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A Novel Role for the Marrow Microenvironment in Initiating and Sustaining Hematopoietic Disease

Aravind Ramakrishnan, MD and **H. Joachim Deeg, MD**
Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA, 98109

1. Abstract

The marrow microenvironment is composed of a complex network of cells and extra cellular matrix that cooperate to regulate normal hematopoiesis. There is growing evidence that microenvironmental defects can contribute to the pathogenesis of hematologic malignancies. Two basic mechanisms could explain the role of microenvironmental defects in the evolution of hematopoietic neoplasms. There is significant 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. More recent studies from mouse models have indicated that a second mechanism involving primary microenvironmental defects can also result in malignancy. We will review the role of the microenvironment in inducing and sustaining hematologic malignancies.

Keywords

hematologic malignancy; hematopoiesis; marrow microenvironment; stromal cells

2. Introduction

Hematologic malignancies are thought to arise from a series of genetic abnormalities in a stem or progenitor cell that lead to uncontrolled growth. Although the primary defect leading to disease is thought to occur in a stem or progenitor cell, data from the past few decades have implicated the marrow microenvironment in the pathogenesis of these neoplasms.

The marrow microenvironment consists of a complex structure of both non-hematopoietic and hematopoietic cells, extracellular matrix as well as soluble and membrane bound factors that cooperate to support normal hematopoiesis. It was known as early as the 1960s, based on experiments with the SL/SLd (“steel” mutant) and W/Wv (white spotted mutant) mice that normal hematopoiesis could not occur without a supportive environment.[1] The SL/SLd mouse develops severe anemia due to a lack of kit-ligand on stroma cells and cannot be cured by hematopoietic stem cell transplantation. However, normal hematopoiesis can be restored by implanting a normal spleen in the peritoneum.[2, 3] In contrast, the W/Wv mouse, which has a similar phenotype, is deficient in c-kit on hematopoietic stem cells and can be cured by stem cell transplantation from a normal donor.[4, 5] We also know from studies in patients with Hodgkin disease that heavily irradiated areas of the skeleton may no longer be

Corresponding author: H. Joachim Deeg, Member, Clinical Research Division, Fred Hutchinson Cancer Research Center, Professor, University of Washington School of Medicine, 1100 Fairview Avenue N., D1-100, P.O. Box 19024, Seattle, Washington 98109-1024, United States, Phone: (206) 667-5985, Fax: (206) 667-6124, jdeeg@fhcrc.org.

able to support hematopoiesis.[6] These early studies revealed the crucial role of the microenvironment in normal hematopoiesis.

In vitro studies of the microenvironment over the last several decades have mostly relied on the long-term marrow culture system, first reported by Dexter.[7] When cells from marrow aspirates are cultured under appropriate conditions, a complex adherent layer of stromal cells forms at the bottom of the flask that recapitulates the in vivo microenvironment and is able to support hematopoiesis for many weeks.[7, 8] The adherent stromal layer is composed of fibroblast-like cells, adipocytes, endothelial cells, macrophages, lymphocytes, osteoclasts and extracellular matrix.[9, 10] Although osteoblasts are not always considered as part of the adherent stromal layer of the Dexter culture, they are found in the medullary cavity, and recent studies have revealed that these cells also play a critical role in supporting hematopoiesis.[11–14] This in vitro system has allowed for the dissection of the components of the microenvironment and the study of the complex contact dependent and contact independent interactions that occur between the stromal compartment and hematopoietic stem cells that regulate stem cell fate decisions.[15]

3. The Role of the Microenvironment in Hematologic Malignancies

Extensive research has revealed that the microenvironment also plays a prominent role in sustaining hematologic malignancies.[16, 17] In many cases this is 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 cytokines to induce functional changes that support the malignant population.[18] The recognition of this dysregulation has led to successful therapeutic targeting of such aberrant signaling in the microenvironment with various drugs including thalidomide and lenalidomide.[19]

In other disorders such as chronic myeloid leukemia (CML), clonally derived hematopoietic cells that normally are part of the microenvironment, e.g. macrophages, interact abnormally with other components of the environment to induce functional disturbances that result in a survival advantage of the malignant clone.[20] 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.[21–24] 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 disturbances that are reversible.

Until recently, there has been little evidence to support the role of primary stromal abnormalities in the pathogenesis of hematologic neoplasms. There are a few reports of chromosomal abnormalities in stromal cells in patients with myelodysplasia (MDS).[25–27] However, only recently has there been definitive evidence based on studies in mouse models that abnormal primary stroma function can induce hematologic neoplasia, i.e. evidence for a malignancy-inducing microenvironment.

4. Interactions of Neoplastic Cells with Stromal Elements Can Alter the Microenvironment to Sustain Malignant Cells

The best studied example of this form of microenvironmental dysregulation is multiple myeloma. The pathophysiology of myeloma is determined not only by genetic abnormalities that occur in the clonal plasma cells, but also by the bidirectional complex interactions with the bone microenvironment that lead to the development of skeletal lesions.

Myeloma cells can adhere directly to stroma or extracellular matrix through interactions with numerous adhesion molecules such as VLA-4 and ICAM-1.[28, 29] These interactions lead to the activation of PI-3 kinase/Akt and NF-κB pathways.[30–32] The net result of activation of these pathways is an increase in expression of genes involved in cell proliferation and anti-apoptotic pathways. These interactions also trigger the stromal components to release a variety of cytokines such as IL-6, IGF, IL-1, SDF-1, VEGF,[33, 34] TNFα, and RANKL to name a few.[19, 29, 33–35] These factors alter the bone marrow cytokine milieu in the direction of being more supportive to the neoplastic cells. Thus, a paracrine loop is established between the microenvironment and myeloma cells.[18, 19, 29, 33–35]

The interaction between myeloma cells and stroma is also thought to play a role in the uncoupling between bone formation and bone resorption leading to the development of lytic lesions. The balance between bone formation and resorption is controlled in large part by the balance of two molecules, RANKL and osteoprotegrin (OPG).[36–38] RANKL activates osteoclastogenesis, whereas OPG functions as a decoy and inhibits the formation of osteoclasts. Wnt signaling regulates the differential activation of these molecules.[39] It has recently been shown that myeloma cells inhibit Wnt activation in the microenvironment by the release of soluble DKK1, which causes an increase in the concentrations of RANKL and a decrease of OPG production. This results in increased activation of osteoclasts and bone destruction.[40] Thus, the microenvironmental dysregulation that occurs in myeloma plays a critical role in the formation of lytic lesions.

This type of functional microenvironmental dysregulation can also be found in myeloproliferative disorders (MPD) and MDS. In chronic idiopathic myelofibrosis (CIMF), the malignant clone induces a polyclonal fibrotic reaction of the stroma that plays a critical role in the pathophysiology of the disease.[41] The mechanism that is currently proposed to explain this scenario is that clonal hematopoietic cells, mainly megakaryocytes and monocyte/macrophages, secrete cytokines that induce a polyclonal stromal reaction that leads to fibrosis. The stromal elements are not part of the malignant clone.[42] Mouse models have implicated elevated levels of TPO, TGF-β, and low levels of the transcription factor GATA-1 in the pathogenesis of myelofibrosis.[42–46] In contrast to myeloma, high levels of stromal-derived OPG have been implicated in osteosclerosis.[47] A similar situation is found in MDS. Studies from our Center have implicated elevated TNFα levels in the microenvironment and increased apoptosis in the marrow.[48, 49] Engraftment of clonal MDS-derived precursors in NOD/SCID was also dependent on stromal support.[50]

Thus, in a variety of diseases, stroma function is altered by signals from the malignant clone, which apparently serve to support propagation of the clone. It is also evident that agents that target the microenvironmental dysregulation can be very effective in the treatment of these disorders. For example, the immunomodulatory (Imid) family of agents has significant activity in all of these diseases and highlights the importance of targeting the microenvironment.[51, 52] Other recent findings have led to the identification of new potential targets for therapy in myeloma such as RANKL,[53] Wnt signaling and IGF signaling,[19] TNFα signaling in MDS,[54] and TGFα and PDGF signaling in MPD.[42] An improved understanding of microenvironmental dysregulation should identify other potentially effective targets for treatment.

5. Hematopoietic Components of the Microenvironment Dysregulate Stromal Function

Many hematologic malignancies are derived from an abnormal stem cell. Thus, they generate clonally-derived progeny including monocytes/macrophages that can lead to a

dysfunctional microenvironment. This was first demonstrated in CML. Bhatia et al. showed that stroma derived from patients with CML did not provide optimal support for normal hematopoietic cells.[20] 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 hybridiziation (FISH), it was determined that stromal macrophages were all bcr-abl positive and were directly responsible for the selective advantage of clonal bcr-abl cells to proliferate through a contact-dependent mechanism.[20]

A similar scenario exists in MDS and MPD. 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. We have also determined that these cells contribute to the high levels of TNFα in the microenvironment.[55] Furthermore, the clonally-derived MDS monocytes respond abnormally to stromal signals. For example, MDS monocytes fail to upregulate MMP9 expression when exposed to stromal signals.[56] The inducible MMP9 levels were inversely correlated with marrow cellularity. MMP9 has been implicated in the cleavage of SDF1 from the microenvironment and may facilitate the egress of hematopoietic cells from the marrow to the peripheral blood.[57, 58] 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 principle role in the pathogenesis of the fibrotic reaction by secreting cytokines such as PDGF, FGF and TGFα.[41, 42]

6. 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.[25, 59] However, these studies must be interpreted with caution as they did not fully account for clonal macrophage contamination, which can often resemble fibroblasts in cultures.[60] 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 the disorders under discussion indicates that intrinsic stroma function is intact or that alterations are reversible in the majority of patients. However, there have been reports of patients who are unable to achieve engraftment despite numerous attempts at stem cell transplantation[61] as well as cases of donor cell-derived leukemia,[62–64] and one may speculate that these patients represent groups that do indeed have an underlying stromal defect.

Only in the past few years, three mouse models have been described which show that primary stroma abnormalities can induce a malignancy in the hematopoietic compartment. In the first model, conditional loss of IκBα, the inhibitor of NFκB, resulted in a disorder similar to chronic myelomonocytic leukemia (CMML) with components of MDS and MPD, which in turn resulted in the death of mice within a week of birth. These findings could not be replicated when IκBα was conditionally deleted in just the myeloid population; thus, constitutive activation of NFκB in myeloid cells did not lead to malignancy. However, it was not clear from this study whether the loss of IκBα was necessary in both the microenvironment and stem cell compartments to develop the disease.[65]

Simlarly, Walkely et al. demonstrated that conditional deletion of the Retinoblastoma gene (RB) resulted in a myeloproliferative disorder in mice. They also showed that this was a

result of interactions between myeloid cells and the microenvironment. The defect had to be present in both hematopoietic cells and the microenvironment to initiate disease.[66]

The final model, reported by the same group, may be the most compelling. In this report, deletion of the Retinoic Acid Receptor γ (RARγ) in mice resulted in a chronic myeloproliferative disorder. Transplant studies revealed that RARγ-hematopoietic cells functioned normally when transplanted into normal mice. However, transplantation of normal hematopoietic cells into the RARγ-microenvironment resulted in a myeloproliferative disorder in the transplanted cells. TNFα was implicated in the pathogenesis of this MPD as the disorder was partially abrogated when TNFα null stem cells were transplanted into the RARγ-microenvironment.[67] These studies therefore showed that a single microenvironmental defect was sufficient to generate a myeloproliferative disorder.

7. EXPERT OPINION

Evidence from research conducted over the last few decades has clearly implicated abnormalities of the marrow microenvironment in the pathophysiology of hematologic malignancies. In the past, 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, recent evidence suggests that focusing therapeutic strategies on the microenvironmental abnormalities can be extremely effective. The Imid family of agents has changed the treatment paradigm in diseases such as myeloma and MDS and highlighted the importance of targeting the microenvironment.[51, 68] It may also very well be that other agents such as hypomethylating agents that have activity in a number of myeloid disorders act through modifying the microenvironment. In the solid tumor literature, tumor associated stroma can acquire aberrant methylation patterns due either to direct contact with or via factors secreted by the malignant cells.[69, 70] Thus, in diseases such as MDS, hypomethylating agents may impart their beneficial effects in part by acting on the stroma as well as the malignant clone. This may partially explain why responses to hypomethylating agents are not always correlated with reactivation of tumor suppressor genes in hematopoietic cells.[71, 72] Further studies focusing on the stroma will be necessary to answer these questions.

The fact that there are components of the microenvironment that are derived from the hematopoietic clone does have therapeutic implications in the setting of stem cell transplantation. With the recent advances in reduced-intensity conditioning regimens, older patients are able to undergo transplant with low-intensity conditioning regimens.[73] These regimens may not be effective at eradicating the abnormal macrophages, and the new stem cells may arrive in an environment that still provides an advantage to the malignant clone. This may partially explain the higher rate of relapses in patients undergoing stem cell transplants with low-intensity conditioning regimens for diseases such as MDS.[74–76] Further research is needed to determine if targeting these hematopoietic-derived microenvironmental components during conditioning will benefit transplant outcomes.

Also of interest are the recent reports of abnormalities in the stroma that lead to malignancies of the hematopoietic compartment. Although historically, hematologic malignancies are thought to arise from a stem or progenitor cell abnormality, there may be groups of patients that have a primary stromal defect leading to the hematologic malignancy. Mouse models have implicated stromal abnormalities in RARγ, RB and IκBα in the development of MPDs. Further research is necessary in patients with MPDs to determine if

these genes are dysregulated in the stromal compartment. One may also speculate that there will be patients with abnormalities in both the stem cell and stromal compartments.

If primary stromal defects are identified in humans and implicated in the initiation of malignancy, this clearly will have great impact on the treatment strategies offered to patients. These patients would not be good candidates for allogeneic hematopoietic stem cell transplantation as this modality cannot correct a stromal abnormality; instead, therapies targeted at correcting the underlying stromal compartment would be necessary. Only further study of the stromal compartment in patients with these disorders will reveal whether primary stromal abnormalities exist.

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