A Workshop on the Marrow Microenvironment and Hematological Malignancy

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Abstract

The Division of Cancer Biology of the National Cancer Institute hosted a workshop on hematological malignancies and the marrow microenvironment in Rockville, Maryland in the fall of 2002. There were 22 invited participants from the United States, Canada, and Europe, and the workshop was organized into disease-specific sessions. The sessions were designed to explore the basic science and therapeutic applications related to the stromal and nonstromal components of the marrow in malignant hematological diseases.

Introduction

Hematological malignancies are clonal disorders resulting from the neoplastic transformation of progenitor cells. Similar to their normal counterparts, transformed hematological progenitor cells remain dependent on signals from the microenvironment for survival and proliferation during their malignant progression. These cells can also induce reversible changes in the marrow stroma that will further foster the development of malignant cells. This dynamic reciprocal interaction between the microenvironment and the malignant hematological cells continues throughout disease progression.

Malignant progression of tumor cells is driven by intrinsic events such as activation of oncogenes, loss of tumor suppressor genes, and maintenance of telomere length and function. Although the intrinsic cellular defects causing malignant transformation have been investigated extensively, the factors in the microenvironment enhancing tumorigenesis have not been clearly defined. They are, however, increasingly recognized as critical factors for the tumorigenic process. The stromal cells in the adjacent tissue must provide a supporting microenvironment for the survival and proliferation of the malignant transformed cells in the early stage of tumorigenesis.

The Division of Cancer Biology of the National Cancer Institute sponsored a recent workshop in Bethesda, Maryland on September 23–25, 2002 to address the role of the microenvironment in hematological malignancy in a focused manner. The workshop was organized into several sessions focused on particular hematological malignant diseases.

MM

MM affected 14,400 new patients in the United States in 2001, with 50,000 total patients, and the disease remains incurable despite conventional high-dose chemotherapy. K. Anderson (Dana-Farber Cancer Institute, Boston, MA) presented a novel therapy to improve patient outcome based on targeting the MM cell as well as its BM microenvironment. In vitro experimental and in vivo animal models were used to characterize mechanisms of MM cell homing to BM as well as marrow stromal cell interactions (e.g., cytokines, angiogenesis) promoting MM cell growth, survival, drug resistance, and migration in the BM microenvironment. Several promising biologically based therapies can target the MM BM microenvironment and thereby overcome classical drug resistance in vitro. Thalidomide and its more potent immunomodulatory analogues, proteasome inhibitor PS-341, and As203 have inhibited human MM cell growth in vitro, decreased angiogenesis, and prolonged host survival in vivo SCID mice. These laboratory studies have already been translated into Phase I and II clinical trials to evaluate their clinical utility and toxicity. Immuno-modulatory analogues and PS-341 demonstrated a marked clinical anti-MM activity even in patients with refractory relapsed MM.

One of the characteristic signs of MM is bone lysis. An experimental model has been developed for studying this bone lysis by growing human primary MM cells in a human bone microenvironment in SCID mice (J. Epstein, University of Arkansas Medical School, Little Rock, AR). MM growth was associated with increased OC numbers and a marked reduction in osteoblasts, resulting in bone lysis. This suggests that MM cells proliferated in association with OCs. Inhibition of OC activity is associated with increased MM cell apoptosis and reduction in tumor burden. In the cellular interaction between MM and OCs, IL-6 and osteopontin appear to be important cytokines in cancer-related bone manifestations. Both cytokines are highly produced by OCs, and IL-6 is known to be a growth factor for MM. IL-6 production was induced in the coculture with OCs and MM. Because the neutralizing antibodies specific to osteopontin and IL-6 reduced the number of viable MM, both cytokines are required for survival and proliferation of MM cells in the microenvironment of bone.

The roles of adhesion molecules (e.g., VLA5, VLA4, and CD44) and the receptors for SDF-1, CXCR4 in MM were presented (Y. Gazit, University of Texas Health Sciences Center, San Antonio, TX). The expression of VLA4, VLA5, and CXCR4 on CD38+CD138+ MM cells was compared with that in peripheral blood stem cells collected from MM patients. A 2-fold decrease in the expression of adhesion molecules and a 3-fold decrease in the expression of CXCR4 concomitant with a >5-fold decrease in plasma SDF-1 was found. Similarly, in a mouse xenograft model, human MM cells adhered to mouse stromal cells via VLA5 molecules, and this interaction resulted in tumor formation and bone destruction in these animals. Tumor formation and bone destruction could be enhanced by MIP1α and substantially decreased in mice implanted with MM cells stably transfected with MIP1α antisense constructs that decreased VLA5 expression.

In addition to the positive effect of the BM stromal cells on MM cell survival and growth (L. Hazlehurst, H. Lee Moffitt Cancer Center, Tampa, FL), MM cells’ adherence to fibronectin in the extracellular matrix may facilitate the development of drug resistance. Cell adhesion confers resistance to melphalan-induced apoptosis in pri-
Lymphocytic Leukemia and Lymphoma

CLL cells accumulate in BM over many years because these malignant B cells do not die like their normal counterparts. The role of T cells and accessory cells in the survival of CLL cells has been investigated (F. Caligaris-Capio, University of Torino, Torino, Italy). Survivin, a member of the inhibitors of the apoptosis family of proteins, was addressed in these studies. Survivin-positive CLL cells had an increased proliferation and extended survival in vitro. Resting CLL cells do not express survivin, but survivin expression is induced by activated CD4+ T cells via CD40 ligand-CD40 interaction. T-cell-attracting chemokines CCL17 and CCL22 are involved in the cellular interaction between CLL cells and T cells.

Leukemia cells appear to participate in building a microenvironment advantageous to the proliferation of ALL cells. Time-lapse video microscopy revealed that leukemia cells in Matrigel migrate toward established capillary-like structures of BMECs (A. Cardoso, Dana-Farber Cancer Institute). Interestingly, the leukemia milieu promoted the differentiation of mesenchymal stem cells into stromal-like cells. Both leukemia-stimulated BMECs and leukemia-differentiated mesenchymal stem cells promoted leukemia cell survival. These observations suggest that leukemia may recruit and stimulate other cells from the BM microenvironment, which provide survival signals to the leukemic cells. In vivo experiments such as Matrigel plug assays and xenografts in NOD/SCID mice showed that the recruitment of BMECs by ALL was accompanied by intense neovascularization.

The role of increased BM vascularization in the microenvironment in the development of childhood ALL was studied by analyzing the relationship between bFGF levels and usual indicators of prognosis in a series of 23 pediatric patients (P. Schneider, University Hospital of Rouen, Rouen, France). The urinary levels of bFGF are elevated >10-fold in children with leukemia versus controls. These results suggest that angiogenesis plays an important role in ALL, as shown by an elevated average level of angiogenic cytokines.

The survival mechanism of ALL cells in the BM microenvironment was studied in vitro using two established cell lines isolated from pre-B-cell ALL (T. LeBien, University of Minnesota, Minneapolis, MN). These cell lines require the presence of the stromal cells to survive. In their absence, these cell lines die within 24–48 h. The cell death was characterized by mitochondrial degradation associated with apoptosis, and the earliest detectable event was caspase 2 activation. Interestingly, inhibitors of caspase 9 promoted apoptosis of these cells after removal from stromal cells. The potentiation of cell death by caspase 9 inhibitors may be a physiological corollary of endogenous inhibition of caspase 9. The consequence of this inhibition would then be activation of a compensatory death pathway that amplifies the death of these cells after withdrawal from microenvironmental signals.

The survival of T-ALL in the BM microenvironment was investigated because ex vivo survival of lymphoblasts on BM stromal cells predicted a more favorable outcome for children with T-ALL (R. Larson, University of New Mexico, Albuquerque, NM). LFA-1 and intercellular adhesion molecule 1 were required but not sufficient for ex vivo leukemic cell survival on stromal cells. Gene microarray studies to identify genes responsible for survival were presented from 21 primary T-ALL patients.

The effects of stromal cell on the development of resistance to apoptosis induced by chemotherapeutic drugs in B-lineage acute lymphoblastic leukemia cells was investigated by L. Gibson (West Virginia University, Morgantown, WV). The efficiency of chemotherapy in B-lineage leukemic cell lines was compared in the presence or absence of BM stromal cells. JM-1, SUP-1315, and RS4 B-lineage leukemic cell lines cleaved Bcl-2 to its 23-kDa form when exposed to either 1-β-d-arabinofuranosylcytosine or VP-16. Coculture of leukemic cells with BM stromal cells during treatment resulted in reduced levels of cleaved Bcl-2 protein, consistent with enhanced survival. Stromal cells engineered to express high levels of human vascular cell adhesion molecule 1 were more effective than matched controls in reducing both caspase 3 activation and Bcl-2 cleavage during 1-β-d-arabinofuranosylcytosine or VP-16 exposure. These observations suggest that the BM microenvironment has a protective effect on leukemic cells by inducing resistance to drug-induced apoptosis.

Finally, there is evidence that stromal cells themselves have mutations in the p53 gene in new and relapsed patients with ALL (A. Narendran, Hospital for Sick Children, Toronto, ON, Canada). This mutation in p53 was associated with increased secretion of VEGF by these cells. Thus, mutations in the microenvironment stromal cells themselves can induce a microenvironment conducive to leukemic cell growth, survival, and resistance to treatment.

B-cell lymphoma is one of the most common B-cell malignancies in the United States. Almost all B-cell lymphomas originate in the peripheral lymphoid follicles including the GC. The GC is a specialized microenvironment where B-cell lymphomas originate through somatic mutation and translocation. FDCs are stromal cells located in the GC derived from non-BM origin. The generation and blast transformation of B-cell lymphoma occur in close contact with FDCs, which is analogous to MM in BM. In the early stage of malignant transformation, lymphoma cells require FDCs for survival and blast transformation.

Recently, the FDC signaling molecules for GC B-cell growth have been identified (Y. S. Choi, Ochsner Clinic Foundation, New Orleans, LA). A novel protein of 232 amino acids, termed 8D6, is a growth factor for GC B cells and lymphoma cells of GC origin because a monoclonal antibody specific to 8D6 inhibits the proliferation of these cells. More importantly, 8D6 protein synergizes with CD44 protein of FDCs to stimulate cellular proliferation. The combination of monoclonal antibodies specific to these two proteins inhibited the growth of the Burkitt lymphoma cell line L3055 in vitro and lymphoma formation in vivo explants in nude mice. Furthermore, FDCs were shown to produce antiapoptotic factor to prevent apoptosis of L3055 (L. Li, Ochsner Clinic Foundation). L3055 cells of GC origin require FDCs for survival and growth. L3055 cells express BAFF receptors. BAFF prevented the induction of apoptosis in the absence of FDCs. These data indicate the critical role of FDC signaling molecules in B-cell lymphomagenesis in the lymphoid follicles. C. Ambrose (Biogen) has reviewed the recent discovery of antiapoptotic factors BAFF/Blys and APRIL. Their interaction with three receptors (BAFFR, TAC1, and BCMA) was discussed. These novel cytokines of tumor necrosis factor family are not usually produced by B or T cells, but by macrophages and dendritic cells. Apparently, FDCs also produce them.

Myeloid Leukemias

The role of the BM microenvironment in CML was also discussed. CML progenitor cells have reduced integrin-mediated adhesion and migration in response to SDF-1. These cells fail to respond to integrin-mediated inhibition of proliferation. The underlying mechanism of these abnormal properties was investigated by transducing Bcr/Abl genes into hematopoietic progenitor cells (R. Bhatia, City of Hope MARROW MICROENVIRONMENT AND HEMATOLOGICAL MALIGNANCY

mary MM cells by reducing DNA damage. The data show that β1 integrin-mediated cell adhesion represents a unique form of de novo resistance distinct from drug exposure-acquired resistance, suggesting potential new therapeutic approaches to overcome this form of drug resistance.
Normal human CD34+ progenitor cells transduced with wild-type Bcr/Abl-containing vectors demonstrated increased proliferation, reduced adhesion, abnormal mobility on fibronectin, and reduced fibronectin-mediated proliferation inhibition compared with cells transduced with control vectors. These abnormalities in growth regulation are similar to those seen in malignant progenitors from CML patients. Introduction of a kinase-inactivated Bcr/Abl dominant negative gene reversed abnormal proliferation, but only partially reversed defects in integrin function.

MMPs and natural tissue inhibitors of metalloproteinases regulate not only the turnover of the extracellular matrix in CML and AML but also regulate proliferation, adhesion, and migration (A. Janowska-Wieczorek, University of Alberta, Alberta, Canada). Normal unstimulated marrow stem/progenitor (CD34+) cells do not secrete MMPs, but precursor cells of various lineages (normal CFU-GM, BFU-E, and CFU-Meg-derived cells) secrete pro-MMP-9, and normal stromal cells secrete pro-MMP-2. On the other hand, AML blasts express both pro-MMP-9 and pro-MMP-2, as well as MT-MMPs (MT1- MT2-, MT4-, MT5- and MT6-MMP). Primary human Bcr-Ab1 (CML) cells and murine FL5,12 cells transfected with Bcr/Ab1 gene exhibited not only high secretion of VEGF but also MMPs, and had high angiogenic potential in vivo. This suggests that MMPs were constituents of the marrow microenvironment that might be involved in intercellular cross-talk in hematopoiesis. Stimulation of angiogenesis by angiogenic factors including MMPs may play an important role in the pathogenesis of CML.

The effect of AML cells on T-cell function in the microenvironment was addressed (A. Buggins, Kings College of Medicine and Dentistry, London, United Kingdom). The tissue culture supernatants from AML cells inhibited T-cell activation and Th-1 cytokine production, preventing activated T cells from entering the cell cycle. AML tissue culture supernatants contained none of the immunosuppressors described to date (e.g., gangliosides, nitric oxide, TGFβ, IL-10, VEGF, or prostaglandins). The secretion of AML factors into the leukemic microenvironment may promote disease progression with the most marked effect in patients with a high disease burden, as opposed to minimal residual disease.

Cell Biology of the Microenvironment

The role of cell surface shedding in intercellular signaling was investigated (N. Dainiak, Bridgeport Hospital, Yale University, New Haven, CT). Signaling molecules are released in the extracellular microenvironment on the surface of plasma membrane-derived SVs. These SVs are enriched in selected proteins and contain greater levels of cholesterol and sphingomyelin than the parent membrane. Factors released in SVs include Fas, macrophage colony-stimulating factor, transforming growth factor β, and FLT-3 ligand. Because the release of proapoptotic and growth-promoting factors on SVs may afford cells the opportunity for long-range communication, these SVs could play a role in signaling in the microenvironment through exfoliation from leukemic cells or from stromal cell membranes.

Physiological genomics is required to characterize changes in gene expression related to alterations in cell function at the single cell level in stroma from both normal individuals and leukemic patients. B. Seshi (H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL) reported the feasibility of isolating single marrow stromal cells selected on the basis of morphology by laser microcapture/microdissection, isolating the RNA, and analyzing the transcriptome with a gene chip consisting of 12,625 probe sets. Using this technology, it was possible to identify a putative mesenchymal progenitor cell that simultaneously expresses transcripts for osteoblasts, fibroblasts, muscle cells, and adipocytes. This technology should be useful in determining the changes in specific stromal cells associated with different hematological malignancies.

A strategy for immortalization of BM-derived mesenchymal cells was presented (D. Campana, St. Jude’s Research Hospital, Memphis, TN). The mesenchymal cell lines were generated by transducing primary human BM mesenchymal cells with human TERT. Transduced cells expressed TERT protein and showed increased telomerase activity. TERT+ mesenchymal cells proliferated at a rate similar to that of primary mesenchymal cells and could differentiate into osteoblasts and chondrocytes. Because their capacity to support leukemic cells and cord blood CD34+ cells was equal to that of primary mesenchymal cells, these cell lines can be used to investigate the role of stromal cells in the microenvironment during the progression of various human hematological malignancies.

Conclusion

This workshop covered a broad spectrum of hematological malignancies such as MM, CLL, CML, ALL, and lymphomas. Understanding the role of the microenvironment in the development and progression of hematological malignancies has implications for understanding drug resistance and improving diagnosis and therapy. This will require increasing amounts of research in several important areas: (a) obtaining functional definitions of stromal cells and immunocytes involved in disease progression; (b) identification of the signaling molecules involved in the cellular interactions; (c) gathering proteomic and genomic data from the microenvironment; and (d) discovering potential therapeutic targets in the microenvironment. Ultimately, this research will lead to development of experimental models that will allow an in-depth understanding of the role of the microenvironment in the progression of hematological malignancies. An increased understanding of the role of the microenvironment in disease pathogenesis will help identify new therapeutic targets. Therapies targeting both the tumor cell and its microenvironment offer great potential to overcome conventional drug resistance and improve patient outcome.
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