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Myeloid Malignancies and the Marrow Microenvironment: Some Recent Studies in Patients with MDS

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Abstract

There is growing evidence for a role of the hemopoietic microenvironment in the pathophysiology of myelodysplastic syndromes (MDS). Effects of various cytokines on the marrow microenvironment of patients with MDS have been studied. Autoimmunity, i.e. a reaction of autologous T lymphocytes against components of the marrow is also operative in a proportion of patients with MDS. The negative feed-back loop that controls tumor necrosis factor (TNF) α levels in healthy individuals is apparently disrupted in MDS due to auto-amplification signals involving TNF α and interleukin (IL-32). IL-32 mRNA levels in primary adherent cells from patients with MDS are 14- to 17-fold higher than in controls. In contrast, cells from patients with chronic myelomonocytic leukemia (CMML), a myeloproliferative disorder with low TNF α levels, express IL-32 at only 1/10 the level observed in controls. Damage in the microenvironment may occur secondary to oxidative stress, which may also lead to accelerated shortening of telomeres. This is, indeed, true for hematopoietic cells in MDS marrow, but telomere length in marrow stroma does not differ from that in age-matched healthy individuals. Nevertheless, the stroma shows functional aberrancies. Stroma-derived signals facilitate apoptosis in clonal hematopoietic cells but not in normal CD34+ cells. Thus while stroma dysfunction is likely due to signals derived from the hematopoietic clone rather than being intrinsic, it does affect clonal death or survival, respectively. Therefore, signals exchanged between both compartments could serve as targets for therapeutic interventions.

Keywords

Marrow stroma; MDS; Telomere; IL-32

INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal diseases of hematopoietic stem/precursor cells. The etiology and pathophysiology of those disorders are areas of intense investigation.

MDS is characterized by pancytopenia resulting from ineffective hematopoiesis; the bone marrow is typically hypercellular. Arrested or disordered maturation occurs in one or more cellular lineages. Approximately one-third of MDS patients ultimately develop acute myeloid leukemia (AML). The incidence of MDS increases with age (median age at diagnosis 70 years or older), and is higher in males than females. There are more than 10,000 new cases of MDS in the United States each year. MDS has been considered a cell autonomous disorder. However,

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as the regulation of hematopoiesis is largely dependent upon the function of the microenvironment, questions have been raised in regards to a role of the microenvironment (including the stroma) in the pathophysiology of MDS.

Several non-transplant therapies induce clinically useful responses in a proportion of patients with MDS, but to achieve treatment success more predictably, a better understanding of the pathophysiology is needed. Insights into the disease mechanism(s) should allow for the identification of targets for specific therapeutic interventions. A prominent feature of early/low grade MDS is excessive programmed cell death (apoptosis), while advanced MDS is characterized by apoptosis resistance. Numerous studies have shown abnormal expression of cytokines and apoptosis regulating signals in the marrow microenvironment of patients with MDS. The fact that MDS progresses to acute myeloid leukemia in a proportion of MDS patients renders MDS a useful model for studying the potential contributions of the hemopoietic microenvironment to leukemic transformation. Recent investigations have also raised questions in regards to cell migration patterns in the marrow of patients with myeloid disorders. We will review some aspects of the role of the microenvironment in inducing and sustaining hematologic malignancies.

The Role of the Microenvironment in Hematologic Malignancies

Extensive research has revealed that the microenvironment plays a role in sustaining hematologic malignancies [1,2]. In many cases this effect is related to a reversible functional disturbance caused by interactions of the neoplastic clone with the stromal components. For example, in myeloma, the neoplastic plasma cells communicate with the environment through cell/cell contact as well as cytokines to induce functional changes that support the malignant population [3]. The recognition of this dysregulation has led to successful therapeutic targeting of aberrant signaling in the microenvironment with drugs such as thalidomide and lenalidomide [4].

In other disorders such as chronic myeloid leukemia (CML), clonally derived cells that normally are part of the microenvironment, such as macrophages, interact abnormally with other components of the environment to induce functional disturbances that result in a survival advantage of the malignant clone [5]. Results of marrow transplantation in animals and humans have clearly demonstrated that the components of the microenvironment that are derived from hematopoietic precursors are replaced by cells of donor origin while stromal cells remain of host origin [6–10]. Also, the fact that stem cell transplantation is a curative procedure for hematopoietic neoplasms suggests that, in the majority of cases, defects in the microenvironment that contribute to the pathogenesis/pathophysiology of hematologic malignancies are functional in nature and reversible.

Until recently, there has been little evidence to support the role of primary stromal abnormalities in the pathogenesis of hematologic neoplasms. A few reports have suggested chromosomal abnormalities in stromal cells in patients with MDS [11,12]. However, only recently has there been definitive evidence based on studies in mouse models that primary stroma abnormalities can induce hematologic neoplasia, i.e. evidence for a malignancy inducing microenvironment.

TNF α and IL-32 Cross-talks in the Marrow Microenvironment

The marrow microenvironment is composed of a complex network of cells and extracellular matrix that cooperate to regulate normal hematopoiesis. Two basic mechanisms could explain the role of microenvironmental defects in the evolution of hematopoietic neoplasms. There are substantial data to support the first mechanism, in which the malignant hematopoietic clone induces reversible functional changes in the microenvironment that result in improved growth

conditions for the malignant cells. We previously characterized gene expression changes that occurred in the stroma cell lines, HS5 and HS27a, derived from normal marrow in response to TNF α exposure [13], known to be up-regulated in the bone marrow of patients with MDS [14]. We devised an *in vitro* model to characterize the impact of the microenvironment on normal and MDS-derived hematopoietic cells in the presence and absence of (exogenous) tumor necrosis factor (TNF) α . We and others have used the leukemia-derived cell lines, KG1a and ML1, as models of apoptosis resistant and apoptosis sensitive clones, respectively [15]. Previous experiments showed that contact of stroma and hematopoietic cells was required for TNF α to trigger apoptosis in hematopoietic cells. Our data are in agreement with this observation in that non-adherent KG1a cells became apoptotic only if contact with stroma took place during or exposure to TNF α . Since one of the genes upregulated in stroma by TNF α was interleukin (IL)-32, we were interested in a potential contribution of this cytokine to dysregulation of apoptosis. Goda *et al.* had shown that upregulation of IL-32 induced apoptosis, whereas down-regulation resulted in proliferation [16], a pattern similar to that observed by us in stroma cells particularly from patients with low-grade/early-stage MDS (high levels of IL-32/apoptosis) and chronic myelomonocytic leukemia (CMML) (low levels of IL-32/no apoptosis), respectively. These results are not in conflict with the concept that stroma contact protects leukemic cells [17]. In fact, KG1a cells remaining in stroma contact also remained viable; however, the data indicate that the final outcome was dependent upon the milieu in which the interactions occurred.

To further define the mechanism that resulted in lower rates of apoptosis in KG1a cells co-cultured with HS5 stroma cells in which we inhibited IL-32 expression by RNA interference, we also determined patterns of other cytokines that are dysregulated in the marrow of patients with MDS at various disease stages [18–20]. For example, Bellami *et al.* [21] suggested that neutralization of vascular endothelial growth factor (VEGF) led to suppression of transcription of TNF α . Such a model is supported by our data, which show that silencing of IL-32 in stroma resulted in a significant decrease in the secretion of VEGF. In primary stroma (and hematopoietic) cells from patients with CMML, lower steady state levels of IL-32 mRNA, as expected, were associated with lower levels of TNF α and VEGF production. Molnar *et al.* [22], also showed lower levels of TNF α and VEGF in the marrow of CMML patients as compared to healthy controls and patients with other subtypes of MDS, consistent with the hypothesis of an autoamplification loop of TNF α and IL 32. IL-32 was initially reported as a cDNA without known function(s) in IL-2 activated NK and T cells [23], both cell types involved in the pathophysiology of MDS [24]. Immunosuppressive therapy (e.g. with anti-thymocyte globulin) or immunomodulation (e.g. with thalidomide or lenalidomide, which interferes with T- and NK cell function) have shown therapeutic benefits in some patients with MDS, but not with CMML [25].

The *in vivo* relevance of IL-32 dysregulation for MDS remains to be proven; it may provide an important link between the immune system (both innate [NK] and adaptive [T cells]) and dysregulated hematopoiesis [14], and between hematopoietic cells and the microenvironment. Intriguing in this context are observations by Roth *et al.*, who showed that phenotypic and functional maturation of NK cells required intimate interactions with stroma cells [26].

These findings provide evidence for a role of IL-32 in the pathophysiology of clonal myeloid diseases and provide objective molecular data for a distinction between CMML and MDS, supporting the reclassification of CMML as a distinct disorder [27].

Hematopoietic Components of the Microenvironment Dysregulate Stromal Function

Hematologic malignancies have been linked in several reports to an abnormal progenitor stem cell or leukemic stem cell. Thus, they generate clonally derived progeny, including monocytes/macrophages that can lead to a dysfunctional microenvironment. This has been shown in both

AML and CML. Work by Mayani and colleagues first suggested that macrophages may play a role in the functional abnormalities seen in stroma from patients with AML [12,28]. They showed that there was no difference between the supportive capacity of pure marrow fibroblast layers from patients with AML and healthy controls. However, when stromal layers containing both fibroblasts and macrophages were compared, the healthy controls were able to support hematopoiesis better. Mayani and colleagues concluded that macrophages derived from the leukemic clone were contributing to the abnormal microenvironment [12,28]. Bhatia *et al.* showed that stroma derived from patients with CML did not provide optimal support for normal hematopoietic cells [5]. In contrast, growth of CML cells on CML-derived stroma was significantly better, suggesting that the microenvironment in CML was more supportive for the malignant clone. Using fluorescent activated cell sorting (FACS) and fluorescent in situ hybridization (FISH), stromal macrophages were shown to be *bcr-abl* positive and were directly responsible for the selective advantage of clonal *bcr-abl* cells to proliferate through a contact dependent mechanism [5].

A similar scenario may exist in MDS. Using FISH in MDS patients with cytogenetic markers, we determined that the percentage of clonally marked monocytes closely approximates the percentage of abnormal cells on routine marrow cytogenetics [29]. We have also determined that these cells contribute to the high levels of TNF α in the microenvironment [24]. Furthermore, the clonally derived MDS monocytes respond abnormally to stromal signals. For example, MDS monocytes fail to upregulate matrix metalloproteinase (MMP)9 expression when exposed to stromal signals [29]. The inducible MMP9 levels were inversely correlated with marrow cellularity. MMP9 has been implicated in the cleavage of stroma derived factor (SDF)1 from the microenvironment and may facilitate the egress of hematopoietic cells from the marrow to the peripheral blood [30,31]. Based on our data, one could speculate that lack of inducible MMP9 levels in MDS monocytes could contribute to the hypercellularity often seen in this disease. In MPD, clonally derived megakaryocytes and macrophages are thought to play a central role in the pathogenesis of the fibrotic reaction by secreting cytokines such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and transforming growth factor (TGF) β [32,33].

Primary Stromal Defects Can Induce Hematologic Disease

Until recently, little was known about the potential of primary microenvironmental defects in the induction of hematopoietic diseases. There have been some reports of chromosomal abnormalities noted in stromal cells from patients with hematologic neoplasms, and in some cases, the same abnormality was found in both the stroma and the malignant clone [11,12]. However, these studies must be interpreted with caution as they did not fully account for clonal macrophage contamination, which can be present in fibroblast cultures [34]. There have also been numerous conflicting reports on stromal function as either being normal or abnormal *in vitro*. The fact that hematopoietic cell transplantation is curative in many of these disorders suggests that underlying stromal integrity is normal. However, there have been reports of patients who are unable to achieve engraftment despite numerous attempts [35] and cases of donor cell-derived leukemia [36–38], and one may speculate that these patients represent groups that do indeed have an underlying stromal defect.

Only in the past few years, compelling evidence from mouse models has been presented showing that primary stroma abnormalities can induce a malignancy. In an elegant approach Rupec *et al.* showed that, conditional loss of I κ B α , the inhibitor of NF κ B, resulted in a disorder similar to CMML with components of MDS and myeloproliferative neoplasms, which resulted in the death of mice within a week of birth. These findings could not be replicated when I κ B α was conditionally deleted in just the myeloid population; thus, constitutive activation of NF κ B in myeloid cells did not lead to malignancy. It was not clear from that report whether

the loss of I κ B α was necessary in both the microenvironment and stem cell compartments to develop disease. A follow-up paper reported by the same group addressed this matter in a more compelling way. They reported that deletion of the Retinoic Acid Receptor γ (RAR γ) in mice resulted in a myeloproliferative disorder which continued for the entire lifespan of the mice. Secondary transplant studies revealed that RAR γ negative hematopoietic cells functioned normally when transplanted into normal mice. However, transplantation of normal hematopoietic cells into the RAR γ -microenvironment resulted in a myeloproliferative disorder in the transplanted cells. TNF α was implicated in the pathogenesis of this myeloproliferative neoplasm as the disorder was partially abrogated when TNF α null stem cells were transplanted into the RAR γ -microenvironment [2]. These studies demonstrated that a single microenvironmental defect was sufficient to generate a myeloproliferative disorder, supporting once again our previous findings [39], which show that abnormal expression of IL-32 in stroma cells could lead to protection of leukemic cells after co-culture.

Other Markers of Stroma Abnormality: No Evidence of Telomere Shortening in Marrow Stroma in MDS

Previous reports showed telomere shortening in hematopoietic cells derived from MDS patients [40–44], possibly related to the effect of Reactive Oxygen Species (ROS). In an attempt to identify potential effects of ROS on stroma, we examined telomere length in stroma. We compared telomere length in 38 patients with MDS to 13 age-matched controls by analyzing telomere-to-centromere ratios and telomere-to-DNA ratio, with both approaches produced nearly identical results [45]. Telomere length in MDS patients (all stages of the disease) measured by q-FISH was not significantly different from that in age-matched controls ($p=0.39$). A second technique, quantitative polymerase chain reaction (q-PCR), also failed to show a statistically significant difference of telomere lengths between these two groups ($p=0.47$). Telomere lengths progressively declined with age by approximately 1%/year in both MDS and control groups. The slopes of the regressions were not statistically significantly different ($p=0.99$, interaction test). These findings do, of course, not exclude a role of the stroma in the pathophysiology of MDS, but they argue against enhanced turn-over or genetic instability in those cells; they do not appear to be subject to increased proliferation or oxidative stress that is thought to underlay the telomere attrition of MDS hematopoietic cells [42].

SUMMARY

Evidence from the last few decades has implicated abnormalities of the marrow microenvironment in the pathophysiology of hematologic malignancies. The abnormalities in the microenvironment were thought to be generated via interactions with the clonal hematologic disorder and that underlying stromal function was normal. Thus in general, treatment strategies have been focused on the eradication of the stem or progenitor cell from which the malignancy arose. However, the bone marrow microenvironment facilitates the survival, differentiation, and proliferation of hematopoietic cells, and serves as a safe haven not only for normal but also malignant hematopoietic cells, thereby offering protection of malignant cells against chemotherapeutic agents. However, recent data indicate that dependent upon the overall milieu in the marrow, stroma may also convey signals that enhance apoptosis in otherwise resistant leukemic cells. This function of stromal cells may be triggered by defects within hematopoietic progenitors that lead to abnormalities in cell-cell interactions, cell signaling or cell cycling.

SCIENTIFIC SECTION: HEMATOLOGIC MALIGNANCIES

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