The bone marrow microenvironment in Waldenstrom macroglobulinemia

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***Abstract*:** Waldenstrom macroglobulinemia (WM) is a low-grade B-cell lymphoproliferative disorder characterized primarily by specific homing and growth of tumor cells within the bone marrow niches. The progressive growth of tumor cells throughout the bone marrow indicates that the tumor cells are capable of homing and adhering to specific niches that allow growth, survival and drug resistance. In this review we highlight the interaction of the tumor cells in WM and the bone marrow microenvironment including bone marrow stromal cells, endothelial cells and mast cells. Migration, adhesion and downstream activation of signaling pathways leads to cell trafficking and cell dissemination in WM. Future therapeutic agents need to target not only the tumor clone, but also its close interaction with the bone marrow microenvironment.

***Keywords*:** adhesion, bone marrow, cell trafficking, homing, migration, niche, Waldenstrom

Introduction

Waldenstrom macroglobulinemia (WM) is a low-grade B-cell lymphoproliferative disorder characterized primarily by specific homing and tumor growth within the bone marrow niches. Indeed, the progressive growth of tumor cells throughout the bone marrow indicates that the tumor cells are capable of homing and adhering to specific niches that allow growth, survival and drug resistance [Dimopoulos *et al.* 2005, 2000; Ghobrial and Witzig, 2004; Owen *et al.* 2003]. The widespread involvement of the bone marrow with tumor cell homing indicates that there is continuous cell trafficking of WM cells in and out of the bone marrow leading to cell dissemination.

In this review, we focus on mechanisms of cell trafficking in WM and how this leads to cell dissemination and further tumor growth. Many of these mechanisms apply to other low-grade lymphomas and plasma cell dyscrasias.

The bone and bone marrow niches

The bone cavity is a complex architecture of cells and blood vessels that retain developing hematopoietic cells within the bone cavity until they have matured and are released into the vascular system. Mesenchymal stem cells (MSCs) give rise to the majority of marrow stromal cell lineages, including chondrocytes, osteoblasts, fibroblasts, adipocytes, endothelial cells, and myocytes. Prior studies have defined the bone marrow niches into two distinct niches, the osteoblastic niche and the vascular niche [Yin and Li, 2006; Sipkins *et al.* 2005]. However, these separations of two niches are currently being challenged. The close interaction of bone marrow cells allow the development of hematopoeitic cells, their egress into the peripheral blood and their homing and further localization into the bone marrow niches at distant sites. By understanding normal cell trafficking of hematopoietic cells, we can begin to delineate mechanisms of cell trafficking and localization of malignant lymphocytes into the bone marrow niches. Indeed, malignant lymphocytes may hijack most of the normal trafficking processes of normal lymphocytes in their migration and homing into the bone marrow. The interaction of the tumor cell compartment with the cellular compartments of the bone marrow leads to enhanced proliferation and drug resistance.

The cellular elements in the bone marrow that specifically regulate cell trafficking of WM cells include the bone marrow stromal cells (BMSCs), endothelial cells and mast cells (MCs). BMSCs regulate the growth and proliferation of tumor

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cells including WM cells [Ngo *et al.* 2009, 2008; Roccaro *et al.* 2009b, 2009c; Leleu *et al.* 2008]. Coculture of WM cells with stromal cells leads to resistance to therapeutic agents such as bortezomib, fludarabine, perifosine and other proteasome inhibitors [Ngo *et al.* 2009, 2008; Roccaro *et al.* 2009b, 2009c; Leleu *et al.* 2008]. In addition, an *in vivo* tumor model of WM of SCID-human bone (SCID-hu) has shown that the stromal cell compartment is essential for WM tumor growth [Tassone *et al.* 2005]. Bone marrow MCs are commonly found in association with lymphoplasmacytic cells in patients with WM [Santos *et al.* 2006; Tournilhac *et al.* 2006]. Their specific role in cell trafficking is not well defined, but a study by Tournhilac and colleagues has shown that coculture of mast cells with WM cells leads to cell proliferation and expansion [Tournilhac *et al.* 2006]. Finally, endothelial cells are also critical players in the tumor growth of WM cells. Indeed, bone marrow microvessel density is increased in 30–40% of patients with WM [Terpos *et al.* 2009; Anagnostopoulos *et al.* 2007]; and primary WM endothelial cells present with a high expression of ephrin-B2 [Azab *et al.* 2010], an important regulator of cell motility, adhesion and repulsion, thus suggesting the importance of endothelial cells in WM biology.

Homing to the bone marrow

Migration of cells through the blood to the bone marrow niches requires active navigation, a process termed homing. Homing is thought to be a coordinated, multistep process, which involves signaling by SDF-1, activation of lymphocyte function-associated antigen 1 (LFA-1), VLA-4/5, cytoskeleton rearrangement, and activation of MMP2/9 [Lapidot *et al.* 2005; Avecilla *et al.* 2004; Kollet *et al.* 2003]. This process is not completely elucidated in WM. However, several studies have examined the role of chemokines in WM, specifically the chemokine CXCL12 or SDF-1. Chemokines are small chemoattractant cytokines that bind to specific G-protein-coupled seven-span transmembrane receptors that are present on the plasma membranes of target cells [Murphy, 2001; Baggiolini, 1998; Luster, 1998]. They are homologous 8–10 kD proteins that are subdivided into families, the CXC, CC, XCR and CX3C families. More than 50 different chemokines and 20 different chemokine receptors have been cloned [Murphy, 2001]. Chemokines usually bind to multiple receptors, and the same receptor may bind to more than one chemokine [Juarez and Bendall, 2004].

The expression pattern of 13 CXC and CC chemokine receptors (*CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7 and CCR8*) has been examined in WM cells using reverse transcriptase polymerase chain reaction (RT-PCR) and flow cytometry [Ngo *et al.* 2008]. The expression of these receptors in WM samples was variable. However, CXCR4 was highly expressed on WM cells. Specific inhibition of CXCR4 by a CXCR4 inhibitor (plerixafor) or by knockdown leads to significant inhibition of migration, transendothelial migration and adhesion of WM cells *in vitro* [Ngo *et al.* 2008]. Importantly, the addition of plerixafor to WM cells induced chemosensitization to bortezomib when WM cells were cocultured with stromal cells [Ngo *et al.* 2008]

Of the chemokine receptors, the CXCR4 ligand, SDF-1 or CXCL12, is the most extensively investigated [Juarez and Bendall, 2004; Kucia *et al.* 2004]. 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. vascular endothelial growth factor [VEGF]) [Kucia *et al.* 2005; Lapidot and Kollet, 2002; Peled *et al.* 2000]. The most important pathways involved in signaling 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 [Kucia *et al.* 2005; Petit *et al.* 2005; Kucia *et al.* 2004; Zhang *et al.* 2001; Ganju *et al.* 1998]. CXCR4 signaling also involves the Ras-activated signaling pathway and several src-related proteins.

Studies to examine the level of SDF-1 or CXCL12 in WM have shown that the mean expression of SDF-1 in the bone marrow of WM patients was significantly higher compared with that of normal controls [Ngo *et al.* 2008]. Interestingly, a recent study assessed the distribution and the clinical influence of *SDF-1* (−801GA) polymorphism using PCR restriction fragment length polymorphism (RFLP) in a series of 114 WM patients [Poulain *et al.* 2009].

SDF-1 (−801AA) genotype was more frequent in WM patients compared with control subjects.

Adhesion to the bone marrow

There are four major families of cell adhesion molecules (CAMs): immunoglobulin superfamily CAMs, integrins, cadherins and selectins [Poulain *et al.* 2009; Petit *et al.* 2005; Zhang *et al.* 2001]. Previous studies have shown that integrins such as VLA-4 and LFA-1 are highly expressed in WM [Ngo *et al.* 2008]. Adhesion of WM cells to fibronectin, endothelial cells and stromal cells are regulated by VLA-4. VLA-4 also interacts with CXCR4, indicating the complexity and interactions between adhesion and migration. The use of VLA-4 neutralizing antibody in WM led to the inhibition of adhesion to fibronectin, stromal cells and endothelial cells *in vitro* [Ngo *et al.* 2008]. Further studies to examine the interaction of CXCR4 and VLA-4 in WM are warranted and may lead to significant de-adhesion and sensitization to therapy in WM.

Another study using proteomic analysis of WM cells compared with 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.

Downstream signaling of adhesion and migration in WM

Src tyrosine kinase is known to regulate cell adhesion and migration. *Src* was the first oncogene to be discovered [Homsi *et al.* 2007; Alvarez *et al.* 2006; Alper and Bowden, 2005; Ishizawar and Parsons, 2004; Biscardi *et al.* 1999]. It is a non-receptor tyrosine kinase that belongs to a nine-member family of kinases including Src, Yes, Fyn, Lyn, Lck, Hck, Fgr, Blk and Yrk [Gauld and Cambier, 2004; Parsons and Parsons, 2004]. Src has been implicated in cell proliferation, survival, adhesion, migration, invasion, inflammation and angiogenesis. Src is activated by growth factor receptors, cytokine receptors, and FAK [Ram and Iyengar, 2001]. Src interacts with a network of intracellular signaling pathways, including the integrin/FAK pathway, β-catenin/Wnt, RAS-MEK, PI3K–AKT and Janus-activated kinase–STAT pathways [Parsons and Parsons, 2004]. A recent study has shown that Src was overexpressed in WM [Ngo *et al.* 2009]. Inhibition of Src by AZD0530 (Astra Zeneca Inc.) was shown to reduce Src levels in WM, as well as inhibition of SDF1-mediated migration and adhesion of WM [Ngo *et al.* 2009].

The PI3K/Akt/mTOR pathway also plays a crucial role in the regulation of cell trafficking in lymphomas including WM. Key signaling molecules regulating cell polarization and migration are phosphoinositide 3-kinase (PI3K) and the Rho family of small GTPases. While PI3K activity defines the leading edge of the cell, the Rho family GTPases regulate the cytoskeletal remodeling during polarization. Moreover, the PI3K pathway is important in enhancing cell survival by stimulating cell proliferation and inhibiting apoptosis [Hennessy *et al.* 2005; Dancey, 2004; Fresno Vara *et al.* 2004; Pene *et al.* 2002; Cantrell, 2001]. 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, providing the preclinical rational for using Akt or mTOR inhibitors in WM. Indeed, inhibition of Akt by perifosine (Keryx Inc), mTOR by RAD001 (Novartis Inc) or the PI3K/mTOR inhibitor (NVP-BEZ235) have shown activity on WM cells even in the presence of BMSCs [Roccaro *et al.* 2009b]. These inhibitors have also led to significant inhibition of adhesion and migration of WM cells. Prior studies of mTOR inhibitors have shown that these agents can induce tumor mobilization of the malignant cells from the lymph node compartment to the peripheral blood compartment, such as in Mantle cell lymphoma [Witzig *et al.* 2005].

Similar to the PI3K pathway, the NF-kB pathway has been implicated in the regulation of adhesion and resistance to therapy in WM. 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 [Hayden MS, 2004]. Inhibition of the NF-kB pathway by proteasome inhibitors such as bortezomib and other agents such as carfilzomib and Onyx0912 [Roccaro *et al.* 2010a] can inhibit growth of WM cells even in the presence of BMSCs.

Histone deacetylases are another class of agents that has been studied in WM and has shown significant preclinical activity [Roccaro *et al.* 2010b]. Histone deacetylase (HDAC) inhibitors can also regulate migration and adhesion in cancer cells. A recent study has shown that the HDAC inhibitor LBH589 (panobinostat, Novartis) can inhibit adhesion of WM cells to stromal cells and fibronectin [Roccaro *et al.* 2010b].

Finally, miRNAs can regulate adhesion in different tumors. miRNA-155 plays a pivotal role in B-cell malignancies including diffuse large B-cell lymphomas, primary mediastinal B-cell lymphomas and Hodgkin lymphomas. A recent study showed that miRNA-155 is increased in WM tumor cells compared with normal CD19+ cells [Roccaro *et al.* 2009a]. Loss of function of miRNA-155 in WM cells reduced adhesion and migration, and significantly decreased proliferation even in the presence of BMSCs. *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 [Roccaro *et al.* 2009a]. Specific knockdown of miRNA-155 inhibited specific genes regulating cell cycle as well as regulators of adhesion and migration [Roccaro *et al.* 2009a].

In summary, this review has highlighted the interaction of the tumor cells in WM and the bone marrow microenvironment including BMSCs, endothelial cells and MCs. Migration, adhesion and downstream activation of signaling pathways leads to cell trafficking and cell dissemination in WM. In addition, the close interaction of WM cells and their specific homing to the bone marrow allows the cells to have a protective environment that allows proliferation and resistance to apoptosis by therapeutic agents. Future therapeutic agents need to target not only the tumor clone, but also its close interaction with the bone marrow microenvironment.

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Conflict of interest statement

Irene Ghobrial is an advisory board for Millennium, Celgene, Novartis, BMS and Onyx.

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