

952 CXCR4 Antagonist BKT140 Synergizes with Rituximab, Targeting Non Hodgkin Lymphoma (NHL) In Vitro and In Vivo in a Xenograft Model with Bone Marrow Involvement

Program: Oral and Poster Abstracts

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Session: 622. Non-Hodgkin Lymphoma - Biology, excluding Therapy: Clinical Determinants and Microenvironment

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Room 5AB (San Diego Convention Center)

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Background: B-cell Non Hodgkin lymphoma (NHL) represents the most common malignant lymphoid neoplasm, with heterogeneous pathophysiological and clinical presentation. It may show systemic, nodal or extra nodal localization in various sites, including bone marrow (BM). Although anti-CD20 antibody rituximab significantly improved the outcome of NHL patients, the relapsed/refractory rates are still high. Chemokine receptor CXCR4 and its ligand CXCL12 are critically involved in the survival and trafficking of normal and malignant B lymphocytes. Taking together with the fact that interaction of malignant B cells with stromal cells via CXCR4/CXCL12 signaling may provide chemo-resistance, we hypothesized that blockade of CXCR4 may antagonize the survival and spreading of lymphoma cells and restore their chemo-sensitivity.

Results: The effect of CXCR4 antagonist BKT140 on lymphoma cell growth and rituximab-induced cytotoxicity was tested in vitro and in vivo. Inhibition of CXCR4 with BKT140 in CD20-expressing lymphoma cell lines (BL-2, BJAB, Raji, Ramos, SUDHL4, OCI-Ly7) and primary lymphoma cells from patients with BM involvement resulted in significant inhibition of cell growth and in the induction of cell death, respectively. Combination of BKT140 with rituximab significantly enhanced the cytotoxic effect (apoptosis) against the lymphoma cells in a dose-dependent manner ($p < 0.01$). This effect was concurrent with increase in Annexin V binding, loss of $\Delta\psi_m$ and caspase 3 cleavage. These findings indicate that mitochondrial membrane dysfunction and caspase 3 activation are involved in BKT140- and rituximab-mediated lymphoma cell death. Moreover, we were able to show that rituximab induced CXCR4 expression in lymphoma cell lines and primary lymphoma cells, both on mRNA and cell-surface levels, suggesting the possible interaction between CD20 and CXCR4 pathways in NHL. This interaction provides the rationale for CXCR4 targeting in combination with rituximab treatment. Indeed, highly-expressing CXCR4 BL-2 cells were protected by BM stromal cells from rituximab-induced apoptosis, whereas low-CXCR4 BJAB cells were not. The protective effect of stroma was abrogated by BKT140. To evaluate the in vivo anti-lymphoma effect of BKT140 we established a xenograft model of B-cell lymphoma with BM involvement in mice. Human CXCR4-expressing B NHL cell line, BL-2, was subcutaneously implanted into NOD/SCID mice, resulting in the development of aggressive local tumors which specifically spread to the bone marrow. Following the tumor establishment, mice were injected subcutaneously with BKT140, rituximab or with combination of both. BKT140 inhibited the local tumor progression of BL-2 generated tumors. Furthermore, BKT140 treatment significantly reduced the number of BL-2 tumor cells in the BM by 77% ($p < 0.01$) compared to the untreated mice, while rituximab decreased this number only by 20%. Importantly, the combination treatment of BKT140 with rituximab further decreased the number of viable lymphoma cells in the BM, achieving 93% reduction ($p < 0.012$). We found that BKT140 promoted lymphoma cell apoptosis within the bone marrow microenvironment. Furthermore, we found that the number of viable lymphoma cells in the peripheral blood of tumor-bearing animals was also reduced following the treatment with BKT140.

Conclusions: Our results demonstrate potent anti-lymphoma effect of CXCR4-specific antagonist BKT140 in vitro and in vivo. The BKT140-mediated anti-lymphoma effect synergizes with that of Rituximab. Moreover, BKT140 effectively targets lymphoma cells in the bone marrow microenvironment, overcoming the stroma-induced resistance to rituximab. These findings suggest the possible interaction between CD20 and CXCR4 pathways in NHL, and provide the scientific basis for the development of novel combined CXCR4-targeted therapies for refractory NHL.

Disclosures: No relevant conflicts of interest to declare.

See more of: 622. Non-Hodgkin Lymphoma - Biology, excluding Therapy: Clinical Determinants and Microenvironment

See more of: Oral and Poster Abstracts

1801 CXCR4 Promotes the Tumorigenicity of Multiple Myeloma, Including Increased Motility, Clonogenicity, up-Regulation of VLA-4, Protection From Chemotherapy and Aggressive Tumor Development In Vivo

Program: Oral and Poster Abstracts

Session: 651. Myeloma - Biology and Pathophysiology, excluding Therapy: Poster I

Saturday, December 10, 2011, 5:30 PM-7:30 PM

Hall GH (San Diego Convention Center)

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Background: Multiple myeloma (MM) is by large incurable neoplasm of plasma cells, characterized by accumulation in the bone marrow (BM), in close contact to cellular and extracellular matrix (ECM) components. Chemokine receptor CXCR4 is expressed by the majority of patients' MM cells. It promotes myeloma cell migration and homing to the BM compartment, supports the tumor cells survival and protects the myeloma cells from chemotherapy-induced apoptosis. Further investigation is required to define the specific molecular mechanisms regulated by the CXCR4/CXCL12 axis in MM. However, surface CXCR4 is commonly down-regulated in the MM cell lines. In order to overcome this limitation, the aim of the current study was to produce a reliable model for studying the functional role of high CXCR4 in MM by generating MM cell lines with stable expression of surface CXCR4.

Results: To over-express CXCR4, we transduced CXCL12-expressing MM cell lines ARH77 and RPMI8226 with lentiviral vector and generated cell lines with high and stable levels of surface CXCR4. Enhanced CXCR4 expression significantly increased the in vitro survival and growth of the 2 MM cell lines in serum-deprivation conditions ($p < 0.01$). Furthermore, elevated expression of surface CXCR4 prominently increased MM cells motility and promoted CXCL12-dependent transwell migration of the transduced MM cell lines. Highly CXCR4-expressing RPMI8226 and ARH77 cells demonstrated 40% migration in response to CXCL12 (50 ng/ml), versus only 0-5% migration of MM cells with low expression of surface CXCR4 ($p < 0.01$). Furthermore, adhesion of MM cells to either ECM proteins or BMSCs localize the malignant PCs within the BM microenvironment, promote growth and survival of MM cells and play a critical role in myeloma bone disease and tumor invasion. In accordance, we observed induced adhesion of the transfected RPMI8226-CXCR4 cells to ECM components fibronectin and laminin and to BM fibroblasts. Moreover, we found that enhanced CXCR4 not only functionally activates, but rather significantly elevates the surface levels of VLA-4 integrin on the RPMI8226 cells. In addition, we found that CXCR4-expressing MM cells were less sensitive to melphalan- and bortezomib-induced apoptosis, when they were co-cultured with BM fibroblasts. Testing the molecular signaling pathways regulated by CXCR4, we found that elevated CXCR4 increased the basic level of pERK1/2 and pAKT in the MM cells, and promoted their prolonged activation in response to CXCL12 stimulation. Finally, the ability to produce colonies in the soft agar semi-solid culture reflects the tumorigenic capacity of cancer cells and cancer stem cells. Differentiated MM cells thus rarely produce colonies in soft agar. Here, we demonstrate that up regulation of CXCR4 promoted ARH77 and RPMI8226 colony formation, significantly increasing colonies number and size. Lastly, we determined the role of CXCR4 in MM tumor development in vivo. CXCR4-expressing ARH77 and RPMI8226 cells were subcutaneously injected into NOD/SCID mice. CXCR4-expressing cells, but not parental cell lines, produced detectable tumors already 10 days after the injection. Rapid tumor growth was further observed in both CXCR4-expressing cell lines. These findings indicate that CXCR4 provided aggressive phenotype and supported MM growth in vivo.

Conclusions: Taken together, our findings clearly demonstrate the important pathophysiologic role of CXCR4 in MM development and progression. Furthermore, for the first time, we provide the evidence for CXCR4 oncogenic potential in MM, showing that CXCR4 promotes the clonogenic growth of MM cells. Our model may further serve to elucidate CXCR4-regulated molecular events potentially involved in the pathogenesis of MM, and strongly support targeting CXCR4 as therapeutic tool in MM.

Disclosures: No relevant conflicts of interest to declare.

See more of: [651. Myeloma - Biology and Pathophysiology, excluding Therapy: Poster I](#)
See more of: [Oral and Poster Abstracts](#)

Multiple Myeloma and Microenvironment Formation: The Role of CXCR4/CXCL12 Chemokine Pathway

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Abstract 2962

Background: Multiple myeloma (MM) is characterized by clonal proliferation of malignant plasma cells (PCs) in the bone marrow (BM) compartment. Interaction of plasma cells with the BM stromal cells (BMSCs) is critical for homing, growth and drug resistance acquisition of the malignant PCs. However, the functional significance of other cellular components of the MM milieu, which includes osteoclasts and immune effector cells, is less clear. Both MM-derived and stromal cell-produced factors, including cytokines and chemokines, are believed to participate in the cross-talk between the MM and stroma leading to disease progression.

Aim and Results: We hypothesized an important role for CXCL12 (SDF-1) chemokine and its receptor CXCR4 in MM-stroma interactions and microenvironment formation. We now show that MM cell lines ARH77 and RPMI8226 and primary MM cells may produce high amounts of CXCL12 and co-express CXCR4 receptor. Co-culture of the MM cells with BMSCs significantly up-regulated both CXCR4 cell-surface expression and CXCL12 secretion by the MM cells. Enhanced CXCR4 signaling in the MM cells upon the interaction with BMSCs promoted the survival and proliferation of the cells in an autocrine way. Moreover, the paracrine effect of increased CXCL12 production on immune cell migration was tested. We found, that conditioned medium (CM) produced by MM cells cultured with BMSCs specifically attracted increased numbers of CXCR4-expressing PB CD14+ cells. Furthermore, CXCR4 inhibition, using neutralizing antibodies toward CXCR4, inhibited the MM-induced migration of CD14+ monocytes, suggesting the possible role of CXCR4/CXCL12 axis in monocyte recruitment to the site of the disease. We next examined the functional consequence of MM-macrophage interaction. We saw that PB-generated macrophages induced the proliferation of MM cells, even more effectively than BMSCs. Furthermore, co-culture with macrophages strongly increased the expression of various pro-inflammatory and pro-angiogenic factors by MM cells, including CCL2 (MCP-1), CCL4 (MIP1a), IL-1b, IL-8 and VEGF. Interestingly, expression of IL-10 by MM cells was also up-regulated following the interaction with macrophages, suggesting the possible reciprocal effect of MM-produced factors on macrophage phenotype polarization.

Conclusion: Taken together, our findings demonstrate that interaction of MM with BM stromal cells positively regulates the expression of CXCR4 and CXCL12 by MM cells, affecting both MM proliferation and CXCR4-dependent monocyte recruitment. The migrated monocytes may in turn interact with MM cells, support their growth and activate cytokine release, therefore producing favorable pro-inflammatory and pro-angiogenic environment and promoting disease progression. Overall, our data provide the basis for future targeting MM-BMSCs and MM-macrophage interactions with anti-CXCR4 agents as a therapeutic strategy to improve the outcome of patients with MM.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH

Anti-Leukemia and Multiple Myeloma Selective Activity of CXCR4 Antagonist 4F-Benzoyl-TN14003 Involves Apoptotic Death Pathway.

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Abstract 3857

Poster Board III-793

The chemokine receptor CXCR4 and its ligand CXCL12 are involved in the development and progression of a diverse number of hematological malignancies, including leukemia, lymphoma and multiple myeloma (MM). Binding CXCL12 to CXCR4 activates a variety of intracellular signal transduction pathways and effector molecules that regulate cell chemotaxis, adhesion, survival, apoptosis and proliferation. It was previously shown that CXCR4 signaling can directly induce caspase-independent cell apoptosis through the interaction with the HIV gp120 envelope protein. In the present study we investigated the effect of CXCR4 specific antagonists 4F-benzoyl-TN14003 (T140) and AMD3100 on the survival and proliferation of different human hematological cancer cells. Here, we demonstrate that T140, but not AMD3100, exhibits preferential cytotoxicity towards malignant cells of hematopoietic origin, as compared to primary normal cells or solid prostate and breast tumor cells. The in vitro treatment with T140, but not with AMD3100, significantly decreased the number of viable chronic myeloid leukemia K562 cells, acute T cell leukemia Jurkat cells, acute promyelocytic leukemia NB4 and HL60 cells, and four

different MM cell lines (U266, NCI-H929, RPMI8226 and ARH77), demonstrating the highest sensitivity to T140 ($p < 0.01$). Notably, T140 inhibited the growth of freshly isolated leukemia and MM cells obtained from consenting patients. T140 inhibits the growth of MM and leukemic cells by inducing their apoptotic cell death. The apoptotic changes in the cells were associated with morphological changes, phosphatidylserine externalization, sub-G1 arrest, DNA double-stranded breaks, decrease in mitochondrial membrane potential, release of cytochrome c, and caspase 3 activation. The important role of CXCR4 in T140-mediated cell death was confirmed by demonstrating that CXCR4 over-expression in NB4 and K562 cells increased their sensitivity to T140. Furthermore, pretreatment of NB4 and HL60 cells with AMD3100 abolishes the effect of T140 on these cells, indicating the involvement of CXCR4 in T140-induced apoptosis. Importantly, the combination with novel anti-myeloma agent bortezomib significantly augments anti-myeloma activity of T140. The anti leukemic and MM effect of T140 was confirmed in xenograft in vivo tumor models. Subcutaneous (s.c.) or intra-peritoneal (i.p.) injections of T140 (100 or 300 mcg/mouse) significantly reduced, in a dose-dependent manner, the tumor size in immuno-deficient mice that were previously inoculated s.c. with human acute leukemia cells NB4 or MM cells RPMI8226 ($p < 0.01$). Tumors from animals treated with T140 had smaller sizes and weights, larger necrotic areas and high apoptotic scores. Taken together, these data support the unique anti-cancer effect of T140 in hematological malignancies and indicate the potential therapeutic role of T140 in MM and leukemia patients.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH members.