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CML cell trafficking: Influence of the stromal microenvironment

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ABSTRACT

Chronic myeloid leukemia (CML) is a malignancy of hematopoietic stem cells. The disease is characterized by abnormal (excessive) proliferation and aberrant trafficking of transformed progenitor cells. This includes early release of these cells into the bloodstream of patients, which is likely due- at least in part- to adhesion receptor abnormalities that affect the interaction of malignant cells with bone marrow stroma and important extracellular matrix proteins. Aberrant trafficking is also contrarily characterized by homing of transformed cells to stroma in the bone marrow and spleen and other tissue sources characterized by high stromal content. This is a fate that- due to the cytoprotective effect of stroma on drug-treated leukemia cells- can lead to development of minimal residual disease in patients undergoing therapy with targeted inhibitors such as imatinib and nilotinib. This review will briefly discuss these events, as well as novel therapeutic strategies designed to override stroma-associated drug resistance in CML.

CML AND STROMA

CML is a hematologic malignancy caused by the t(9;22) chromosome translocation product and oncogene, BCR/ABL [1]. Aberrant proliferation of CML progenitor cells occurs, as well as untimely liberation of these cells into the bloodstream. The mechanisms underlying the abnormal trafficking and proliferation of CML progenitor cells as compared to untransformed hematopoietic cells have been attributed to altered integrin function or expression, possibly leading to altered adhesion to stroma and fibronectin, an important extracellular molecule of the bone marrow microenvironment [2-9]. BCR-ABL itself may cause integrin defects in CML cells that play a role in aberrant trafficking, and BCR-ABL-altered cellular adhesion may increase cell cycling as well as release of leukemia cells out of bone marrow and into the bloodstream and extramedullary regions [2-9].

In addition to stromal cell interaction with BCR-ABL-expressing cells influencing CML cell trafficking, stromal cells have also been implicated

in drug resistance and minimal residual disease in CML patients treated with tyrosine kinase inhibitors (TKIs), such as the frontline CML therapy, imatinib (STI571; Glivec; Novartis Pharma AG) [10, 11] and the second generation Abl inhibitor, nilotinib (NVP-AMN107-NX; Tasigna, Novartis Pharma AG) [12]. In clinical trials, CML reemerges in over half of the patients who discontinue imatinib therapy after having maintained a complete molecular response (defined as at least a 5 log reduction in BCR-ABL mRNA expression) [13]. BCR-ABL-expressing leukemic stem cells that continue to reside in the bone marrow of tyrosine kinase-treated patients having sustained undetectable molecular residual disease may contribute to patient relapse following cessation of drug treatment [14]. Bone marrow stromal cells, which secrete growth factors and protect leukemia cells from targeted inhibitors, have thus been implicated in the development of minimal residual disease comprised of CD34+ cells that, despite expressing BCR-ABL, are insensitive to ABL inhibitors [15].

Using an in vivo bioluminescence model of CML, we tracked the homing and progression of luciferase-expressing leukemia cell growth, which involved monitoring the relative localization of tumor burden in different tissue sources in untreated mice, as well as that of residual disease in nilotinib-treated NCr nude mice [16]. We found that, in both vehicle-treated and nilotinib-treated mice, leukemia appeared to migrate to stroma-associated tissues, with the regions that showed the highest luminescence (a measure of tumor burden) also being those that were highest in stromal content. These results, which showed a leukemia distribution pattern reminiscent of that observed in CML patients treated with imatinib and nilotinib, support the notion that significant reservoirs for tumor growth are likely tissues that are able to support stem cell development.

In our study, both the spine and the spleen of vehicle-treated control mice were found to have the highest relative tumor burden, whereas only the spine of nilotinib-treated mice showed the highest relative tumor burden. Splenic stroma is a notable source of soluble growth factors associated with hematopoiesis [17] and diseased splenocytes provide viability signals, including IL-2, IL-6, macrophage chemoattractant protein-5 (MCP-5), soluble tumor necrosis factor receptor 1 (sTNFR1), tumor necrosis factor-alpha, and vascular endothelial growth factor-A (VEGF-A), which lead to growth and survival of leukemic cells [18]. Thus, in addition to bone marrow stroma, splenic stroma appears to have a significant influence on the proliferation and viability of leukemic cells. Indeed, splenomegaly, or spleen enlargement, is a characteristic of late-stage CML [19].

Recent studies have identified factors, such as c-Myb-dependent Slug expression [20], which are believed to be essential for the homing of CML to the bone marrow. Other studies have in contrast found proteins, such as Crk-associated substrate lymphocyte type (Cas-L), to have a negative influence on CML infiltration into tissues such as spleen [21]. Signaling pathways and signaling molecules, such as Src, may play a role in supporting leukemic cell survival under hypoxic conditions, which is a feature characteristic of the bone marrow stromal microenvironment and which has an influence on drug sensitivity and leukemic cell progression [22].

The provision of viability signals by the bone marrow stroma, including granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and stem cell factor (SCF), has been found to enhance hematopoietic stem cell proliferation as well as a block in terminal differentiation [23-29]. This led us to investigate the possibility that stromal-secreted cytokines might be able to confer cytoprotection to tyrosine kinase inhibitor-treated BCR-ABL-expressing cells. We cultured imatinib- and nilotinib-treated primary CML cells and a human CML cell line with conditioned media pooled from human stromal cells, and observed that the stromal-conditioned media (SCM) partially protected the leukemic cells from the inhibitory effects of both agents [16]. Our results supported the notion that stromal-mediated chemoresistance in the context of CML is at least in part likely attributed to stromal-derived viability signals. A more in-depth analysis ensued with the aim of identifying specific cytokines capable of mimicking the effects of SCM [16]. In parallel with SCM, we tested the effects of a cytokine cocktail consisting of stromal-derived factor-1 (SDF-1), a bone marrow-secreted chemoattractant that supports stem cell homing, as well as a panel of cytokines secreted in high concentrations from

human stromal cells [30]. Indeed, the cocktail, consisting of SDF-1, interleukin (IL)-6, IL-8, IL-11, GM-CSF, and M-CSF, enhanced the growth of BCR-ABL-expressing cells and partially protected imatinib-treated leukemic cells to a similar extent to SCM. The level of protection conferred by each individual cytokine was less than the complete cytokine cocktail, suggesting that two or more growth factors are likely needed for maximum cytoprotection [16]. Our findings were consistent with other published findings that demonstrated cytokine protection of chemotherapy-treated myeloid leukemia cells [31].

NOVEL THERAPEUTIC STRATEGIES TARGETING STROMAL CYTOPROTECTION OF CML

Novel approaches to overriding stromal-mediated chemoresistance contributing to minimal residual disease in CML patients have been proposed. As we and others have found that viability signals play an integral role in chemoresistance due to the stromal microenvironment, one such strategy involves blockade of apoptotic signaling through inhibitor of apoptosis (IAP) inhibition. We thus investigated the ability of pro-apoptotic agents, such as IAP inhibitors, to potentiate the effects of ABL inhibitors and, in effect, delay or prevent the onset of residual disease. Effective inhibitors of the IAP family of proteins [32, 33] have been developed, such as LBW242 [34], and its structural analog, LCL161, which bind and impede the activity of multiple IAPs. We tested the ability of LCL161 to significantly delay disease recurrence in mice harboring BCR-ABL-positive cells and treated for several weeks with a moderate-to-high dose of nilotinib [35]. Using an in vitro model of stromal-mediated chemoresistance, as well as an in vivo model of progressive and residual disease, we demonstrated the ability of LCL161 to potentiate the inhibitory effects of nilotinib against leukemic disease. Specifically, LCL161 synergized in vivo with nilotinib to lower leukemia burden significantly below the basal suppression exhibited by a moderate-to-high dose of nilotinib. These results support the notion of using IAP inhibitors in combination with ABL inhibitors to suppress or eliminate progressive and drug-resistant/residual disease. LCL161 is being considered for testing in clinical trials for leukemia; early clinical trials in advanced solid tumors are ongoing.

Another putative approach to overriding the cytoprotective effect of stroma on TKI-treated leukemic cells involves the administration of antagonists of the SDF-1 receptor, CXCR4. This receptor is integral to stromal cell:leukemic cell interactions, as a primary function of CXCR4 is to mediate hematopoietic cell migration to bone marrow stroma. CXCR4 expression and signaling are diminished in cells that express BCR-ABL, [36, 37], whereas imatinib or nilotinib inhibition of BCR-ABL kinase activity increases CXCR4 surface expression, an effect prompting CML cells to migrate to bone marrow stroma where they are protected and resistant to TKI therapy [36-38]. As CXCR4 antagonists steer leukemic cells away from cytoprotective stroma, they are potentially effective in potentiating the apoptosis-inducing effects of TKIs like imatinib [39]. Of relevance, plerixafor (AMD3100; Genzyme), an antagonist/partial agonist of CXCR4 and allosteric agonist of CXCR7 [40], has been demonstrated to augment the cytotoxic effects of chemotherapy- or tyrosine kinase inhibitors on stroma-protected acute myeloid leukemia (AML) [41-43], multiple myeloma [44, 45], and CML [38, 46].

In CML, potentiation of tyrosine kinase activity by plerixafor has been demonstrated via measurement of the percentages of human CD19+ cells that engrafted in the BM and spleen of mice following co-cultivation of imatinib-treated BCR-ABL-expressing cells with mesenchymal stromal cells and pre-treatment with plerixafor [46]. In this study, there were substantial reductions in engraftment for cells pretreated with plerixafor followed by secondary treatment with imatinib, which suggests a restoration of ABL inhibitor sensitivity of the cells by plerixafor. We recently tested the synergizing potential of plerixafor in our in vivo assay system [35] that allows monitoring of progressive disease and baseline level (or "residual") disease following treatment with a moderate-to-high dose of nilotinib [47]. We found that leukemic stem cell mobilization into the peripheral blood of mice, induced by plerixafor, enhances the efficacy of nilotinib by inhibiting leukemia recurrence post-nilotinib therapy. Specifically, nilotinib initially effectively reduced leukemia burden in mice, however with continued treatment, nilotinib resistance was evident as tumor burden increased despite prolonged drug

exposure. Plerixafor demonstrated no single agent activity, however combination with nilotinib significantly postponed time to relapse and also significantly extended survival when compared to treatment with nilotinib treatment alone ($p<0.0001$). Plerixafor was thus demonstrated to act synergistically with nilotinib when administered at a well-tolerated dose to inhibit the growth of BCR-ABL-positive leukemia.

In support of our in vivo results, plerixafor was observed in vitro to decrease the migration of BCR-ABL-expressing cells, as well as diminish leukemic cell adhesion to bone marrow stromal cells, fibronectin, and endothelial cells. Plerixafor was also demonstrated in an in vitro co-culture system to reverse the cytoprotective effect of stroma on nilotinib induction of apoptosis of CML cells. All results taken together support the notion that plerixafor interferes with leukemic:stromal cell interaction and in effect sensitizes BCR-ABL-positive cells to tyrosine kinase inhibition.

Our results differed with those of another report [48], which tested the combination of plerixafor with imatinib or the second generation ABL inhibitor, dasatinib (BMS-354825, Sprycel, Bristol-Myers Squibb) [49] in a murine CML retroviral transduction/transplantation model mimicking highly active CML. According to this study, the combination of plerixafor and the TKIs- in contrast to our findings- promoted extramedullary leukemic infiltrations in vital organs, including the brain, with no reduction in leukemia burden observed. Differences in the murine models used are believed to account for the disparate results between the two studies. Irradiation of mice, which was not employed in our CML model, is believed to have damaged the blood-brain barrier in the murine model utilized by Agarwal et al. Of relevance, others have reported that in non-irradiated mice, plerixafor does not cause bone marrow-derived cells to infiltrate the brain [50]. In addition, our model attempted to mimic minimal residual disease and to effectively demonstrate an effect of plerixafor on stem cells. In the Agarwal et al. model, the comparatively high leukemia burden in mice left little margin to show a cooperative effect between plerixafor and TKIs, and this was coupled by plerixafor-enhanced toxicity due to the extramedullary infiltration and migration of leukemic cells into vital organs stemming from irradiation. Finally, differences in in vivo efficacy and stability/half-life between nilotinib, imatinib, and dasatinib may also account for differences in results between the studies. Thus, we conclude that stem cell mobilization combined with TKI therapy is a potentially effective strategy aimed at suppressing or eradicating minimal residual disease in CML patients.

CONCLUSION

The significant role of stroma in CML cell trafficking is apparent from the numerous studies that have been carried out over the past couple of decades. Interference with stroma:leukemia cells interaction due to abnormal adhesion properties likely contributes to enhanced proliferation of leukemic cells and their premature release out of the bone marrow and into the peripheral blood of patients. The homing of CML cells to stroma negatively influences the effectiveness of TKI therapy due to envelopment of leukemic cells in a cytoprotective environment that leads to development of minimal residual disease. Novel approaches to overriding stromal-associated chemoresistance, such as the use of proapoptotic agents in combination with TKIs, and the administration of CXCR4 antagonists with TKIs, show promise in preclinical studies and will hopefully lead to improved CML patient response and survival if developed as anticancer therapeutics.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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