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THE ROLE OF THE TUMOR MICROENVIRONMENT IN HEMATOLOGICAL MALIGNANCIES AND IMPLICATION FOR THERAPY

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1. ABSTRACT

The tumor microenvironment is essential for tumor cell proliferation, angiogenesis, invasion, and metastasis by providing survival signals and a sanctuary site for tumor cells, by secretion of growth factors, pro-angiogenesis factors and direct adhesion molecule interactions. Our knowledge of microenvironment is only now beginning to unfold. In this review, the morphological and molecular characteristics of microenvironment in various hematological malignancies including acute lymphoblastic leukemia, acute myeloid leukemia, myelodysplastic syndrome, lymphoma, chronic lymphocytic leukemia, and multiple myeloma are summarized and the molecular mechanisms of microenvironment contributing to leukemogenesis are elucidated. We also aim to discuss the encouraging preclinical and clinical trials for treatment of hematological malignancies by targeting the tumor microenvironment. Further understanding of the signal transduction pathways between tumor cells and microenvironment will lead to the development of novel targeted therapeutic agents and more effective combination of current drugs for fighting hematological malignancies.

2. INTRODUCTION

In hematological cancer research, the focus on oncogenes and tumor suppressor genes, together with the emergence of powerful biotechnology tools such as microarray, has led to a better understanding of the mechanisms of disease and the discovery of novel targeted therapy (1-3). In contrast, knowledge of tumor cell and host interaction has not been well established. The tumor microenvironment is apparently distinct from that of normal tissue with the characteristic of disordered neovascularization (angiogensis) (4-7).

The role of microenvironment in hematological malignancies has been explored for almost one decade, but the literature is often conflicting and still controversial. In this review, we aim to summarize our current understanding of the cellular and molecular pathophysiology of tumor microenvironment in various hematological malignancies and the development of novel strategies for treatment targeted on tumor microenvironment.

3. PATHOGENESIS OF MICROENVIRONMENT IN HEMATOLOGICAL MALIGNANCIES

3.1 Acute leukemia and myelodysplastic syndrome

3.1.1. Acute lymphoblastic leukemia

It was initially felt that angiogenesis was important for solid tumor development but would not be of such importance in the leukemias. However, in childhood

Table 1. Direct receptor and ligand interaction between leukemic cell and microenvironment.

Disease	Receptor	Ligand	Method	References
B-lineage ALL	VLA-4, VLA-5, CD44	Fibronectin	Immunogold label/ Electron microscopy	16
B-lineage ALL	VLA-4	VCAM-1	Flow cytometry	17
T-ALL	LFA-1	ICAM-1	Flow cytometry	18
AML	VLA-4	Fibronectin	Mouse model/ Flow cytometry	36
CLL	VLA-4, LFA-1	VCAM-1, ICAM-1	Flow cytometry	42

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; VLA: very late antigen; VCAM-1: vascular cell adhesion molecule-1; LFA-1: lymphocyte function-associated antigen-1; ICAM-1: intercellular adhesion molecule-1.

acute lymphoblastic leukemia (ALL), the median microvessels density (MVD) of bone marrow (BM) in ALL patients is significant higher than that seen in the control group when a computer-aided three-dimensional model is used to analyze BM biopsies stained with anti-factor VIII-related antigen (anti-FVIIIrAg), CD31 and CD34. Urinary basic fibroblast growth factor (bFGF), a potent angiogenic factor, is also decreased at the completion of induction therapy (8). The appearance that MVD (hot spot) is high in ALL at presentation and drops to normal at remission has been confirmed by other studies (9,10). To date there has been no association reported between MVD and poor prognosis.

It is also appreciated that the microenvironment plays not only an important role in B and T cell development (11), but also provides survival signals to B-lineage and T leukemic cells (12). Coculture of allogeneic bone marrow-derived stromal layers can inhibit apoptosis in B-lineage leukemic cells in vitro (13,14). Patients with high cell recovery after coculture have four year event-free survival (EFS) of 50 ± 9%, compared to 94 ± 6% in patients with low cell recovery (15).

The precise mechanism of BM microenvironment supporting leukemic cell survival remains elusive. Amongst four main types of BM stromal cells (BMSCs) including fibroblasts, macrophages, adipocytes, and endothelial cells, fibroblasts are identified as the main functional cells which alone provide equal or better survival rate for leukemic B-lineage cells (16). It is generally agreed that direct contact between the microenvironment and leukemic cells is essential, but investigators have not reached a consistent conclusion about the key molecules in the receptor-ligand interaction, including very late antigen (VLA)-4 and VLA-5, CD44, fibronectin (16), vascular cell adhesion molecule-1 (VCAM-1) (17), lymphocyte function-associated antigen-1 (LFA-1), intercellular adhesion molecule-1 (ICAM-1) (18), and possible LFA-3 (19) (table 1). Activation of phosphatidylinositol-3-kinase (PI-3K)/Akt-Bcl2 appears to be an indispensable pathway induced by microenvironment and leukemic cell interaction (20-22). These data demonstrate that the bone marrow microenvironment provides a sanctuary site for leukemic cells and probably contributes to residual disease and reoccurrence of leukemia. Therefore interference in interactions between microenvironment and leukemic cells are an attractive target for novel antileukemic agents.

Recent research on Notch signaling has yielded particularly exciting results. There are two families of Notch ligands–Jagged1, 2 and Delta1, 3, 4. Jagged1 is the dominant Notch ligand expressed on primary BMSCs. Activation of Notch signaling by Jagged1 promotes hematopoietic cells self-renew (23-25). Gain-of-function mutations have been found in about half of human T-ALL (26) and inhibition of Notch signaling using γ-secretase inhibitor are being investigated in a phase I clinical trial for the treatment of patients with T-ALL.

3.1.2. Acute myeloid leukemia and myelodysplastic syndrome

There are ample evidences for BM microenvironment involvement in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Compared to normal controls, MVDs are also significantly higher in BM biopsies from untreated AML adults (27-29) and restored to normal level after achieving complete remission (CR) (27,29). There is a positive correlation between vessel scores and percentage of marrow blasts in AML, but no difference between MVD and French-American-British (FAB) subtypes has been observed (28,29).

bFGF is found to be significantly increased in BM biopsies from AML patients compared to controls, but the expression is not correlated with BM MVD (30). Coculture of AML cell lines with bFGF reveals a dose-dependent increase in proliferation and colony formation of leukemic cells. These data indicates that bFGF autocrine stimulation may have an important role in the pathogenesis of AML. These stimulation effects could be abrogated by the addition of a polyclonal anti-bFGF antibody.

Two studies compared MVD in different types of hematologic malignances (9,31). They reported that MVD in MDS is significantly higher than in controls and infectious disease, but lower than in ALL, AML, myeloproliferative disorder (MPD), and chronic myelogenous leukemia (CML). Interestingly, MVD in MDS is intermediate in levels between normal controls and AML, suggesting that the microenvironment is associated with leukemia progression. Plasma levels of vascular endothelial growth factor (VEGF), bFGF, hepatocyte growth factor (HGF), and tumor necrosis factor (TNF)-alpha in various leukemias and MDS are significantly higher than in normal controls.

Coculture of stromal cells with CD34+ AML cells upregulates the anti-apoptotic proteins Bcl2 and

prevents apoptosis induced by Ara-C (32). However, this association between inhibition of apoptosis and elevated Bcl2 has not been confirmed by another study (33). A number of studies have suggested a possible paracrine pathway between VEGF and its receptors - fms-like tyrosine kinase-1 (FLT1, VEGFR-1) and KDR (VEGFR-2) in AML. Reverse transcriptase-polymerase chain reaction (RT-PCR) detects VEGF transcript in most of patients with *de novo* AML or with secondary AML. FLT1 and KDR expression is detected in about half and one fourth of patients with *de novo* AML or with secondary AML, respectively (34). Another study (35) observed that VEGF and KDR, but not FLT1, are highly expressed in BM biopsies from patients with newly diagnosed AML compared to control patients. Furthermore, the expression of VEGF and KDR is positively associated with BM MVD (VEGF: P =0.024; KDR: P =0.040). In BM obtained in CR, KDR expression returns to normal. Together, these data suggest that KDR would be an attractive target of novel small molecule inhibitors in AML.

AML cell lines cultured on fibronectin-coated plates have higher viability than on BSA- and VCAM-1-coated plates, even with treatment of daunorubicin (DNR) or cytosine arabinoside (Ara-C) (36). VLA-4-specific blocking antibody decreases the cell viability, but VLA-5-specific blocking antibody has no effect. These results imply that the interaction between VLA-4 and fibronectin is necessary for the survival of AML cells. Phosphorylation of PI-3K occurrs 5 minutes after the contact of VLA-4 and fibronectin, followed by phosphorylation of AKT and high expression of Bcl-2. Patients expressing VLA-4 on AML cells have a worse prognosis than patients without expression of VLA-4 (5 year-EFS: 44.4% vs 100%, P =0.0094). In a mouse model, combination therapy of anti-VLA-4 antibody and Ara-C significantly improves survival rate compared to mice treated with Ara-C alone. This study suggests that VLA-4 can be utilized as surrogate marker for monitoring clinical outcome and that combination of anti-VLA-4-specific antibodies with chemotherapy may be effective for elimination of minimal residual disease (MRD) in patients with AML (36).

3.2. Lymphoma and chronic lymphocytic leukemia

3.2.1. Lymphoma

Compared to the large number of studies in acute leukemias, less work has focused on the role of microenvironment in non-Hodgkin's lymphomas (NHL). Immunohistochemistry and morphometric analysis reveal that MVD and macrophage counts are higher in B-cell NHLs than in benign lymphadenopathy (37), and high grade NHL had higher levels than those seen in low grade NHL. In agreement with the increase of MVD, elevation of VEGF and bFGF predicts poor survival rate in NHL (38). The ultrastructural patterns of neovascularization is different in low and high grade B-NHL, with fusions between endothelial and tumor cells closer in high grade NHL (39,40). Taken together, the progress of neovascularization formed by tumor cells and stromal cells from benign, low to high grade B-NHL indicates that tumor microenvironment has an important impact on tumor proliferation, angiogenesis, invasion, and progression.

3.2.2. Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) cells cultured on BMSCs *in vitro* have prolonged survival; otherwise, these cells rapidly progress to apoptosis without direct contact, suggesting an important role for stromal cells in CLL cell survival. (41). Further research showed that the number of CLL cells directly adhesive to stromal cells is higher than that seen for normal cells. Blocking antibodies against ICAM-1 and VCAM-1 inhibits adhesion, indicating a major role for the beta 1 and beta 2-integrins VLA-4 and LFA-1 and their ligands VCAM-1 and ICAM-1 in the interaction between B-CLL cells and stromal cells. Adhesion induces high expression of Bcl2 and prevents apoptosis of CLL cells (42,43). A small proportion of blood cells with stromal cells markers were identified from CLL patients, which were termed "nurse-like cells". These cells secrete stromal cell-derived factor-1 (SDF-1), preventing spontaneous apoptosis of CLL cells. The activation of CLL cells via SDF-1 may represent a new mechanism of microenvironment favoring CLL cell survival (44). To mimic CLL cell-microenvironment interactions *in vitro*, cocultured CLL B cells with a follicular dendritic cell line (HK) show protection against spontaneous apoptosis of CLL cells. The antiapoptotic Bcl2 family protein Mcl-1 is upregulated by direct contact between CLL cells and HK. This protection could be interfered by neutralizing antibody against CD44, but not by antibodies against CD40, ICAM-1 and VCAM-1 (45).

The chemokine receptor CXCR3 is expressed on all CLL cells, partially on other B-cell malignancies, but is absent in normal CD5+ or CD5- B cells (46). The CXCR3 ligand, Mig, is also coexpressed in the majority of CLL patients, but less frequently in other B-cell malignancies (47). In addition to CXCR3, CXCR4 and 5 are also abundantly found in CLL and mantle cell lymphoma (MCL) (48). Because Mig is expressed mainly in stromal cells, it is suggested that microenvironment might selectively attract and home CLL cells through an array of specific chemokine receptors.

In vitro data on CD40 stimulation pointed out that microenvironment factors might stimulate CLL cell upregulated survivin expression, which downregulated apoptosis (49,50). Not only survivin is more intensively expressed on CLL cells, less intensive on ALL cells and a few on normal controls, but also the subcellular localization of survivin is different between ALL and CLL (51).

3.3. Multiple Myeloma

Multiple myeloma (MM) originates from a postswitch memory B cell or plasmacell (PC) and evolves through different stages, from monoclonal gammopathy of undetermined significance (MGUS) to aggressive plasmablastic MM. Since the accumulation of these slow proliferative malignant cells takes place almost exclusively in the BM, the microenvironment can play a critical role for the tumor clone evolution through signals that are still incompletely understood. However, considerable work highlighting the importance of the microenvironment has been performed in MM. Candidates for survival and proliferation of myeloma cells are direct physical contact of

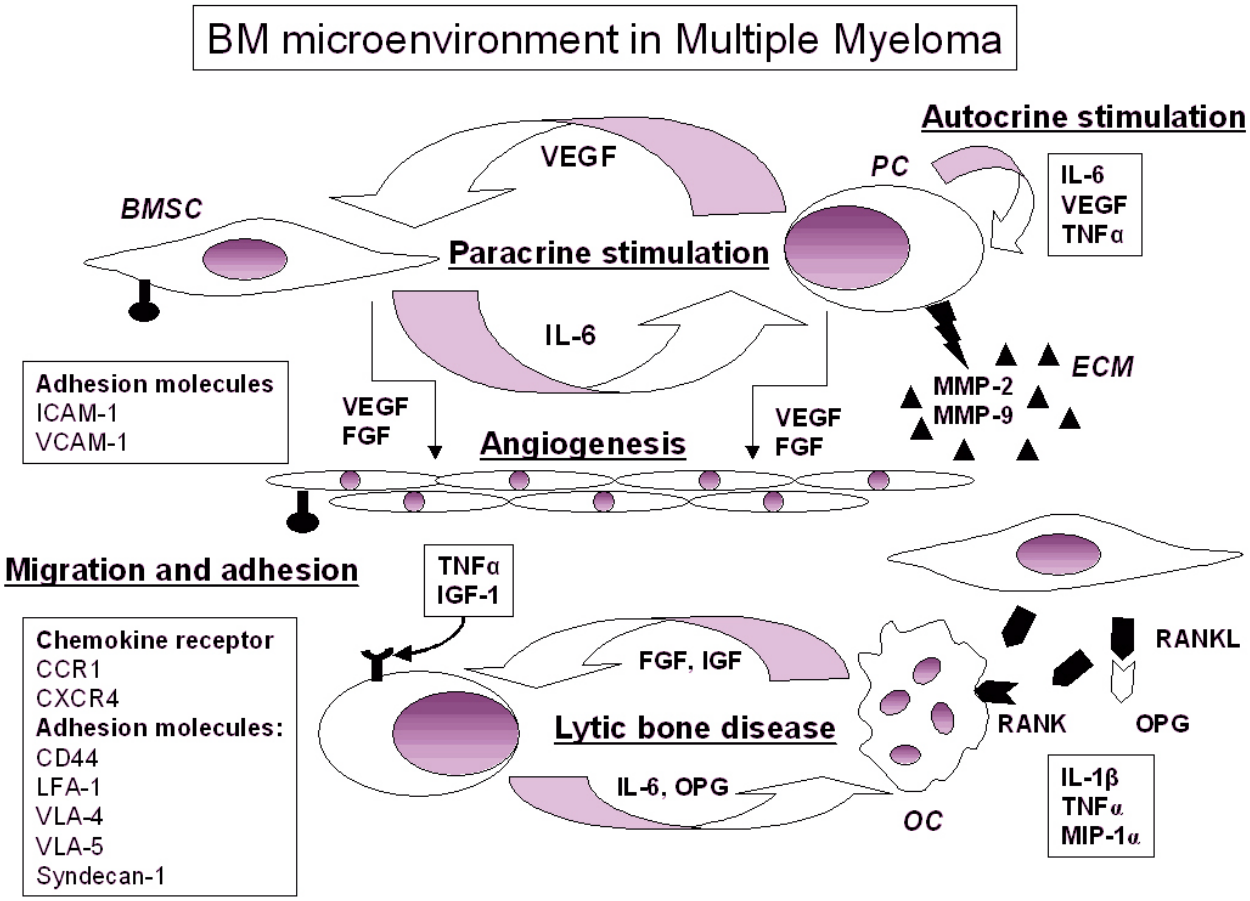

Figure 1. Role of BM microenvironment in multiple myeloma pathogenesis. BMSC: bone marrow stromal cell; PC: plasmacell; ECM: extracellular matrix; OC: osteoclast.

tumor cells with BMSCs and the extracellular matrix (ECM) as well as soluble factors, such as chemokines, growth factors, and cytokines (figure 1).

Primarily, the selective homing of MM cells to the BM compartment involves chemokines and their receptors. Two of these receptors, CCR1 and CXCR4, have been found to be present on the tumor cell surface, but not on normal PCs (48,52). CXCR4 and its ligand, the chemokine SDF-1, are essential for myelopoiesis and B lymphopoiesis, by confining precursors within the BM microenvironment (53). Although mature forms of B cells express CXCR4, they do not migrate towards SDF-1 (54). In contrast, primary MM cells have a functional chemokine receptor, which promotes the migration of tumor cells (52).

After migration, MM cells can survive and proliferate in the BM microenvironment through their interactions with BMSCs and ECM, mediated by several adhesion molecules, including CD44, VLA-4, VLA-5, LFA-1 (CD11a), and syndecan-1 (CD138) (55). By binding MM cells to BMSCs, adhesion molecules can modulate the secretion of cytokines involved in tumor cell proliferation. Indeed, interleukin-6 (IL-6) production by BMSCs is induced by malignant PCs through activation of nuclear-transcription-factor (NF)-kappa B and requires cell-to-cell interaction mediated by VLA-4, VLA-5, LFA-1, and CD44 (56, 57). It has been recently demonstrated that IL-6 upregulates CD44 cell surface expression and modulates the alternative splicing of CD44 mRNA, leading to a vicious circle (58). Furthermore, cell surface molecules are often polarized on MM cells membrane favoring the proteolytic activity of malignant cells on the ECM, and providing a mechanism of tumor cells invasion (59). In particular, syndecan-1, whose expression correlates with a worse prognosis, increases adhesion of MM cells to collagen, promoting bone resorption and tumor invasion (60). Some of the adhesion molecules are also able to potentiate the biological activity of growth factors, such as insulin-like growth factor-1 (IGF-1) (61), HGF (62), FGF (63), by sequestering them in their site of secretion and thus favoring autocrine and paracrine activity.

IGF-1, which is a potent growth and survival factor for MM cells, acts through PI-3K and mitogen-activated protein kinase (MAPK) signaling pathways (64). IGF-1 stimulates adhesion and migration of MM cells and this function is potentiated by a transient association of IGF-1 receptor with VLA-4 (65). HGF-1 was found in the conditioned medium of MM cell lines and freshly isolated

PCs (66). Its biological effects are transduced via the transmembrane tyrosine kinase Met. Uncontrolled activation of Met is oncogenic and has been implicated in growth, invasion and metastasis of a variety of tumors (67). Indeed, HGF-1 has been demonstrated to be a potent MM growth factor, inducing proliferation and inhibition of MM cell apoptosis via MAPK and PI-3K signaling (68). HGF-1 signals are amplified by syndecan-1, which binds HGF-1 and acts as a functional coreceptor (62).

FGF and VEGF are the major regulators of myeloma-associated angiogenesis and they promote tumor growth, invasion and metastasis (69,70). Signaling of FGF is mediated by a family of tyrosine kinase receptors that require the interaction with syndecan-1 on MM cells (63,71). High level of expression of one of these receptors, FGFR-3, has been observed in patients carrying the t(4;14) translocation (72). VEGF acts via Flt-1 phosphorylation (73); both FGF and VEGF activate the extracellular signal-regulated kinase (ERK) and PI-3K signaling cascades (74,75). FGF and VEGF-receptors (FGFR and VEGFR) are predominant on endothelial cells in malignant tissue. Through their receptors, FGF and VEGF promote proliferation and inhibit apoptosis of endothelial cells (69). Moreover, FGFR and VEGFR are expressed on MM cell lines and patients cells, suggesting an autocrine loop, that can be a target of therapeutic intervention (75,76). In MM, these growth factors are produced by tumor cells as well as BMSCs and their expression is regulated by several molecules such as IL-1beta, IL-6, platelet-derived growth factor (PDGF), transforming growth factor (TGF) beta, IGF-1 and TNF-alpha (70,76). On the other side, VEGF and FGF induce an increase in IL-6 secretion by MM cells and BMSCs (76, 77). MM cells also release matrix-metalloproteinase-2 and 9 (MMP-2 and MMP-9), which degrade the ECM, promoting angiogenesis (69). Levels of FGF and VEGF in the lysates of patient MM cells as well as MMP mRNA expression increase according to disease stage. Indeed, BM angiogenesis, measured by MVD, increases with disease progression, from MGUS to advanced MM, and it is considered to be an adverse prognostic factor correlated with a worse EFS and overall survival (78). The observation that MM patients with deletion of chromosome 13q have a high MVD supports this finding (79). Moreover, to confirm the role of these growth factors, high serum levels of VEGF, FGF and HGF were reported to be associated with unfavorable prognosis in MM patients. In particular, increased levels of VEGF in BM compared to peripheral blood (PB) have been observed in MM patients and, in both compartments, its levels were increased compared to normal controls (80).

Among cytokines, IL-6 is the major survival and growth factor for MM cells both *in vitro* and *in vivo* (81); it prevents apoptosis induced by serum starvation, dexamethasone and Fas (82,83). IL-6 triggers at least 2 intracellular signaling pathways, which in turn activate the NF-kappa B/the janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) and the Ras/MAPK pathways. Activation of the Ras/MAPK signaling cascade correlates with the proliferative response of MM cells to IL-6, while the STAT3 pathway provides an important anti-apoptotic signal in tumor cells (84,85). Although MM cells can secrete IL-6, BMSCs are the main source of this cytokine. Malignant PCs secrete cytokines, such as TNF-alpha, TGF-beta, VEGF and IL-1 beta, which further upregulate IL-6 secretion from BMSC (77, 86). MM cells express IL-6 receptor and serum levels of IL-6 and soluble IL-6 receptor are correlated with disease activity (87). In support of this findings, it has been shown that anti-IL-6 monoclonal antibodies inhibit MM cell growth (88). IL-6 interacts also with ECM, binding selectively on various glycosaminoglycans. For example, hayluronan promotes the accumulation of IL-6 in culture medium, favoring its autocrine and paracrine activity. This interaction is probably important to promote the tumor cell resistance against chemotherapeutic agents (89).

The accumulation of MM cells in the BM leads to an increased activity of osteoclasts (OCs), resulting in lytic bone disease in $\geq$ 80% of patients. Since in MM patients, osteoblasts, usually involved in OCs formation, are decreased, it is likely that tumor cells are the major inducers of OCs via osteoclastogenic factors production (90). TNF-alpha is one of the cytokines involved in lytic bone disease. Both MM cells and BMSCs secrete TNF-alpha and high level of this cytokine has been found in patients with bone disease (91). Although the direct effect of TNF-alpha on MM cells proliferation is modest, it promotes survival and growth of tumor cells indirectly, enhancing the paracrine IL-6 transcription and secretion and the expression of adhesion molecules on both MM and BMSCs via NF-kappa B activation (92).

OCs express RANK, a member of the TNF receptor superfamily. After activation of RANK by its ligand, RANKL, differentiation, proliferation and survival of preosteoclasts are enhanced, OCs activation is promoted while OCs apoptosis is suppressed (93,94). The expression of RANKL in MM cells has been controversial. Its main source appears to be BMSCs, and its secretion is induced by direct cell-to-cell contact with MM cells (95). Furthermore, osteoprotegerin (OPG), a soluble decoy receptor of the TNF receptor family, which binds to RANKL to prevent its interaction with RANK has been found to be decreased in MM patients (96). The low levels of OPG are due to the inhibition of gene expression and protein secretion by MM cells and BMSCs and to the binding of OPG to syndecan-1 on tumor cells (97). OCs also support long-term survival and proliferation of PCs, not only by soluble factors production but also by cell-to-cell contact, maintaining a vicious circle with tumor cells (98). Other inflammatory cytokines are involved in the pathogenesis of myeloma bone disease with a RANKL-dependent pathway (IL-1beta, IL-6) (87) or RANKL-independent pathway (macrophage inflammatory protein MIP-1alpha) (99).

Recently, gene expression profiling studies have identified several genes that discriminate normal and malignant PCs. Many of them are involved in adhesion, apoptosis, signaling and transcription. The expression pattern distinguished 4 different subgroups of MM, which are in agreement with the disease stages (100). Microarray

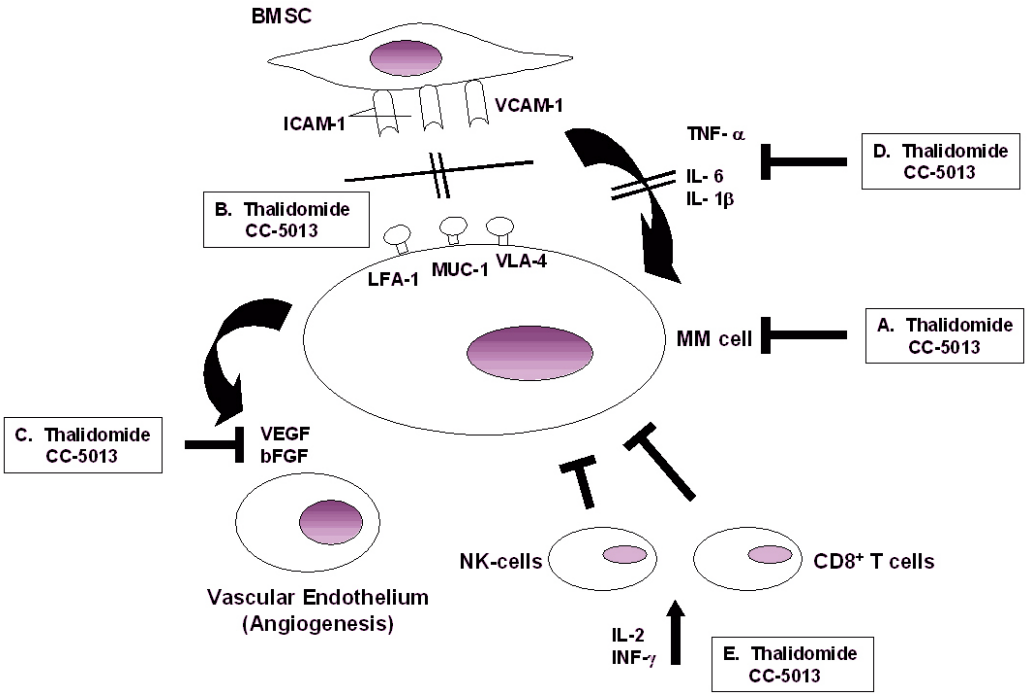

Figure 2. Mechanisms of action of thalidomide and CC-5013 targeting MM cells and the BM microenvironment.(A) MM cell apoptosis and G1 growth arrest. (B) Decreasing MM – BMSC interaction. (C) Inhibiting angiogenesis. (D) Decreasing cytokine activity and production. (E) Inducing host anti -MM immune response.

analysis also provided information about the influence that soluble factors and cell-to-cell contact have on gene expression (101).

In summary, multiple interactions between BM microenvironment and the malignant clone favor migration, growth, survival of MM cells and lead to angiogenesis contributing to evolution of the disease. Gene expression profiling studies continue to contribute to identify the molecular pathways that will provide new targets for the therapy of this still incurable disease.

4. NOVEL THERAPEUTIC STRATEGIES TARGETING THE MICROENVIRONMENT IN HEMATOLOGICAL MALIGNANCIES

Over the last several years, it has become increasingly apparent that effective therapy against cancer may require targeting both the cancer cell and its microenvironment. As described above, the role of the microenvironment in cancer cell progression involves several components including expression of adhesion molecules, interactions between cancer cells and stromal cells, cytokine and growth factor release, and angiogenesis, which taken together, provide multiple targets for novel therapeutic modalities. Many naturally occurring and synthetic inhibitors of angiogenesis and pharmacological agents targeting other components of the microenvironment are currently under preclinical and clinical investigation. Among others, these agents include interferon (INF) alpha, IL-12, thalidomide and its analogues, proteasome inhibitors, arsenic trioxide, VEGF receptor tyrosine kinase inhibitors and anti-VEGF monoclonal antibodies, matrix metalloprotease inhibitors and farnesyl transferase inhibitors.

4.1. Cytokines

Lower values of VEGF and HGF were found in patients with CML treated with IFN-alpha therapy compared to patients receiving alternative agents such as hydroxyurea (102). In CML, INF-alpha restored beta 1 integrin-mediated adhesion of CML progenitors to the stroma, resulting in beta 1 integrin-mediated microenvironmental inhibition of CML progenitor proliferation (103). In hairy cell leukemia, the efficacy of low-dose chronic IFN-alpha administration has been well established (104,105), however in recent years purine analogues have been found to be more effective than IFN-alpha (106) so that mechanisms of action have not been pursued.

IL-12 is a multifunctional cytokine, targeting the tumor vasculature via IFN-gamma and human interferon-inducible protein 10 (IP-10) (107). IL-12 significantly enhances PB mononuclear cell (PBMNC) cytotoxicity and decreases the quantity of leukemia cells in PBMNC of most patients with MDS, CML, and AML in CR, suggesting that IL-12 might be of considerable benefit in the elimination of MRD in patients with hematological malignancies (108).

4.2. Thalidomide and related analogues

Thalidomide has immunomodulatory, antiangiogenic and direct cytotoxic properties and is an established treatment modality for patients with refractory or relapsed MM. The mechanisms of action of thalidomide continue to be understood. Thalidomide directly affects the MM cell, inducing apoptosis or growth arrest (figure 2)

(109). The drug can modulate expression of cell surface adhesion molecules including ICAM-1 and VCAM-1 (110), thus altering MM–BMSC interaction. Angiogenesis inhibition is probably affected through inhibition of bFGF and VEGF activity (111). Thalidomide inefficiently, but selectively, inhibits production of TNF-alpha in a dose-dependent fashion by enhancing the degradation of TNF-alpha mRNA (112,113). Thalidomide also alters the secretion and bioactivity of cytokines secreted into the BM microenvironment by BMSCs such as IL-6, IL-1beta, and TNF-alpha. In addition thalidomide stimulates host anti-MM natural killer (NK) cell immunity (114).

Several groups have evaluated the single agent activity of thalidomide (100 to 800 mg/day) in refractory and relapsed myeloma, demonstrating response rates (complete or partial responses) ranging from 25% to 47% (78,115-118). Thalidomide has also been shown to increase the efficiency of chemotherapy and dexamethasone treatment with response rates of up to 72% in MM and to be an effective agent when used upfront for newly diagnosed MM (119-121). In addition, thalidomide has been evaluated for the therapy of other hematological malignancies. In AML, encouraging results have been observed with a response rate of 25% and with a significant decrease in MVD in BM, accompanied by declining plasma levels of bFGF in responding patients treated with single agent thalidomide in *de novo* and previously treated AML (122). In contrast, a recent study using different response criteria experienced a response rate of only 6%, therefore suggesting, not surprisingly, that thalidomide used alone would not be an effective strategy for poor prognosis *de novo*, relapsed- or refractory-AML (123). In addition, thalidomide in combination with chemotherapy did not result in clinical benefit in patients with poor prognosis AML (124). Some degree of efficacy could be shown in the treatment of MDS by improving cytopenia and achieving independence of transfusion therapy in a subset of patients (125-127). A recent study showed that both low and high risk MDS may benefit significantly from combination therapy with arsenic trioxide and thalidomide, particularly in those patients with high pre-therapy EVI1 (ecotropic viral integration site 1) expression (128). In relapsed and CHOP (cyclophosphamide, adriamycin, vincristine, prednisone**)** resistant MCL, thalidomide in combination with rituximab has marked anti-tumor activity (129) and significant effects are to be expected in other lymphoma subtypes due to the finding that elevated serum levels of VEGF correlate with poor prognosis in patients with NHL (38,130).

Analogues of thalidomide such as the immunomodulatory drugs (ImiDS, Celgene Corporation) and selective cytokine inhibitory drugs (SelCIDs, Celgene Corporation) designed to increase efficacy and to decrease toxicity (e.g. lack of teratogenicity or sedation) have been developed. These analogues have been shown to be up to 50,000 times more potent than the parent molecule at inhibiting TNF-alpha production by PBMC *in vitro* (131). ImiDs also downregulate the constitutive MAPK phosphorylation indicative of IL-6 induced growth stimulation (132) and target the BM microenvironment by enhancing modulation of IL-2 and IFN-gamma levels relative to the levels achieved following treatment with thalidomide (131,133). In addition, there is some evidence that ImiDs play a role in overcoming drug resistance that is characteristic of MM (132). In a Phase I clinical trial of the immunomodulatory drug CC-5013 (Revimid^{TM}), a best response of at least 25% reduction in paraprotein in 17 (71%) of 24 patients with relapsed/refractory MM, including 11 who had received prior thalidomide has been shown (114). In MDS, six (66%) of nine evaluable patients showed hematological benefit after treatment with CC-5013 (134). In both studies the thalidomide analogue was well tolerated (absence of somnolence, constipation, or neuropathy); dose limiting toxicity appeared to be myelosuppression (leucopenia and thrombocytopenia). Given the preliminary efficacy of CC-5013 in MM and MDS, investigation of thalidomide analogues as therapy for patients including these and other hematological malignancies is warranted and such studies are ongoing.

4.3. Proteasome inhibitor

Proteasome inhibitors represent another potential anticancer therapy targeting the MM cell and its BM microenvironment. The proteasome is a multicatalytic proteinase complex responsible for the degradation of most intracellular proteins involved in cell cycle regulation, cell growth, and apoptosis. The novel proteasome inhibitor Bortezomib (VELCADE^{TM} formely known as PS-341), a dipeptide boronic acid analogue, was recently approved for use in patients with refractory and relapsed MM and to date is the only proteasome inhibitor to have entered clinical trials. Bortezomib acts directly in MM cells but also alters cellular interactions and cytokine secretion in the BM milieu to inhibit tumor cell growth and associated angiogenesis, induces apoptosis, and appears to overcome drug resistance (135,136). TNF-alpha secreted from MM cells induces NF-kappa B activation in BMSCs as well as MEK/MAPK and NF-kappa B activation in MM cells, which upregulates the expression of cell surface adhesion molecules (VLA-4, LFA-1, and Mucin-1) on MM cells and their receptors (VCAM-1 and ICAM-1) on BMSCs.In BMSCs NF-kappa B is activated when the proteasome degrades the inhibitor protein IkappaB-alpha. Once released from IkappaB inhibitor, NF-kappa B translocates to the nucleus and activates the transcription of genes that protect the cell from apoptosis and promote cell growth and differentiation. Proteasome inhibition via Bortezomib can block the activity of the transcription factor NF-kappa B, thus inhibiting the TNF-alpha induced expression of cell-surface adhesion molecules and downregulating the production of growth factors including IL-6 in BMSCs (figure 3). The important effects mediated by TNF-alpha on MM cell growth, survival, and migration provide a strong rationale for targeting TNF-alpha and its sequelae. A phase I trial of the proteasome inhibitor Bortezomib in patients with refractory hematologic malignancies showed activity against refractory MM and possibly NHL (137). Phase II studies of Bortezomib in patients with relapsed and refractory MM, demonstrated evidence of antitumor activity and acceptable safety profile (138). The ability of Bortezomib to enhance the antimyeloma efficacy of dexamethasone in vitro (135) suggests therapy using

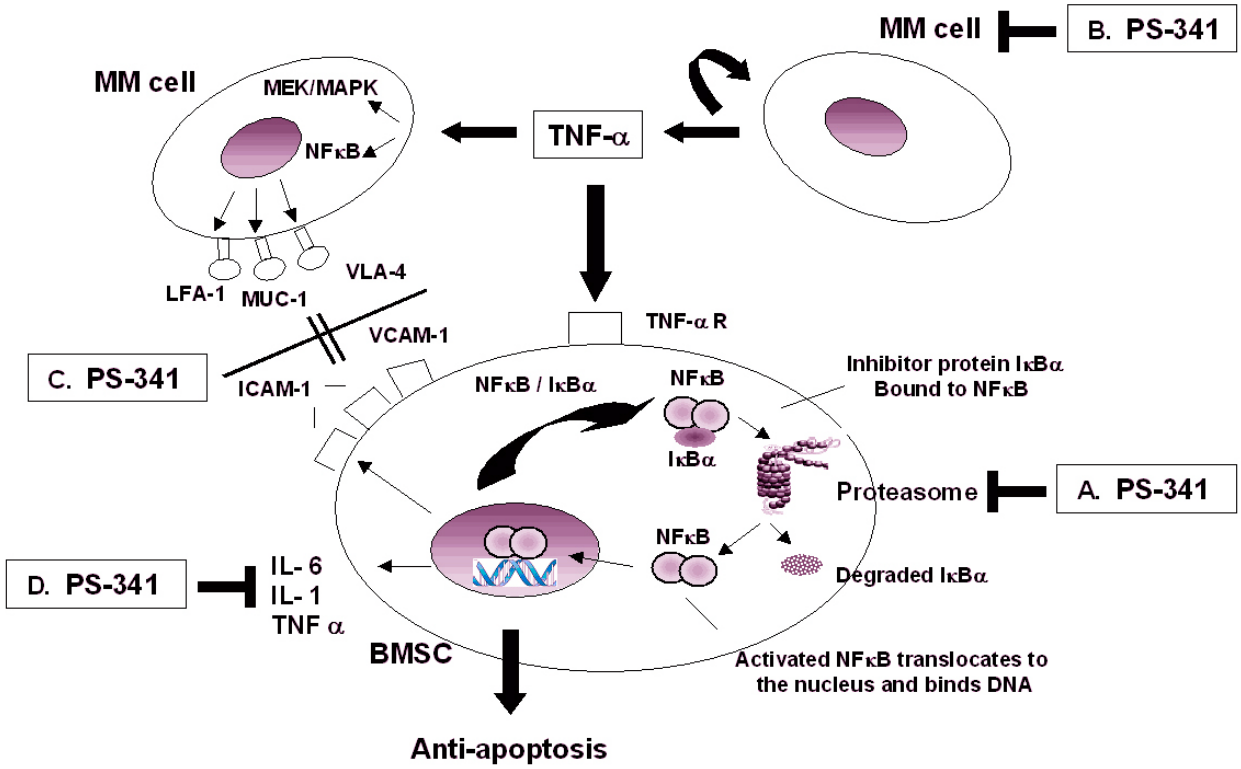

Figure 3. Effects of the proteasome inhibitor PS-341 on MM. (A) Specific and selective inhibition of the 26 proteasome. (B) Direct apoptotic effect on MM cells. (C) Downregulation of cytokines induced expression of adhesion molecules and inhibition of MM cell and BMCS binding. (D) Inhibition of cytokine secretion in the BM-milieu.

combinations such as dexamethasone and Bortezomib might be particularly effective.

4.4. Arsenic trioxide

Recent discoveries have renewed interest worlwide in the use of arsenicals for treating cancer. Arsenic trioxide induces dose- and time-dependent apoptosis of human endothelial cells *in vitro*, inhibits VEGF-induced capillary tubule formation, and decreases leukemic cell VEGF production, suggesting that arsenic trioxide may exert its antileukemic affect in part through inhibition of angiogenesis (139). In patients with relapsed acute promyelocytic leukemia (APL), low doses of arsenic trioxide can induce CR with good tolerability and no evidence of cumulative toxicity in 92% of patients (140). Arsenic trioxide also holds therapeutic promise in the treatment of MM. On an intent-to-treat basis, 23% of patients with advanced refractory MM showed a clinical response with >50% paraprotein reduction (141). In MDS, preliminary results of ongoing phase II studies suggest that arsenic trioxide produces hematological improvement including durable transfusion independence in approximately 30% of patients.

4.5. VEGF receptor tyrosine kinase inhibitors and anti-VEGF monoclonal antibodies

VEGF is considered the most potent endothelial cell activator, strongly suggesting VEGF as a novel therapeutic target. To date, a number of specific VEGF receptor tyrosine kinase (RTK) inhibitors have been developed. SU5416, a VEGFR-1 and VEGFR-2 inhibitor, has been assessed in phase II clinical trials in patients with refractory AML or MDS (142,143). SU5416 showed biologic activity and modest clinical activity in these patients.I It was associated with a partial response or a hematological improvement in four of 55 (7%) patients (142) or with a CR in one (2%) patient and a partial remission in seven patients of 42 (17%) eligible patients (143). Recently, in a phase I study with SU11248 (an oral more broad kinase inhibitor of FLT3, c-Kit, VEGF and PDGF receptors), responses, although longer in refractory AML patients with mutated FLT3, were of short duration. Further evaluation of this compound, e.g. in combination with conventional AML therapy or for treatment of patients with MRD after chemotherapy is warranted. Other receptor antagonists, such as PTK787, are under active clinical research.

Bevacizumab (Avastin), a recombinant anti-VEGF humanized monoclonal antibody, administered in a phase II clinical trial after cytotoxic chemotherapy, yields a favorable overall response (48%) and CR rate (33%) in adults with AML that is resistant to traditional treatment approaches (144).

4.6. Matrix metalloprotease (MMP) inhibitors

Other agents, such as the metalloproteinase

inhibitors and farnesyl transferase inhibitors have limited clinical experience. MMPs are the principal secreted proteinases required for extracellular matrix degradation in a variety of physiological and pathological tissue remodelling processes, including wound healing, embryo implantation, tumor invasion, metastasis, and angiogenesis (145-147) and inhibition of MMPs has been postulated to block tumor invasion and metastasis. A leading MMP candidate for antiangiogenic therapy in hematologic malignancies is the synthetic gelatinase inhibitor, AG3340 (Prinomastat^{TM}), which, because of its selectivity, has limited clinical toxicity (148).

4.7. Farnesyl transferase inhibitors (FTIs)

The FTIs inhibit *ras* protooncogene activation, an upstream transcriptional regulator of VEGF essential for endothelial cell proliferation. Several FTIs are under investigation including R115777, which has shown activity in AML, Ph-positive CML, CMML, MDS, and myelofibrosis and Schering 66336, which has shown activity in CMML and MDS. In a phase I clinical trial of the orally administered R115777, 29% of patients with refractory and relapsed acute leukemias showed a clinical response, suggesting that inhibitors of FT may have important clinical antileukemic activity (149).

4.8. TNP-470

TNP-470 is a derivative of fumagillin, a naturally occuring substance which inhibits endothelial cell growth (150). Phase I and II trials of TNP-470 have been reported to show efficacy in cervical cancers (151) and Kaposi's sarcoma (152). *In vitro* data with TNP-470 shows moderate cytotoxic effects on human leukemia and myeloma cell lines (153) and antitumor activity on murine T-cell lymphoma/leukemia cells (154), suggesting a therapeutic benefit in hematological malignancies.

The angiogenesis inhibitors and therapeutics targeting molecular markers represent a novel and promising approach for cancer treatment, modulating cell-cell interaction between normal and malignant cells. Preclinical and clinical data strongly suggest that antiangiogenic and pharmacological agents targeting other components of the microenvironment work additively or even synergistically with established treatment modalities as conventional cytotoxic agents, radiation, and immunologic approaches. It will therefore be a future challenge to integrate antiangiogenic and biologic anticancer agents in currently existing treatment protocols to improve the outcome of therapy.

5. PERSPECTIVE

The evidences presented here demonstrate that the microenvironment plays a major role in tumor formation and progression, although the role of specific adhesion molecules in such interactions remain somewhat controversial. The elucidation of the molecular mechanisms underlying the signal transduction pathways between malignant cell and microenvironment is critical in answering the role of microenvironment in promoting tumor cell proliferation, invasion and survival and in identifying those interactions which can best be targeted for therapeutic benefit. The use of powerful cDNA microarry to profile global transcription changes after direct contact between tumor cell and microenvironment will likely lead to identification of signatures of gene expression required for growth and survival factors. The rapid development of proteomic technology, like nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry, provides exciting outlook for characterization of the structure and qualitative and quantitative map of signal transduction cascades. Taken together, this emerging knowledge will lay the basis for the discovery of novel therapeutic targets and lead to the development of novel therapeutic agents for curing hematological malignancies.

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