that MDS progression is related to accumulation of blast cells with increased Bcl-2 expression and decreased apoptosis. Conceivably, as the disease progresses, immature hematopoietic cell population with decreased apoptosis may arise from a background of increased apoptosis in early MDS.

The most puzzling question involves the molecular biological lesions leading to increased apoptosis in MDS. Possible candidates include cytokine abnormalities, the Fas/Fas-ligand system, cell cycle abnormalities, caspase activities and marrow stromal abnormalities<sup>[11,12]</sup>.

### **Leukemic transformation of MDS in the context of apoptosis**

In contrast to excessive apoptosis in ineffective hematopoiesis, leukemia is a disease associated with increased cell survival and proliferation. As in many other human malignancies, acute leukemia cells have a decreased ability to undergo apoptosis in response to at least some physiological stimuli<sup>[13]</sup>. The leukemic transformation of MDS imposes a paradox in that apoptosis, once excessive in the preleukemic stage, is no longer in operation to keep cells from uncontrolled growth. One of the final steps along the pathway of multistep carcinogenesis is the process of neoplastic transformation of the preneoplastic clone during which the clone has undergone additional genetic alterations, resulting in loss of tumor suppressor gene function and/or activation of growth promoting gene. Clearly, the concept of malignancy strongly implies that an increased threshold to apoptosis may be a crucial step in multistep leukemogenesis.

Cancer develops when cells accumulate in an uncontrolled manner because malignant cells proliferate at a faster rate than normal or they live longer than normal. Cancer may result from the combination of both. These growth and survival advantages are considered to arise from somatic DNA mutations due to carcinogenic stimuli such as irradiation or genotoxic agents. Normal cells can detect DNA damage, which will be repaired. If unrepaired, cells may commit suicide by apoptosis, thereby sacrificing themselves to eliminate dangerous genetic information and safeguard the host. Many genes implicated as inducers or repressors of apoptosis are originally known to be involved in the development of malignancy. Most well known is the induction of apoptosis by wild-type p53 and inability to induce apoptosis by a mutated p53 gene. A mutated p53 gene occasionally found in advanced stages of MDS and post-MDS leukemia are consistent with this notion<sup>[13]</sup>. The p53 -/- knockout mouse provides a good model to indicate impaired apoptosis in a variety of malignancies<sup>[13]</sup>. Cells from these mice are resistant to apoptosis induced by irradiation or genotoxic agents, although apoptosis by other stimuli is not altered. The loss of tumor suppression gene function not only relates to inactivation of apoptosis, but also correlates with tumor aggressiveness<sup>[13]</sup>. The interferon regulatory factor-1 gene (IRF-1) mapped to 5q31.1 has been shown to be deleted at one or both alleles in patients with MDS or AML with 5q chromosomal aberrations. As a transcription factor, IRF-1 activates the expression of interferon, leading to negative effects on cell growth. Thus, it is a candidate tumor suppressor gene.

At the heart of the issue is the question when and how default apoptosis occurs in the progression of the MDS clone. Of relevance is the observation that cells labeled by the ISEL method in advanced stage of MDS were mostly immature erythroid, myeloid and megakaryocytic cells, sparing the most immature blast cells<sup>[8]</sup>. We have also confirmed these findings (unpublished). It is clear that, biologically, MDS is a blend of increased proliferation and accelerated apoptosis<sup>[14]</sup>. Taken together, these findings are consistent

with the hypothesis that leukemic transformation from MDS is associated with a reduction in cell death, and that blast cells in advanced MDS probably survived DNA-damaging insults. Is escape from apoptotic control strong enough for leukemic transformation of MDS? Probably overriding the apoptotic control mechanism is a very critical step, but cells that have escaped from normal controls of cell survival or death may not necessarily be dangerous by themselves, unless coupled with signals or events that stimulate such cells to proliferate<sup>[13]</sup>. Oncogene transformation may lead to a dysregulation of genes that control cell survival or death. We propose that two steps, escape from apoptotic control and growth promotion, represent key biological processes involved in the paradigm of leukemic evolution of MDS. Whether the two processes occur in an orderly and successive fashion or they are separate processes independent from each other is unknown.

### **Unresolved questions and directions for future study**

The following questions have to be asked: Is apoptosis in MDS predominantly seen in progenitor cells or maturing cells as well? Is apoptosis seen in clonal cells or residual normal cells as well in MDS? What are clinical implications of increased apoptosis in MDS? Is apoptosis central to MDS? Clearly, we need to develop a new method that will enable us to more sensitively and specifically detect cells destined to die via apoptosis. It is hoped that with further understanding of these issues and questions, we may be able to define the molecular sequence of events in MDS from excessive apoptosis in preleukemic stages to default apoptosis in the final leukemic transformation and to establish new treatment modalities.

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