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Role of Chemokine Receptor4 (CXCR4) Expression in Acute Myelogenous Leukemia

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Abstract:

Acute myeloid leukemia (AML) is a hematopoietic clonal malignancy that is characterized by uncontrolled proliferation of hematopoietic stem cells (HSCs) and progenitors without the capacity to differentiate into mature cells. The treatment and prognosis of AML patients depend on accurate cytogenetic and genetic examinations. Despite the fact that various novel reagents have demonstrated clinical activities, long-term outcomes of AML patients remain poor, which is mainly due to resistance to chemotherapy and disease relapse post-chemotherapy. CXC chemokine receptor 4 (CXCR4) expression on acute myeloid leukemia (AML) cells correlated with stromal cell derived factor-1α (SDF-1α) and retained hematopoietic progenitors and leukemia cells within the bone marrow microenvironment.

Keywords: Chemokine Receptor4 Expression, Acute Myelogenous Leukemia, CXCR4.

Introduction:

Chemokines are small peptides with molecular weights of 8-12 KDa, which are secreted by multiple types of cells, such as immune cells, stromal cells and tumour cells. Chemokine receptors are seven-transmembrane G-protein-coupled receptors (GPCRs), and one receptor can bind to multiple chemokines. Conversely, one chemokine can recognize several receptors. CXCR4 was first discovered as a cofactor facilitating the entry of human immunodeficiency virus (HIV) into CD4+ T cells and was then classified into GPCR subfamily. CXCR4 is widely expressed in many types of cells, including haematopoietic stem cells (HSCs), T lymphocytes, B lymphocytes, monocytes, macrophages, epithelial cells, endothelial cells and neurons. Stromal-derived factor 1 (SDF-1), also known as CXCL12, is the only ligand for CXCR4 but can also bind to CXCR7. After the engagement of SDF-1 and CXCR4, many intracellular pathways are activated, including RAS-MAPK, PI3K-AKT-mTOR and JAK-STAT, which then regulate chemotaxis, gene expression and cell survival. Sdf1 or Cxcr4 homozygous mutations in mice resulted in embryonic lethality, and the development of B lymphocytes and myeloid cells was severely impaired **(1).**

Other defects, including cardiac ventricular septal defect and defective formation of large vessels supplying the gastrointestinal tracts, were found. HSCs express high levels of CXCR4 and can migrate from the foetal liver to the bone marrow (BM) along with the SDF-1 gradient participating in the transformation of haematopoietic sites in different stages of individual development. After birth, SDF-1 secreted by stromal cells recruits HSCs into the BM niche to regulate quiescence or proliferation. CXCR4 contributes to lung alveolar

regeneration after pneumonectomy. SDF-1 expression is upregulated after tissue injury, promoting the migration of CXCR4+ adult stem cells to injury lesions to protect or repair infarcted cardiac and ischaemic cerebral tissues. The treatment of ischaemic diseases by mobilized tissue-committed stem cells was reviewed by Kwon et al. 16 CXCR4 is a co-receptor for the entry of HIV type 1 (HIV-1) into CD4+ T cells, which was prevented by SDF-1. Therefore, increasing efforts have been made to develop new CXCR4 antagonists to control HIV infection (reviewed by Zhang et al). CXCR4 also plays important roles in the development, invasion, angiogenesis, epithelial–mesenchymal transition and maintenance of stemness of tumour cells and targeting CXCR4 is a potential therapeutic strategy for treating malignant tumours **(2,3).**

Role of CXCR4 in AML

- **CXCR4 participates in homing and residence of AML cells in BM.**

CXCR4 was critical for murine BM engraftment by human severe combined immunodeficient repopulating stem cells. Human cells pretreated with CXCR4 antibodies impeded engraftment and in vitro CXCR4-dependent migration to SDF-1 of CD34+CD38-/low cells associated with in vivo engraftment and stem cell function. CXCR4 expression influences the engraftment of autologous stem cells in patients undergoing auto-HSCT. Significantly faster haematologic recovery was found in patients who received transplanted CD34+ cells that showed high spontaneous and SDF-1-induced migration. Therefore, SDF-1/CXCR4 plays a critical role in homing to and retention in the BM of normal HSCs **(2).**

Like normal HSCs, CXCR4 is also closely associated with the migration of AML cells. Higher SDF-1-induced migration was observed in AML for CD34+ BM-derived cells than in paired CD34+ peripheral blood (PB)-derived cells, and a lower percentage of circulating leukaemia blasts in patients with a relatively high level of SDF-1 induced migration indicated the role of CXCR4 in the anchoring of leukaemia cells in the BM.Monaco and his collages that evaluated the engraftment of AML cell into NOD/SCID mice reported that there was No correlation between CXCR4 expression and engraftment was found, and anti-CXCR4 antibody failed to block the engraftment of AML cells. Concurrently, CXCR4-dependent engraftment of AML cells into NOD/SCID mice has been reported. Although AML cells from some patients did not express cell surface CXCR4, intracellular CXCR4 expression was detected in all samples. Pretreatment of human AML cells with neutralizing CXCR4 antibodies blocked their homing to the BM and spleen of NOD/SCID/β2Mnull mice and treating mice previously engrafted with AML cells with antibodies against CXCR4 resulted in a dramatic decrease in leukaemia cell levels in a dose- and time-dependent manner. Subsequently, a debate on whether engraftment of AML cells into mouse BM was dependent on SDF-1/CXCR4 between these two groups was published. The opposite observations may be associated with different mice used and if newly expressed CXCR4 was inhibited **(1).**

Recently, a murine MLL-AF9-driven AML model was used to evaluate the engraftment of leukaemia cells into mouse BM. The deletion of cxcr4 in AML cells eradicated leukaemia cells in vivo, but their homing to the BM was not impaired. Furthermore, SDF-1 is dispensable for the development of leukaemia in mice. Thus, CXCR4 signalling may play an essential role in AML stem cells, preventing differentiation independent of SDF-1. Using high-resolution 2-photon and confocal intravital microscopy of mouse calvarium BM, chemoresistant MLL-AF9 AML cells were found to become less motile and unaffected by AMD3100. Therefore, there may be other factors that regulate the homing and retention of AML cells within the BM. However, whether such phenomena possess leukaemia-type specificity remains unclear **(3).**

- **CXCR4 expression and its regulation in AML**

AML cells exposed to low oxygen partial pressure showed upregulated expression of CXCR4, and the underlying mechanisms involved alteration of lipid rafts. NPM1 is one of the most common mutated genes in AML, and increased CXCR4 expression was observed when NIH3T3 cells were transfected with plasmids encoding NPM1 mutation A with enhanced migration and invasion abilities. AML blasts with mutated NPM1 displayed significantly higher CXCR4 expression than those without. However, no significant correlation between NPM1 mutation and CXCR4 or phosphorylated CXCR4 (pCXCR4) expression was observed in the BM specimens of untreated AML patients. CEBPA mutations consist of unilateral and bilateral mutations, whereas only bilateral mutations indicate a favourable prognosis **(4).**

N-terminal CEBPA mutations may impair CXCR4 expression, as only CEBPA p42 can recognize the CXCR4 promoter by chromatin immunoprecipitation assays. FLT3-ITD mutation is an indicator of poor prognosis for patients with AML and associates with upregulated CXCR4 expression in a series of studies. The downstream pathways may involve STAT5 and Pim-1. Epigenetic regulation of CXCR4 expression by miR-146a has been reported in patients with different subtypes of AML. Chemotherapy-induced upregulation of CXCR4 expression was observed in both AML cell lines and clinical samples, which may represent a mechanism of treatment-induced resistance in AML. Accordingly, the expression of CXCR4 in AML is regulated by multiple mechanisms, indicating a complicated role of CXCR4 **(1).**

- **Relationship between CXCR4 expression and prognosis of AML**

The unfavourable prognostic indication of CXCR4 expression in AML has been well documented in many studies. AML patients with <20% CXCR4+/CD34+ cells had significantly superior OS and relapse-free survival (RFS) than those with ≥20%. In a prospective study, patients with AML were divided into groups with low, intermediate or high levels of CXCR4 expression, as determined by CXCR4 mean fluorescence intensity ratio thresholds of <5, 5-10 and ≥10, respectively, which resulted in significantly different outcomes. AML patients with normal karyotype showed higher percentages of CXCR4+ cases than those without, and high CXCR4 expression predicted poor prognoses in multivariate analysis. A combination of CXCR4 and VLA-4 expression can divide AML patients into different groups with various prognoses. In paediatric patients with AML, high CXCR4 expression indicated an unfavourable prognosis only in the low-risk group. Taken together, CXCR4 expression levels show prognostic indications in AML and may be a potential marker for re-stratifying the prognosis of patients with AML **(2).**

- **Targeting CXCR4 in treatment of AML**

CXCR4 small molecular antagonist AMD3100/AMD3465 The first generation of CXCR4 antagonist AMD3100 inhibited the migration of AML blasts induced by SDF-1 and their proliferation in vitro and reversed the enhanced engraftment of AML blasts into NOD/SCID mice mediated by SDF-1. Tavor et al found that AMD3100 could significantly inhibit proliferation and induce apoptosis in multiple AML cell lines and upregulate the expression of CD15 and CD11b. AMD3465 is the second generation of CXCR4 antagonist that can inhibit the migration of AML cells induced by SDF-1 and multiple intracellular signalling pathways responsible for cell survival. AMD3465 partially reversed the protective effects of stromal cells on leukaemia cells in vitro. AMD3465 alone or combined with granulocyte colony-stimulating factor (G-CSF) mobilize leukaemia cells from the BM and render them killed by chemotherapeutic drugs or sorafenib in leukaemic mice, leading to reduced leukaemia burden and prolonged survival. In a similar study of a murine APL model, AMD3100 also reversed the drug resistance of AML cells mediated by stromal cells in vitro and reduced leukaemia burden and prolonged survival of leukaemic mice when used with chemotherapy **(1).**

Cocultivation of FLT3-ITD mutated AML blasts or haematopoietic progenitor cells (HPCs) on BM stromal cells resulted in a strong proliferation advantage compared with FLT3-wide-type AML blasts, and addition of AMD3100 to the co-culture significantly reduced the proliferation of FLT3-ITD mutated cells, but did not affect FLT3-wide-type cells. AMD3100 promoted the death of leukaemia cells with high CXCR4 expression and reduced NOG leukaemia-initiating cells but had no efficacy when AML cells did not express CXCR4. This suggests that CXCR4 expression levels may be a potential marker for identifying candidates who can benefit from CXCR4 antagonists. A triple combinational therapy using AMD3100 and anti-PD-L1 plus chemotherapy was investigated in a mouse AML model. Noticeable benefits of triple combinational therapy could be achieved to eradicate leukaemia blasts that transformed into prolonged survival of mice. The frequencies of regulatory T cells (Tregs) and myeloid-derived suppressor cells in the PB of mice treated with triple combinational therapy consistently decreased. Collectively, conventional chemotherapeutic drugs, kinase inhibitors or immune checkpoint inhibitors are potential strategies to be combined with CXCR4 antagonists to enhance the eradication of AML **(2).**

New peptide or antibody antagonists of CXCR4 These antagonists not only inhibit SDF-1 or stromal cell-induced chemotaxis of leukaemia cells, but also impair the proliferation or induce death of leukaemia cells

directly. Thus, when used alone or in combinational therapies, CXCR4 antagonists were found to significantly inhibit the growth of leukaemia cells and prolong the survival of leukaemic mice. It is worth noting that LY2510924 and PF-06747143 have entered phase I clinical trials. Although some of these antagonists were suggested to be more potent than AMD3100, further preclinical and clinical studies are needed to confirm it **(2).**

Other strategies that target CXCR4 Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK), is used to treat Waldenström's macroglobulinaemia, mantle cell lymphoma and lymphoblastic leukaemia, which also inhibits SDF-1 induced AKT and MAPK activation, leading to the inhibition of the migration and proliferation of leukaemia cells. Downregulation of CXCR4 expression by small interfering RNA (siRNA) is a potential strategy to treat many diseases, including AML. Lipopolymer/siRNA complexes are used to decrease CXCR4 expression, resulting in the inhibition of AML cell proliferation and chemosensitization. Dual-function polycation (PCX)/siRNA nanoparticles can simultaneously inhibit CXCR4 expression and deliver siRNAs that target key oncogenes in AML cells. Monomethyl auristatin E conjugated with the CXCR4-targeted protein nanoparticles could be utilized to kill CXCR4+ AML cells and to reduce leukaemia burden in mice without the severe toxicity of classical AML therapeutic drugs **(5).**

CXCR4 is potential target for immunotherapy The frequencies of Tregs in PB and BM of AML patients were higher than those in healthy controls. Increased CXCR4 expression robustly promoted the migration of Tregs towards BM, which played critical roles in immunosuppression of conventional T cells through proliferation inhibition, apoptosis promotion and suppression of IFN-γ production. Using a murine MLL-AF9 AML model, blocking CXCR4 was found to reduce Treg accumulation in the leukaemia haematopoietic microenvironment and promote anti-leukaemic effects of CD8+ T cells, and delay leukaemia progression **(6-9).**

CXCR4 and differentiation syndrome Differentiation syndrome is a common complication of APL. Differentiated APL cells expressed high levels of CXCR4, and SDF-1 secreted by lung cells could help these cells migrate to lung tissues, which was reduced by pretreatment with an anti-CXCR4 antibody. Therefore, targeting CXCR4 may provide the basis for potential prophylaxis or treatment of differentiation syndrome **(2).**

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