The microenvironment in hairy cell leukemia: pathways and potential therapeutic targets

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Abstract

Hairy cell leukemia (HCL) cells accumulate and proliferate in the spleen and the bone marrow. In these tissue compartments, HCL cells interact with accessory cells, matrix proteins, and various cytokines, collectively referred to as the 'microenvironment.' Surface receptors expressed on HCL cells and respective stromal ligands are critical for this cross-talk between HCL cells and the microenvironment. Chemokine receptors, adhesion molecules (integrins, CD44), the B cell antigen receptor (BCR), and CD40, expressed on the HCL cells, are likely to be critical for homing, retention, survival, and expansion of the neoplastic B cells. Some of these pathways are now targeted in first clinical trials in other mature B-cell malignancies. We summarize key aspects of the cellular and molecular interactions between HCL cells and their microenvironment. Also, we outline future prospects for therapeutic targeting of the microenvironment in HCL, focusing on CXCR4 and kinase inhibitors (Syk, Btk, phosphatidylinositol 3-kinase [PI3K]) that target B cell receptor signaling.

Keywords: *Hairy cell leukemia (HCL), microenvironment, CXCR4, B cell receptor (BCR)*

Introduction

Hairy cell leukemia (HCL) is a chronic B-cell malignancy of mature neoplastic B cells with hairy protrusions, characterized by splenomegaly and bone marrow infiltration and marrow fibrosis, causing cytopenias [1]. Over the past few years, there has been a paradigm shift in B cell malignancies and other cancers from primarily studying the malignant cells and their abnormal cytogenetics and signaling networks, toward also emphasizing the importance of the microenvironment in tumor progression [2,3]. This is based on compelling evidence suggesting that cross-talk with accessory stromal cells in specialized tissue microenvironments, such as the bone marrow and secondary lymphoid organs, favors disease progression by promoting malignant B cell growth and drug resistance [4,5]. Therefore, disrupting the communication of malignant B cells with their milieu is an attractive novel strategy for treating B-cell malignancies. This approach has been systematically studied in diseases that share some biological features with HCL, such as chronic lymphocytic leukemia (CLL) [2], and there is strong evidence that some of these pathways also could become attractive therapeutic targets in HCL.

Chemokine receptors and adhesion molecules in hairy cell leukemia

Chemokines and their receptors organize the recruitment and positioning of cells at each stage of the immune response, a system critically dependent upon coordination to get the right cells to the right place at the right time [6]. Chemokine receptors expressed on HCL cells are thought to function in a similar fashion, regulating the trafficking of hairy cells (HCs) between blood, lymphoid organs, and the bone marrow, and within sub-compartments within these tissues, in concert with adhesion molecules and other guidance cues. HCs express high levels of CXCR4 chemokine receptors (CD184) [7–9], while other B cell chemokine receptors that are critical for entry and positioning in lymph nodes (CXCR5, CCR7) are not expressed in HCL [9] (Figure 1), explaining the low propensity of HCL for lymph node involvement.

HCs also express various adhesion molecules that cooperate with the chemokine receptors in tissue homing and retention [10–12], such as integrins and CD44. Among the different integrins, $\alpha_4\beta_1$ (CD49d) and $\alpha_5\beta_1$ (CD49e) are critical for HCL cell adhesion to vascular cell adhesion molecule-1 (VCAM-1;

Figure 1. Expression of chemokine receptors and adhesion molecules on HCL cells. These overlay histograms depict fluorescence intensities of CD19/CD11c/CD103-positive, CD19-gated HCL cells, stained with monoclonal antibodies (mAbs) toward the antigens that are displayed above or below each of the histograms. The light gray histograms depict the fluorescence intensities of HCs stained with respective isotype control mAbs for comparison with the specific mAbs (dark gray). HCL cells express CXCR4, CD49d, CD44, and CD40, but are negative for CXCR3 and CXCR5.

CD106) and fibronectin (FN) on stromal and endothelial cells, and as part of the extracellular matrix [13–15] (Figure 2). $\alpha_M\beta_2$ (CD11b), $\alpha_X\beta_2$ (CD11c), and $\alpha_E\beta_7$ (CD103) are also expressed on HCL cells and are important for immunophenotyping of HCL (CD11c, CD103), but their function is less defined. The $\alpha_V\beta_3$ integrin functions as a receptor for vitronectin (VN) and platelet/endothelial cell adhesion molecule-1 (PECAM-1; CD31) and is important in HC motility. CD44 is expressed on HCs and functions as a receptor for hyaluronan [16]. HCs binding to hyaluronan via CD44 enhance autocrine production of FN and fibroblast growth factor-2 (FGF-2) [17], which, along with HC-derived transforming growth factor- β 1 (TGF- β 1) [18] is involved in the development of the marrow fibrosis typically seen in HCL.

Functionally, CXCR4 and VLA-4 are critical for migration, adhesion, and retention of normal hematopoietic progenitors within the marrow [19]. Neoplastic B cells utilize these molecules to access and parasitize within protective marrow niches that normally are occupied by hematopoietic progenitors [20]. Marrow stromal cells (MSCs) constitutively express the ligands for CXCR4 and VLA-4 (CXCL12, and VCAM1 and FN, respectively; Figure 2), and contact between MSCs and malignant B cells induces cell adhesion-mediated drug resistance (CAM-DR), a primary drug resistance mechanism that may account for minimal residual disease after conventional therapies. Given the high

Figure 2. Cellular and molecular interactions between HCL cells and their microenvironment. HCL cells express CXCR4 chemokine receptors and adhesion molecules for adhesion to MSCs, endothelial cells, and extracellular matrix. These pathways are critical for homing and retention within the marrow and possibly also the spleen. In these tissues, HCL cells can also interact with other accessory cells, such as T cells, and may become activated via the BCR. It is currently unknown whether BCR-related kinases (Syk, Btk, PI3K) play a role in BCR signaling in HCL; however, these kinases and CXCR4 are currently targeted in first clinical trials in patients with other mature B-cell malignancies. Cross-talk between HCL cells and their microenvironment leads to activation of downstream pathways in HCs, such as mitogen activated protein (MAP) kinases and the nuclear factor κ B (NF κ B) pathway.

affinity of HCL cells for homing and retention within the marrow, and the high expression of CXCR4 and VLA-4 integrins on HCL cells, we speculate that these molecules induce HC adhesion to MSCs and thereby retention and protection of HCs in the marrow. Ongoing functional *in vitro* studies will determine the relevance of CXCR4 and VLA-4 for MSC adhesion and drug resistance in HCL.

Importance of B cell antigen receptor in hairy cell leukemia microenvironment

The very *raison d'être* of mature B-cells is their antigen (Ag) receptor. It follows that in mature B-cell malignancies the concept of the microenvironment as a regulator of malignant B-cell growth is tightly linked to the possible role of Ag stimulation [21]. Chronic B cell antigen receptor (BCR) stimulation by latent microbial or auto-Ag can trigger the development and expansion of malignant B-cells. The majority of HCL cases display mutated immunoglobulin variable region genes (M-HCL) [22] and a restricted set of BCR with immunoglobulin (Ig) heavy chain variable (V) gene sequences that are identical or stereotyped in subsets of patients [23–27], suggesting that these BCRs bind similar antigens

that are relevant to the pathogenesis of HCL. A minority of cases are unmutated (UM-HCL) [22] and apparently more responsive to BCR triggering, whereas the BCR of M-HCL may be less sensitive to stimulation by antigen [22]. The issue becomes how we might translate these BCR-related insights into therapeutic approaches. The possibility of targeting signal transduction pathways activated in HCs by microenvironmental interactions that activate the BCR exists, considering the impressively rapid progress in the field of BCR-related signaling inhibitors. In patients with CLL, inhibition of BCR signaling is currently emerging as a promising new therapeutic approach, using specific spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (Btk), or phosphatidylinositol 3-kinase (PI3K) inhibitors. This raises the question whether subgroups of patients with HCL, such as those with UM-HCL, may be candidates for alternative treatments with these new, targeted agents.

T cell interactions with hairy cells

Immunodeficiency in patients with HCL is thought to be related, at least in part, to T cell dysfunction [28,29]. Interestingly, T cells from the blood and spleen of most patients with HCL are clonally expanded and show a restricted and skewed repertoire of the T-cell receptor β family [30], suggesting activation and expansion of oligoclonal T cells in response to the HCL clone. Such T cells can recognize and then become activated by autologous, CD40-activated HCs [31]. The key question remains whether such HC-reactive T cells suppress or stimulate the HCL clone. The fact that CD40 crosslinking is a very potent stimulus for HC proliferation [32] indicates that interactions between HCs and CD40 ligand (CD154) expressing T cells in tissue microenvironments would result in disease progression, rather than suppression (Figure 2).

Targeting the microenvironment in hairy cell leukemia: CXCR4 and B cell receptor signaling

Overall, we are only starting to learn which pathways deliver critical survival and drug-resistance signals in the complex interactions that occur between mature B-cell malignancies and their microenvironments [2]. In this context the bone marrow is a tissue of crucial importance, given that the marrow is a common site of minimal residual disease (MRD) and the source of relapses in patients with B-cell tumors such as HCL. One possible explanation is that, while conventional treatment eliminates the bulk of clonal elements, residual HCs lurk in protective niches where they receive signals from

accessory cells that promote survival and drug-resistance. It is reasonable to suggest that these niches may have similarities with niches that normally protect hematopoietic progenitors, including the presence of stromal cells, T cells, and endothelial cells [20]. Given the importance of CXCR4 for malignant B-cell adhesion to MSCs [33], and more generally, its significance for cancer stem cell homing to protective niches [34], CXCR4 antagonists (Plerixafor/AMD3100, or T140 analogs) could be useful for mobilizing HCs for a more effective exposure to anticancer drugs. However, this approach would also co-mobilize normal hematopoietic progenitor cells and expose them to cytotoxic drugs outside their protective niches, which might result in increased toxicity. Ongoing and future clinical trials using CXCR4 antagonists will have to determine whether malignant B-cells have the same mobilization threshold as normal stem cells. Combinations of CXCR4 antagonists with drugs that target predominantly the malignant cells, such as anti-CD20 monoclonal antibodies, could help avoid this potential hazard.

An alternative, similarly exciting therapeutic approach is related to BCR signaling. BCR signaling is increasingly recognized as a central patho-mechanism in B-cell malignancies, including CLL [21,35] and diffuse large B-cell lymphoma (DLBCL) [36]. New targeted agents that interfere with BCR signaling, such as Syk and Btk inhibitors, are entering the clinical stage and show promising results in first clinical trials in patients with CLL and other B-cell malignancies [37,38]. Because of these emerging new therapeutic options it is important to identify patients who may benefit from these new approaches and/or become candidates for treatment at an early stage of their disease. Some data suggest that UM-HCL cells are more responsive to BCR stimulation [22] and hence could be more responsive to therapeutic approaches targeting the BCR.

Collectively, new therapeutic approaches that target the cross-talk between HCL cells and their microenvironment, rather than the HCs alone, could become a clinical reality in the near future. Targeting either CXCR4 chemokine receptor or BCR signaling could be explored in HCL, given that effective agents are already in clinical use in patients with other B-cell malignancies. The question will become how to best explore the activity of these drugs (i.e. in untreated or treated patients, alone or in combination with standard therapy, or for treatment of residual disease). After establishment of purine nucleoside analogs as standard therapy for HCL, such disease biology-oriented approaches are the next logical step for improving the outcome in HCL. Hopefully, such

efforts will help eliminate residual disease, and thereby improve the outcome of patients with HCL.

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