A paradigm shift in myelodysplastic syndromes

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A poorly defined transforming event(s) affects the pluripotential bone marrow (BM) stem cell in myelodysplastic syndromes (MDS), conferring a growth advantage upon it which leads eventually to monoclonal hematopoiesis. The progeny of this transformed ancestor undergo recognizable albeit dysplastic maturation. We propose that this picture is further complicated by a variety of cytokines, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and interleukin 1β (IL-1β) which exert a dual effect on the diseased cells. The immature CD34+ cells are stimulated to proliferate, while their later differentiated daughters are induced to undergo apoptosis accounting for the clinical syndrome of pancytopenia despite hypercellular BMs. Studies directed at measuring the rates of proliferation and apoptosis as well as the levels of TNF-α, TGF-β and IL-1β confirm this hypothesis and are presented in greater detail. A novel approach towards MDS therapy emerges as a result of this paradigm shift based upon the premise that anti-cytokine therapy would prevent excessive intramedullary apoptosis and result in improved cytopoenias as well as cause a slowing down of the diseased precursor cell proliferation resulting in resumption of polycyonal hematopoiesis. Because a number of cytokines function through common lipid second messengers, interruption of this pathway should theoretically cause disruption in the signalling of a cascade of cytokines.

Keywords: myelodysplastic syndromes (MDS); tumor necrosis factor alpha (TNF-α); transforming growth factor beta (TGF-β); interleukin 1β (IL-1β); apoptosis

Introduction

The myelodysplastic syndromes (MDS) are indolent clonal disorders which predominate in the elderly and which clearly involve a diseased pluripotential hematopoietic stem cell since monoclonality of at least the non-lymphoid cells is the rule.1-5 The clinical presentation poses an apparent paradox in that the majority of patients have variable cytopenias despite hypercellular marrows. Approximately 30% patients evolve into acute leukemia which is commonly of the myeloid variety, although lymphoid transformation has also been clearly, albeit rarely, demonstrated in these patients.6-10 Abnormalities of chromosomes 5 and 7 are rather common followed by those affecting chromosomes 8, 20 and 17.11-14 The only treatment option for the majority of MDS patients continues to be supportive care since various therapeutic approaches ranging from the use of vitamins and growth factors to intensive chemotherapy have failed to benefit a substantial number of patients.15-17 Given this depressing clinical scenario, efforts have been focused at understanding the biology of MDS better with the hope of developing objective and rational treatment approaches assigned to reverse the lesion(s). Our own studies combined with those of others over the last 10 years have allowed us to develop a new model for the pathogenesis of MDS which we believe constitutes a paradigm shift in these disorders. The purpose of this article is to summarize these studies and present the new paradigm.

MDS is a stem cell disease

Highly sophisticated biochemical and molecular studies have repeatedly demonstrated that the bone marrow in MDS patients is monoclonal.18-22 This monoclonality should not be confused with the monoclonality of malignancy. For example, de novo standard risk primary acute myeloid leukemia (AML) is a monoclonal disease, but here the clonality refers to only the leukemic cells or blasts, while the residual hematopoietic cells are usually not clonal.24 In MDS on the other hand, the BM consists of cells belonging to erythroid, myeloid and megakaryocytic lineages (as well as probably the B lymphocytes) at all stages of differentiation (albeit dysplastic) but all of which are the descendants of one single diseased parent stem cell. In other words, the transforming event in the stem cell conferred upon it a growth advantage so that the entire ‘feeder’ compartment of pluripotential cells was overwhelmed by the progeny of the transformed cell (see Figure 1). In this sense, MDS is closer to the chronic phase of chronic myeloid leukemia (CML) where the (normally) differentiated

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Figure 1 Killman’s model of normal hematopoiesis has been utilized to describe the paradigm shift in myelodysplastic syndromes. Note that the ‘initial event(s)’ in MDS affects a stem cell in the ‘feeder’ compartment while commonly encountered cytogenetic abnormalities affecting chromosome 5, 7, 8 etc are later evolutionary events.
erythroid, myeloid and megakaryocytic cells are all marked by the pathognomonic Philadelphia (Ph') chromosome. In CML, the initial 'event' appears to be the Ph' translocation since the BM resumes a polyclonal nature upon disappearance of this cytogenetic abnormality in response to interferon therapy. In MDS on the other hand, cytogenetic abnormalities such as those affecting chromosomes 5 or 7 appear to be epiphenomena or secondary events since they often involve a proportion of cells in a marrow which is otherwise monoclonal. In MDS therefore, the first consequence of the transforming event is monoclonality and cytogenetic abnormalities represent disease evolution while in CML, the Ph' translocation may be the 'transforming event' since there is no evidence of monoclonal hematopoiesis upon eradication of the Ph' chromosome. The model of normal hematopoiesis proposed by Killman is particularly relevant in this setting (Figure 1).

Nature of the transforming event is obscure and ambiguous in MDS

Since there are certain cytogenetic abnormalities which occur in MDS with a regular frequency, it is not unreasonable to look for genic abnormalities associated with the most commonly involved areas of the affected chromosomes. In MDS, the interstitial deletion involving 5q31.1 with its associated loss of the IRF-1 gene is one such abnormality. The 5q31.1 deletion is present in 30% MDS patients and 50% AML patients who developed leukemia secondary to a preceding myelodysplasia. Located at this site is the transcription enhancer/tumor suppressor IRF-1 gene which can also be functionally inactive due to accelerated 'exon skipping' despite the presence of a normal gene. It is also possible that exaggerated exon skipping in the allele containing the normal IRF-1 superimposed upon the deleted IRF-1 allele accounts for the evolution of MDS to AML. Conversely, overexpression of IRF-2 can suppress the effects of IRF-1. The problem with implicating the IRF-1 in the 'transforming event' is that this deletion was detected in only 20–80% of the hematopoietic cells in MDS patients correlating with the percentage of blasts rather than the 100% of the cells expected on the basis of the monoclonal nature of the disease. In other words, a genetic abnormality which only affects a proportion of the MDS cells instead of all the monoclonal cells by definition cannot be a candidate for the transforming event.

Inappropriate expression of another gene, the esotropic virus integration site 1 gene (Evi-1) associated with retrovirally induced myeloid leukemias in mice has also been associated with AML and MDS in humans. Aberrant expression of Evi-1 conferred a non-responsiveness in murine BM progenitor cells to the effects of erythropoietin, an observation particularly relevant to MDS since the erythroid cells appear to be universally most diseased in these disorders. Because abnormalities of Evi-1 are not found in all MDS patients, once again it is difficult to consider this gene as being somehow involved in the initial 'transforming event'. Other molecular lesions which have been described include mutations of ras, fms, p53 as well as deregulation of other as yet unidentified tumor suppressor genes.

In summary therefore, MDS are clearly stem cell disorders although the nature of the initial event is unknown. Given that these complex syndromes are highly heterogeneous in their clinical presentations, it is likely that the initial events will parallel this heterogeneity being different from one group of MDS patients to another, if not varying amongst individuals. Whatever the initial event is in MDS, its consequences are very well appreciated. The affected cell develops a growth advantage over its neighbors eventually leading to monoclonal hematopoiesis. Once the entire BM is descending from a single parent cell, the likelihood of additional mutations accumulating in one of its daughters is very great. Further, the evolved and additionally mutated cell will have a greater chance of survival and future proliferation in a monoclonal setting as compared to a polyclonal one because all the surrounding sister cells share in the same 'initial event' and thus are not very different from it. The expansion (and perhaps further mutations) of one such clone is probably responsible for the eventual evolution to acute leukemia. The key consequence of the stem cell defect in MDS then is monoclonality which predisposes these patients to frank malignancy. In fact, the definition of pre-leukemia should perhaps be rendered more objective by restricting the term to individuals in whom monoclonality (in the absence of a substantial number of blasts) can be documented.

What causes the clinical syndrome of MDS?

The apparent paradox of variable cytopenias in the presence of generally cellular marrows in these patients could be accounted for by increased proliferation of hematopoietic cells being cancelled by an equally increased rate of intramedullary apoptosis. Thus, despite frantic activity in the marrow compartment, the peripheral blood would remain devoid of functional hematopoietic cells. In fact, our own studies directed at measuring the in vivo rates of proliferation and programmed cell death have demonstrated the above hypothesis to be quite true. By infusing MDS patients with thymidine analogues iodo- and/or bromodeoxyuridine (IUDR, BrdU) as previously described, we found that the total cell cycle time (Tc) in 120 MDS patients was a median of 35 h while the labeling index (LI) was 26.1%. Figure 2 shows a double-labeled slide in an MDS patient where large numbers of cells can be seen synthesizing DNA. Thus, MDS are clearly actively proliferative disorders with no dearth of S-phase cells in their bone marrows. Unfortunately, the rapid proliferation is countered by rapid and excessive cell death.

![Figure 2](image-url)
Apoptosis in the BM biopsies of MDS patients was measured using the technique of in situ end labeling (ISEL) of fragmented DNA\(^{40-44}\) and found to be very high as compared to normal or AML marrows.\(^{43,44}\) Among 102 MDS patients studied by ISEL, approximately half showed 30-50% cells undergoing apoptosis in their biopsies. Figure 3 shows a typical MDS BM biopsy. Double-labeling for proliferation (IUdR/BrdU) and apoptosis (ISEL) simultaneously showed large numbers of S-phase cells to be apoptotic (Figure 4) raising the question of ‘antonymous’ death in MDS.\(^{43}\) Thus, while the BM in MDS patients is packed with proliferating cells, they are also in the process of concomitantly committing suicide. This may account for the paradox of pancytopenia despite hypercellularity of the marrow.

**Could dual-acting cytokines be responsible for the excessive cell-birth and cell-death in MDS bone marrows?**

A variety of cytokines such as tumor necrosis factor alpha (TNF-\(\alpha\)), transforming growth factor beta (TGF-\(\beta\)) and interleukin 1 beta (IL-1\(\beta\)) along with IL-1\(\beta\) converting enzyme (ICE) can have dual effects of stimulating proliferation and inducing apoptosis in hematopoietic cells.\(^{46-48}\) It is conceivable that one or more of these cytokines are causing the stimulation of early CD34\(^+\) progenitor cells in the BM while inducing apoptosis in their maturing progeny. Indeed, we have demonstrated higher than expected levels of all three cytokines in MDS patients.\(^{49}\) What is the source of these cytokines? Either they are being produced by the transformed cells themselves as a mode of autostimulation or they are being secreted by the ‘normal’ monocytes/macrophages in the body’s attempt to contain the expanding monoclonal population of transformed cells. Whatever the source, their effect is detrimental at two levels since they end up stimulating the offending premature CD34\(^+\) blasts while destroying the maturing CD34\(^-\) cells.

**A paradigm shift**

Figure 5 presents the new paradigm. A poorly defined initial event(s) leads to unrestrained growth of the abnormal cell eventually overwhelming the entire BM as demonstrated by studies of ‘monoclonality’. Presence of cytokines whose source also remains unexplained at the moment, confounds the picture further by stimulating the early precursors and destroying the later forms most likely accounting for the clinical syndrome of pancytopenia and hypercellular bone marrows. The commonly encountered cytogenetic abnormalities probably represent later evolutionary events since these are not usually present in every BM cell. When MDS patients evolve to AML, there are distinct additional events, i.e., loss of differentiation in the leukemic clone and therefore loss of apoptosis. Implicit in the above is of course the loss of cytokine-induced effects and therefore an assumption of more autonomous growth by the leukemic clone. Treatment outcome of such secondary AML patients is distinctly inferior to primary AML probably because the cell involved is decidedly more primitive in the former (belonging to the ‘feeder’ pool) as compared with the latter (belonging to the ‘committed’ myeloid progenitor).

**Novel therapies resulting from the new paradigm**

By the time MDS patients present to their physicians with the full-blown clinical syndrome, several additional abnormalities have probably already followed the initial transforming event. Attempts to reverse each of the genic events may be some
way in the future. The clinical symptoms however may be a result of the confounding actions of cytokines and therefore one could attempt to neutralize some of these effects with the expectation of clinical benefit. In the past, this approach of biotherapy in MDS has been restricted to the use of ‘viability factors’ such as interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage CSF (GM-CSF) and erythropoietin or their combinations. These trials have resulted in selective and variable benefits in a number of MDS patients.50–52 Unfortunately, the responses have been mostly transient and often the side-effects preclude prolonged trials.50–52 Chemotherapeutic trials have been equally toxic and unimpressive and could only be attempted in patients with excess blasts.15 Combinations of growth factors and chemotherapy have been tried with similar results.53 Obviously, these approaches may be refined further in the future when newer agents such as thrombopoietin and chemotherapy such as topotecan are introduced into clinical trials in MDS patients. We propose a novel approach to MDS therapy based on the new paradigm. Why not try anticytokine therapy which would neutralize the noisy cytokines thereby producing a dual effect of ameliorating cytopenias (by suppressing apoptosis) and decrease the proliferation of the transformed clone leading to resumption of polyclonality? What are the candidate agents in this area?

First, a cascade of cytokines including TNF-α, TGF-β and IL-1β act via the phosphatidic acid (PA) → diacylglycerol (DAG) lipid signalling pathway.54–59 Interruption of this second messenger system by drugs such as pentoxifylline57 and ciprofloxacin58 or lisophylline59 could be tried, Second, specific inhibition of individual cytokines could be attempted by use of soluble TNF-α receptor or IL-1β receptor antagonists given to patients in vivo. Finally, a combined ‘multi-modality’ approach using viability factors and chemotherapy alternating with the anticytokine approach may be a far more valuable strategy than using any one of the above alone. The idea is that a whole new area of clinical trials has been opened up by the construction of this new paradigm. Further therapeutic advances will occur only if we do not throw the baby out with the bath water and make sure that parallel detailed biological studies are conducted with the trial of every new agent(s) in order to define the reasons for response and non-response.

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