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Targeting the bone marrow in Waldenstrom Macroglobulinemia

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Abstract

Waldesntrom Macroglobulinemia (WM) is a low-grade B cell lymphoma characterized by widespread involvement of the bone marrow with lymphoplasmacytic cells. In about 20% of patients, the malignant clone also involves the lymph nodes and induces hepatosplenomegaly. The mechanisms by which the tumor cells home to the bone marrow and preferentially reside in the marrow niches are not fully elucidated. In this review, we examine the role of the bone marrow microenvironment in the regulation of cell growth, survival and cell dissemination in WM. We also summarize specific regulators of niche-dependent tumor proliferation in WM. These include chemokines, adhesion molecules, Src/PI3K/Akt/mTOR signaling, NF-kB activation, and miRNA regulation in WM. Targeting these pathways in clinical trials could lead to significant responses in this rare disease.

Keywords

Waldenstrom; cell trafficking; homing; niche; bone marrow; adhesion

Introduction

Waldesntrom Macroglobulinemia is a low-grade B cell lymphoma characterized by widespread involvement of the bone marrow with lymphoplasmacytic cells. In about 20% of patients, the malignant clone also involves the lymph nodes and induces hepatosplenomegaly[1-3]. The mechanisms by which the tumor cells home to the bone marrow and preferentially reside in the marrow niches are not fully elucidated. However, many studies have shown that the microenvironment plays a crucial role in tumor cell proliferation, survival, and drug-resistance in WM.

The bone marrow niche is a term that was first coined by Schofield who indicated that the bone marrow microenvironment structures are essential for the long-term maintenance of a stable hematopoetici stem cell pool[4]. The BM microenvironment consists of hematopoietic and non-hematopoietic cells, as well as an extracellular and liquid compartment organized in a complex architecture of sub-microenvironments or “niches”[5]. At least 2 distinct niches have been identified in BM: the osteoblastic niche and the vascular niche[6,7]. The stroma of

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the bone marrow is composed of cells of various lineages, including osteoblasts, endothelial cells, fibroblasts, and adipocytes, but the relative contributions of each lineage have remained elusive.

Genetic and epigenetic events in the tumor clone of WM lead to its oncogenesis. WM is thought to arise from B cells in the germinal center that were arrested between the stages of somatic hypermutation and isotype class switching[8,9]. Deletion of the long arm of chromosome 6 (6q-) is the most frequent cytogenetic abnormality in WM. In 102 WM patients, the incidence of 6q21 deletion was found to be 7% by conventional cytogenetics and 34% when analyzed by fluorescent in situ hybridization (FISH). Patients with deletion of 6q had worse prognostic features that correlated with poor prognostic features of WM. In addition, patients with smoldering WM who displayed the abnormality showed a trend to an earlier requirement of therapy[10]. Other cytogenetic abnormalities include trisomy 4, trisomy 5, monsomy 8 and deletion 20q[11-13]. However, these events may not be sufficient, and a permissive microenvironment has been suggested to be required for frank malignancy to emerge[14]. Examples of microenvironmental contributions to neoplasia include a necessary mast cell contribution to Nf1-induced neurofibromas[15] and mesenchymal cell alteration of epithelial tumor growth kinetics[16]. Changes in a tissue microenvironment have also been suggested to precede and promote the initiation of genetic events by creating a 'pre-malignant' state characterized by disruption of quiescence-inducing signals or increases in proliferative signaling[17]. This has been validated experimentally with altered TGF-β signaling in tissue fibroblasts[18] and with myeloid progenitor expansion after RAR-γ deletion in bone marrow or Rb deficiency in haematopoietic and microenvironmental cells[19]. In addition, the relevance of niche-mediated oncogenesis has become evident with studies showing that the microenvironment plays an integral role in the development of myeloproliferative syndromes[14].

Although no such evidence of niche-mediated oncogenesis is present in WM, several studies have shown that the interaction of WM cells with their microenvironment leads to tumor proliferation, survival and drug-resistance. In this review, we describe studies that demonstrate the critical role of the bone marrow niche in regulating tumor progression in WM.

Cell trafficking in WM

WM represents a spectrum from early asymptomatic monoclonal gammopathy of undetermined significance (IgM-MGUS), where there are small numbers of lymphoplasmacytic cells in the bone marrow (<10%), to active WM with anemia, hyperviscosity and widespread disease[20,21]. In a similar plasma cell dyscrasia such as Multiple Myeloma, studies have demonstrated the presence of a small number of circulating plasma cells in over 70% of patients with MM[22]. The number of circulating cells in the peripheral blood increased with progression of the disease, and was an independent unfavorable prognostic marker[22]. This data implies that progression of these plasma cell dyscrasias occurs through the continuous trafficking of the malignant cells to new sites of the BM.

The CXCR4/SDF-1 axis and migration in WM

Migration of cells through the blood to the bone marrow niches requires active navigation through the process of homing. Homing is thought to be a coordinated, multistep process, which involves signaling by stromal derived factor (SDF-1), activation of lymphocyte function–associated antigen 1 (LFA-1), VLA-4/5, and activation of MMP2/9[23-25]. SDF-1 is chemokine that has been extensively studied in the regulation of homing and migration of hematopoietic cells and lymphocytes. The receptor of SDF-1 is CXCR4. Recently, a new

receptor was discovered that is activated by SDF-1, namely CXCR7 (RDC-1)[26]. SDF-1 is primarily produced by stromal cells. The major biological effects of SDF-1 are related to the ability of this chemokine to induce (a) motility, (b) chemotactic responses, (c) adhesion, and (d) secretion of MMPs and angiopoietic factors (e.g., VEGF)[27-29]. SDF-1 has been reported to induce firm adhesion and migration by inducing activation of integrins LFA-1, VLA-4, and VLA-5 on stem cells[27,29-31]. CXCR4 is expressed on the surface of normal cells such as hematopoeitic stem cells and T and B lymphocytes, as well as malignant cells such as breast cancer cells and lymphoid malignancies[32-36]. CXCR4 knockout mice die neonatally due to multiple developmental defects, with lymphopoiesis and myelopoiesis dramatically defective in these fetuses[37]. Plasma cells in chimeric mice reconstituted with CXCR4-deficient fetal liver cells were found in elevated numbers in the blood, and failed to accumulate in the BM, indicating an important role of CXCR4 in the homing of malignant plasma cells[38]. The most important pathways involved in signaling that regulate migration or adhesion include activation of the MAPK p42/44–ERK-1, PI3K–AKT, PKC, NF-kB pathways, focal adhesion components such as focal adhesion kinase (FAK), paxillin, Nck, Crk, Crk-L, protein kinase C (PKC), phospholipase C (PLC), and calcium flux[29,39-41,42].

We previously showed that WM cells express high levels of chemokine and adhesion receptors, including CXCR4 and VLA-4[43]. We showed that CXCR4 was essential for the migration and trans-endothelial migration of WM cells under static and dynamic shear flow conditions, with significant inhibition of migration using CXCR4 knockdown or the CXCR4 inhibitor plerixafor[43]. Similarly, CXCR4 or VLA-4 inhibition led to significant inhibition of adhesion to fibronectin, stromal cells, and endothelial cells[43]. Decreased adhesion of WM cells to stromal cells by plerixafor led to increased sensitivity of these cells to cytotoxicity by bortezomib. To further investigate the mechanisms of CXCR4-dependent adhesion, we showed that CXCR4 and VLA-4 directly interact in response to SDF-1. We further investigated downstream signaling pathways regulating migration and adhesion in WM, including the PI3K and ERK pathways and found that these pathways are activated in response to CXCR4 activation[43].

Another study was recently published that examined the distribution and the clinical influence of the SDF-1 (CXCL12) (-801GA) polymorphism in a series of 114 WM patients[44]. CXCL12 (-801AA) genotype was more frequent in WM patients compared with control subjects ($p = 0.01$). On the other hand, CXCL12 (-801GG) patients had a shorter median survival after initiation of first line therapy than remaining patients that did not have CXCL12 (-801GG), ($p = 0.01$). In conclusion, the CXCL12 (-801GA) polymorphism may either be associated with a high incidence of WM or influence clinical outcome.

CXCR4 is not the only receptor for CXCL12 (SDF-1). The more recently identified CXCR7 receptor has been identified in other hematological malignancies, specifically multiple myeloma. Its role in WM is currently being evaluated. These studies indicate that CXCR4 inhibitors may be used in the therapy of patients with WM. The preclinical data showing that inhibition of CXCR4 with plerixafor leads to sensitization of tumor cells to therapy with bortezomib indicate that this strategy may be used in clinical trials. The use of plerixafor in chemosensitization therapy in clinical trials of AML and multiple myeloma are ongoing and the results are promising.

Adhesion receptors and WM

Proteomic analysis of WM cells compared to normal CD19+ cells has shown that adhesion – related proteins are upregulated in WM. These included Integrin b3 (CD61), MDC9 (ADAM), JAM-1, Mena, Maspin, LAR, annexin II, p62 lck ligand, pp120 src substrate, CLA-1 (CD36), RPTPb, nexilin,contactin, and tensin.

Previous studies presented by our group and others have shown that VLA-4 and LFA-1 are highly expressed in WM and that adhesion to fibronectin, endothelial cells and stromal cells are regulated by VLA-4. We also showed that VLA-4 interacts with CXCR4, indicating the complexity and interactions between adhesion and migration.

Src tyrosine kinase is known to regulate cell adhesion and migration[45]. A recent study has shown that Src was overexpressed in WM[45]. Inhibition of Src by AZD0530 (Astra Zeneca Inc) was shown to inhibit the basal activation of Src in BCWM1, as well as to inhibit the SDF1-induced activation of Src and other cytoskeletal-related proteins and polymerization of actin, indicating an important role of Src in the signaling cascade downstream of CXCR4 leading to an increase of migration and adhesion of WM[45]. Inhibition of CXCR4 by plerixafor or inhibition of Src results in similar effects of inhibition of the chemotaxis. Therefore, Src inhibition may be a good target for the development of therapeutic agents in WM.

The PI3K/mTOR pathway

Tumorigenesis results from synergistic interactions of a complex of signal transduction processes, including multiple onco-proteins and tumor suppressors such as Ras, Myc, PKB/Akt, Her-2/Neu, p53 and PTEN[46-48]. Overexpression of Akt plays an important role in the initiation and progression of malignancies. The PI3K pathway is important in enhancing cell survival by stimulating cell proliferation and inhibiting apoptosis[49-53]. Regulators of this pathway include PTEN, HSP90, P18, P15, P19, P16 and P21[54,55].

There is no evidence that there are activating mutations in the PI3K/Akt/mTOR pathway in WM. Therefore, activation of this pathway may be due to external stimulation though the bone marrow microenvironment such as stimulation through IGF-1 or SDF-1. Previous studies have shown activation of Akt and mTOR in WM. Inhibition of Akt by perifosine (Keryx Inc)[56], mTOR by RAD001 (Novartis Inc) or the PI3K/mTOR inhibitor (NVP-BEZ235)[57] have shown activity on WM cells even in the presence of bone marrow stromal cells.

Based on the preclinical studies, a phase II trial of single agent perifosine was conducted in patients with relapsed or refractory WM[58]. The study showed that of the 37 patients, 4 achieved partial response (11%), 9 minimal response (24%), and 20 showed stable disease (54%). The median progression-free survival was 12.6 months. Gene expression profiling of samples obtained from patients before and after therapy with perifosine showed that there were 162 genes significantly changed in expression in response to perifosine. These included reduced expression of several genes involved in the adhesion and migration processes, as well as regulators of the NF-kB pathway.

RAD001 (Everolimus, Novartis Inc) has shown preclinical activity in WM. In additon, it inhibited adhesion of WM cells to stromal cells and enhanced activity of rituximab and bortezomib (unpublished data from our lab). A Phase II trial of single agent RAD001 in patients with relapsed WM was conducted in 50 patients[59]. The response rate was 42% (all PR) with 28% MR for an overall clinical benefit rate of 70%. The estimated PFS at 6 and 12 months is 75% (95%CI: 64-89%) and 62% (95%CI: 48-80%)[59]. Prior studies of mTOR inhibitors have shown that these agents can induce tumor mobilization of the malignant cells from the lymph node compartment to th e peripheral blood compartment, such as in Mantle cell lymphoma. Similar observations were seen with PI3K delta inhibitors in chronic lymphocytic leukemia (CLL), indicating that inhibitors of this pathway can induce mobilization of the tumor clone. Mobilization of tumor cells to the peripheral blood can make them more sensitive to therapeutic agents that are usually more active on tumor cells that are not adherent to stroma. We did not examine peripheral mobilization of WM cells in

the RAD001 study, but future studies of mTOR inhibitors and other inhibitors of this pathway will examine the effect of these agents on mobilization of the tumor cells to the peripheral blood and their sensitization to therapeutic agents while they are in circulation.

In a recent study using the PI3K/mTOR inhibitor NVP-BEZ235, Roccaro et al showed that bone marrow stromal cells (BMSCs) triggered increase of 52% in proliferation of WM cells, which was inhibited by NVP- BEZ235 in a dose-dependent manner[57]. Adherence of BCWM.1 cells to BMSCs induced phosphorylation of Akt and mTOR; conversely, NVP-BEZ235 abrogated BMSC adhesion- induced phosphorylation of Akt and mTOR in WM cells, indicating that NVP-BEZ235 exerts its antitumor activity even when WM cells were in contact with BM milieu. Similarly, NVP-BEZ235 targeted rictor and raptor in WM cells, even when cultured in presence of BMSCs, which induced up-regulation of raptor, but not rictor. NVP-BEZ235 induced significant inhibition of adhesion to either fibronectin or BMSCs. NVP-BEZ235 also inhibited migration of WM cells in response to SDF-1. NVP-BEZ235 inhibited phosphorylation of focal adhesion kinase, paxillin, and cofilin; important proteins that act as key regulators of adhesion and cell migration . Finally, NVP-BEZ235 resulted in a significant inhibition of homing of WM cells to the BM in vivo.

The NF-kB pathway

The NF-kB pathway in malignant transformation, progression and resistance: NF-kB comprises a family of transcription factors that regulate the transcription of hundreds of genes involved in inflammation, innate immunity, cell growth and apoptosis [60]. Inhibition of the NFkB pathway by proteasome inhibitors such as bortezomib and other agents such as NPI-0052m carfilzomib and Onyx0912 can inhibit WM cells even in the presence of bone marrow stromal cells[61,62]. Bortezomib is known to inhibit adhesion of plasma cells in MM. Studies using bortezomib alone or in combination with rituximab have shown significant activity with over 80% response rate even in relapsed or newly diagnosed WM[63-65]. Future studies using carfilzomib are planned in WM.

Epigenetics in the bone marrow of WM

miRNA 155 in WM. miRNA-155 plays a pivotal role in B-cell malignancies including diffuse large B-cell lymphomas, primary mediastinal B-cell lymphomas, and Hodgkin lymphomas[66]. We found that miRNA-155 is increased in WM tumor cells compared to normal CD19+ cells[66]. Therefore we studied the functional role of miRNA-155 in WM by examining proliferation, adhesion, and migration in miR-155 LNA knockdown WM cells compared with controls (control probe–transfected BCWM.1 cells or nontransfected BCWM.1 cells). Our studies have shown that knocking down miRNA-155 in WM cells reduced the adhesion and migration *in vitro*; and significantly decreased proliferation even in the presence of bone marrow stromal cells[66]. *In vivo* studies confirmed significant inhibition of homing and proliferation of miRNA-155 knockdown WM cells; decreased IgM secretion; and a significant survival benefit in mice. We further confirmed that specific knockdown of miRNA-155 inhibits specific genes regulating cell cycle as well as regulators of adhesion and migration. Together, these studies confirm that miRNA-155 is a critical regulator of proliferation, cell cycle regulation, migration and adhesion[66]. Further studies to use miRNA 155 in the treatment of patients with WM are ongoing.

In summary, we show that the bone marrow microenvironment is a critical regulator of cell growth, survival and cell dissemination in WM. Targeting this niche-dependent tumor growth is important in the design of future clinical trials in this disease. Specific targets that can be used for clinical trials include chemokine receptors, adhesion molecules, downstream signaling targets such as Src, PI3K/Akt/mTOR, or specific epigenetic changes such as miRNA regulation

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Table of novel agents targeting migration and adhesion.

Pathway or Target	Therapeutic agent	Clinical or preclinical testing	Reference
CXCR4	Plerixafor	Preclinical testing	43
VLA-4 and LFA-1	VLA-4 antibodies	Preclinical testing	43
Src	AZD0530	Preclinical testing	45
PI3K/Akt/mTOR pathway	Perifosine, RAD001, BEZ-235	○ Phase II trial of perifosine showed an ORR of 35% including MR in relapsed or refractory WM. ○ Phase II trial of RAD001 showed an ORR of 70% including MR in relapsed or refractory WM. ○ NVP-BEZ-235 completed preclinical testing.	56, 57, 58, 59,
NF-kB	Bortezomib, NPI-0052, carfilzomib and Onyx 0912	○ Bortezomib single agent or in combination with rituximab has completed phase II clinical trials in WM with an ORR of about 80-90% including MR in newly diagnosed or relapsed WM. ○ NPI-0052, Carfilzomib and Onyx-0912 completed preclinical testing.	61-65
miRNA	Anti-miR-155 LNA	Preclinical testing	66