

## The biology of myelodysplastic syndromes: unity despite heterogeneity

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### Abstract

Myelodysplastic syndromes (MDS) traditionally have been grouped together as a disease entity based on clinical phenomena seen in association. Despite the similarities, there is great heterogeneity among the syndromes. Recent insights have shown, however, that there exists a biologically cohesive theme that unifies and thereby validates the conceptual interconnectedness. The first suggestion that such a relationship existed where biology could directly explain the observed cytopenias was the finding of excessive premature apoptosis of hematopoietic cells in MDS marrows. This apoptosis was mediated by paracrine as well as autocrine factors implicating both the seed and the soil in the pathology of the disease. Pro-inflammatory cytokines in the marrow microenvironment were mainly the paracrine mediators of apoptosis, but how the clonal cells committed suicide because of autocrine stimulation had remained a mystery for more than a decade. It has been shown now that deregulation of ribosome biogenesis can initiate a stress response in the cell through the p53 signaling pathway. Congenital anemias had been associated with mutations in ribosomal protein genes. The surprise came with the investigation of 5q- syndrome patients where haplo-insufficiency of the ribosomal protein gene *RPS14* was found to be the cause of this MDS subtype. Similar ribosomal deregulation was shown to be present in all varieties of MDS patients, serving as another unifying characteristic. In addition to these findings, there are other DNA-related abnormalities such as uniparental disomy, mutations in the *TET2* gene, and epigenetic phenomena that are associated with and occur across all types of MDS. This paper summarizes the themes unifying this heterogeneous group of diseases.

### Introduction

While an improved understanding of the biology helps us comprehend disease manifestations better, the converse is also true

in that clinical observations can sometimes provide critical clues into the underlying biology of the disease. MDS exemplify this. These syndromes, which predominate in the elderly, are characterized by the presence of a variable cytopenia, with a third of the cases progressing to acute myeloid leukemia (AML). The initial, as yet poorly understood lesion strikes an early hematopoietic stem cell that develops a growth advantage over its normal neighbors and leads to clonal expansion and eventual monoclonal hematopoiesis. Detailed studies of cell cycle kinetics revealed that the marrow is quite actively proliferative with more cells in S-phase and cycling faster than even normal marrows. The presence of cytopenias in the background of such active proliferation was difficult to explain initially. This paper summarizes the mechanism of the underlying cytopenias in MDS, which turned out to be caused by a premature intramedullary apoptotic death of hematopoietic cells. Apoptosis, therefore, was the first biologic characteristic to be found in different sub-categories of MDS patients, albeit in varying degrees, thus unifying the disparate group of diseases through biology for the first time. Since then, several additional unifying biologic features have been described and are discussed here.

### Excessive apoptosis in the myelodysplastic syndromes cell or the seed

It was recognized early through a number of sophisticated studies performed in female African-American patients heterogeneous for the enzyme G6PD that MDS is a clonal disease. Cell cycle studies using *in vivo* infusions of the thymidine analogues bromodeoxyuridine and iododeoxyuridine further demonstrated the highly expanded nature of the clone to be the result of excessive proliferation of hematopoietic progenitors in the marrow.<sup>1-3</sup> How such an expanded clone could produce peripheral cytopenias remained a mystery for some time. It was only in the 1990s when the phenomenon of programmed cell death through the peculiar mode of apoptotic cellular suicide had been described that the paradox of cytopenia in the presence of a cellular marrow of MDS patients was resolved finally. It appeared that the excessive proliferation in the marrows of MDS patients was matched by an equally excessive intramedullary apoptosis of hematopoietic cells.<sup>4-6</sup> This apoptosis was seen in all FAB categories of patients, although it was higher in those with lower risk disease and proportionately lower in patients with increasing percentage of blasts. Furthermore, it was shown that the excessive apoptosis was largely

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cytokine mediated with a number of pro-inflammatory cytokines being over-expressed in the marrows of these patients; such as tumor necrosis factor alpha (TNF $\alpha$ ), transforming growth factor beta (TGF $\beta$ ), and interleukin 1 beta (IL1 $\beta$ ).<sup>6-9</sup> A second important paradox at this point was the presence of clonal expansion and monoclonality in cells that appeared to be prone to premature 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.<sup>6</sup> The cells are stimulated further to proliferate through the effects of pro-inflammatory cytokine TNF $\alpha$ . This eventually leads to a monoclonal hematopoiesis. Interestingly, as the daughter cells mature, the very cytokines driving the proliferation of the progenitors can exert a dual action in that apoptosis is induced in the maturing progeny. The propensity to undergo apoptosis is not inherited uniformly by all the daughter cells; rather there is a spectrum of sensitivity to the pro-inflammatory cytokines. The cells most sensitive to apoptosis are the ones undergoing premature death in the marrow while those most resistant to it are the cells that actually make it out to the blood. This has been supported by showing that granulocytes in MDS patients, although clonal in nature, are more resistant to apoptosis than granulocytes obtained from normal, healthy donors.<sup>10</sup>

To summarize, it seems that an early hematopoietic progenitor develops a growth advantage for a poorly understood reason and clonal expansion results, eventually leading to monoclonality. Daughters of this transformed clone have an unequal tendency for premature apoptotic death in the presence of increased amounts of a cascade of pro-inflammatory cytokines whose master switch appears to be TNF $\alpha$ . Thus the clinical manifestations of the disease arise from the contributions of both the

cell (seed) and its microenvironment (soil). MDS must be viewed, therefore, as a disease of the seed and soil, a concept which is now gaining credence in other malignancies as well and which becomes critical when designing therapeutic approaches, especially those directed at changing the natural history of the disease. Apoptosis, therefore, was the first biologic characteristic present in all subtypes of MDS.

## Abnormal soil or a pro-inflammatory bone marrow micro-environment

While a molecular or genetic lesion can convert a hematopoietic cell into an MDS seed, it cannot thrive *in vivo* unless it is received by a complementary soil that is especially prepared to nourish it. There is ample evidence from many sources that the bone marrow microenvironment in MDS mimics a pro-inflammatory milieu with increased angiogenesis, infiltration by immune regulatory cells, and most importantly, an abundance of pro-apoptotic cytokines such as TNF $\alpha$ , IL1 $\beta$ , TGF $\beta$ , etc.<sup>6-9</sup> Once again, these changes have been described with varying intensities in all different types of MDS patients, be they low or high risk, and constitute a second unifying theme for these disorders.

## Defective biogenesis

A third unifying theme that has been discovered in the last few years relates to defective ribosomal biogenesis. This interesting story started with a study of the most common chromosomal abnormality in MDS patients that affects chromosome 5 with two regions of deletions in the long arm. Patients with 5q-syndrome have been recognized as a distinct clinical entity for 35 years. These individuals present with the more telomeric of the two deleted regions on 5q, anemia, normal or high platelet counts, no increase in blasts, a marrow filled with clusters of hypoblasted micro-megakaryocytes, rarely transform to AML, and have long survival. While the deletion on 5q can be variable in length, a 1.5MB region flanked by marker D5S413 and the gene *GLRA1*, and containing 40 genes, called the Commonly Deleted Region (CDR), is missing universally in these patients.<sup>11</sup> Not surprisingly, a search for candidate genes that could be the cause of 5q-syndrome has focused on the CDR. Two ribosomal genes, *RBM22* and *RPS14*, are located within the CDR but while no mutation in the normal allele was found, Boulwood *et al.* noted that their expression was reduced (haplo-insufficiency).<sup>11,12</sup> Ebert *et al.* used

RNAi to silence one gene at a time in the CDR in normal bone marrow CD34<sup>+</sup> cells,<sup>13</sup> and showed that it was the knock-down of a single gene, *RPS14*, which resulted in the apoptosis of erythroid cells while preserving megakaryopoiesis. Equally impressive was the finding that when the expression of *RPS14* was forced in the diseased cells of 5q-syndrome patients, it rescued the phenotype. *RPS14* is a universal structural protein of the 40S ribosomal subunit that is essential for the cleavage of the 30S precursor rRNA molecule into the 18S/18S rRNA molecule. Haplo-insufficiency of *RPS14* in MDS with del(5q) leads to impaired processing of the 18S ribosomal RNA with subsequent disruption of the small 40S subunit. Furthermore, haplo-insufficiency of *RPS14* also explains the paradox of anemia and high number of platelets in that rapidly dividing cells such as erythroid precursors apoptose with the slightest disruption in ribosomal biogenesis, causing anemia, while slowly proliferating ones like megakaryocytes could accumulate the 50% reduced dose and continue proliferation (high number of platelets). One caveat in this defective ribogenesis story is that it does not explain the initial clonal expansion, only the apoptosis in the progeny of the MDS stem cell.

More recently, expression of multiple genes associated with ribosome biogenesis was found to be decreased in CD34<sup>+</sup> cells from del(5q) patients when compared to that from refractory anemia (RA) patients with normal karyotypes, as well as with healthy controls.<sup>14</sup> Another study shows that a proportion of non-del(5q) patients have low *RPS14* expression when compared to normal controls and that these levels are similar to that of 5q-syndrome MDS patients.<sup>15</sup> These findings clearly indicate that defective ribogenesis is not limited to the 5q-syndrome MDS patients but is a more widespread finding across the various subtypes and therefore constitutes another theme that links the syndromes of MDS through biology. The generality of defective ribogenesis described here is supported by the finding of congenital anemias such as dyskeratosis congenita, cartilage-hair hypoplasia, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome.<sup>16-20</sup>

## The molecular basis of excessive apoptosis in myelodysplastic syndromes: role of *p53*

The most prominent clinical characteristic of MDS, a variable cytopenia, has been explained by a propensity of the maturing hematopoietic cells in the bone marrow to undergo premature apoptosis. While apoptosis is at least partially mediated through pro-

inflammatory cytokines found in the microenvironment, the clonal cells themselves also manifest the tendency to apoptose outside of the abnormal soil. Thus both paracrine and autocrine factors contribute to the excessive apoptosis in MDS. While the cytokines represent the paracrine influence, an interesting new genetic candidate has emerged as the autocrine mediator of apoptosis, as a result of the defective ribogenesis story. The tumor suppressor gene *p53* is well recognized as exerting nodal control over multiple pathways including apoptosis. The ribosome stress response refers to the increased expression of *p53* as a direct result of defective ribogenesis.<sup>21-23</sup> Barlow *et al.* found that elevated levels of *p53* drive the excessive apoptosis and dysplastic morphology in the erythroid compartment of mice lacking the *RPS14* gene through deletion of 5q.<sup>24</sup> The cytopenias in MDS thus may be the result of deregulated ribosomal biosynthesis leading to activation of *p53* and excessive apoptosis.

## Mutations in *TET2* in myeloid diseases

It was hypothesized that the earliest lesion that leads to the initial clonal expansion may be common across several myeloid diseases including MDS, myeloproliferative disorders (MPD), and AML. It was found that somatic mutations in the *TET2* gene were present in 15% of patients with various myeloid cancers (19% in MDS, 12% in MPD, and 24% in AML).<sup>25,26</sup> This gene is present on chromosome 4q and its precise function is unknown; however, wild-type *TET2* appears to control the balance between survival, apoptosis, and differentiation in normal hematopoiesis. In a study published in 2009, mutations or defects in gene structure were identified in 23% of MDS patients.<sup>27</sup> While patients with mutated *TET2* did not differ significantly in clinical or hematological presentation from patients with wild-type *TET2*, the five-year overall survival was 77% in mutated versus 18% in un-mutated patients, indicating that *TET2* mutations are associated with a good prognosis. A lower rate of transformation to AML in mutated patients might account for this survival advantage. In a multivariate analysis, *TET2* mutation emerged as an independent good prognostic factor while the absence of *TET2* mutation was associated with a five-fold increase in the risk of death.<sup>27</sup> Prospective studies might establish the value of *TET2* mutational status as an important parameter for prognostic stratification in MDS. Thus *TET2* mutation unifies not only the syndromes of myelodysplasia but also several myeloid malignancies.

## Uniparental disomy

Small genetic lesions such as uniparental disomy (UPD) could not be detected until high density single nucleotide polymorphism (SNP) arrays were developed. When MDS patients were studied, it was found that 10-15% of those with apparently normal karyotypes had regions of DNA that were derived from only one parent. This phenomenon has been termed UPD.<sup>28-30</sup> In addition, small genetic lesions such as amplifications and deletions, which had remained undetected by cytogenetics or FISH analyses, were also discovered. While UPDs were constitutional and not limited to the clonal cells, the amplifications and deletions turned out to be acquired. These data suggest that individuals who are born with constitutional UPDs may be predisposed toward genetic instability and an increased risk for developing MDS. Once again, this biologic feature transcends the various subtypes of MDS and is found in patients with both low and high risk disease.

## Epigenetics

Genetic mutations and structural changes are not the only causes for a cell to become cancerous. The last few decades have demonstrated the extraordinary role played by epigenetics, so that today it is believed that a mixture of both genetic and epigenetic changes participate in the transformation process.<sup>31,32</sup> Thus while epigenetic silencing of tumor suppressor genes through hypermethylation is found in a variety of cancers, epigenetics plays a more prominent role in MDS where only 10% of patients actually present with identifiable genetic lesions and ~50% respond to hypomethylating drugs. MDS is known to be a disease of the elderly, and repetitive stem cell division that occurs during aging leads to an accumulation of epigenetic damage to the DNA, making the cell susceptible to neoplastic transformation. Unlike genetic damages, it is sometimes possible to reverse epigenetic changes, be they hypermethylation or histone deacetylation. Two hypomethylating agents are FDA approved for the treatment of MDS and provide support for the contribution made by epigenetics in the pathology of these disorders.<sup>32</sup>

## Concluding remarks

### Unity despite heterogeneity

As the name itself implies, MDS is not a single disease entity but consists of a collection of syndromes that generally have been divided into those that are at high risk of transform-

ation to acute leukemia versus those that prove fatal through an increasing profundity of cytopenias. Biologic heterogeneity naturally parallels the clinical diversity. Over the years, however, the principle underlying the need to collect these syndromes under one title has been vindicated as more and more biologic features common to all types of MDS have been identified. Apoptosis, the first link in this thematic cohesion, accounts for the cytopenias that underlie the clinical manifestations of the syndromes. Paradoxically enough, this propensity to undergo a premature programmed death is not a property of the MDS progenitor, because if it were, there would be no clonal expansion to begin with. Rather, most of the apoptotic signals are mediated through the cytokines in the marrow microenvironment. It has been shown that it is only when the daughters of this MDS progenitor cell mature and begin to express the appropriate cytokine receptors that premature apoptotic death is induced in the hematopoietic cells.<sup>33</sup> 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 apoptosis did not affect earlier progenitors. Because the rate of apoptosis is inversely related to the risk of transformation, this strategy worked best in patients with lower risk MDS where anti-TNF agents like thalidomide, lenalidomide, infliximab, and etanercept have been found to be effective in improving the cytopenias in a subset of patients. It is important to note that even high risk patients have the same incidence of apoptosis in the maturing cells as lower risk patients; it is only the immature blasts that are not dying, which accounts for the lower overall incidence of apoptosis in this group.

A totally unexpected area of research has been inaugurated with the discovery of defective ribogenesis in both congenital and acquired anemias. In MDS, it was *RPS14* haplo-insufficiency that was found to be causative for the 5q- syndrome. This was quickly followed by studies identifying abnormal ribosomal gene expression in all subtypes of MDS. Defective ribogenesis has been known to cause the stress response inducing apoptosis via *p53* up-regulation. Finally, we have a molecular explanation for the autocrine-induced apoptosis in MDS clonal cells. Both *TET2* mutations and UPD are indicative of genetic instability whose presence renders the affected cell prone to both initiation as well as progression of MDS.

One of the hottest areas of research in cancer is epigenetics, which clearly affects the ability of a cell to differentiate and die. It acquired a particular significance in MDS because of the effectiveness of hypomethylat-

ing drugs in improving the cytopenias in these patients. Initially it was thought that silenced methylated tumor suppressor genes were being reactivated through hypomethylation resulting in apoptosis of clonal cells and regeneration of normal hematopoietic cells. However, despite multiple attempts to identify both specific genes targeted by the hypomethylating drugs as well as global methylation patterns, it has not been possible to correlate gene methylation status with therapeutic outcome. Nonetheless, the observation that these agents are effective in all subtypes of MDS once again underscores the biologic unity despite the clinical heterogeneity of MDS.

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