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Role of CXCR4 in Multiple Myeloma

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Abstract:

CXCR4 is a pleiotropic chemokine receptor which acts through its ligand CXCL12 to regulate diverse physiological processes. CXCR4/CXCL12 axis plays a pivotal role in proliferation, invasion, dissemination and drug resistance in multiple myeloma (MM). Apart from its role in homing, CXCR4 also affects MM cell mobilization and egression out of the bone marrow (BM) which is correlated with distant organ metastasis. Aberrant CXCR4 expression pattern is associated with osteoclastogenesis and tumor growth in MM through its cross talk with various important cell signalling pathways. A deeper insight into understanding of CXCR4 mediated signalling pathways and its role in MM is essential to identify potential therapeutic interventions. The current therapeutic focus is on disrupting the interaction of MM cells with its protective tumor microenvironment where CXCR4 axis plays an essential role. There are still multiple challenges that need to be overcome to target CXCR4 axis more efficiently and to identify novel combination therapies with existing strategies.

Keywords: CXCR4 in Multiple Myeloma, Cancer.

Introduction:

CXCR4 (C-X-C motif chemokine receptor 4) is a widely studied chemokine receptor due to its significant role in immune response, hematopoiesis, developmental processes as well as in pro-tumorigenic functions. CXCR4 expression is ubiquitous in different hematopoietic cells. CXCR4 is also expressed in different non-hematopoietic cells. CXCR4 can bind to CXCL12, CD4 and CD74, among which CXCL12 or stromal cell-derived factor 1α (SDF-1α), acts as an exclusive endogenous ligand for CXCR4. Apart from CXCR4, CXCL12 can also bind to its second receptor CXCR7. Contrary CXCR4, CXCL12 is not expressed in hematopoietic cells but rather it is expressed and secreted in different non-hematopoietic tissue sites, most prominently in brain, lung, liver, stromal, endothelial cells and BM where it chemo attracts CXCR4- expressing hematopoietic stem cells (HSCs), thus playing a critical role in the homing of these cells in the BM microenvironment. The expression of CXCL12 in BM ensures the retention of the hematopoietic stem cells until they are needed elsewhere in the body. Therefore, inhibiting CXCL12-CXCR4 interaction can liberate HSCs from the BM niche into circulation **(1).**

CXCR4-CXCL12 interaction activates a variety of extra and intracellular signalling pathways, thus contributing to

different vital biological processes. Upon agonist stimulation, CXCR4 is rapidly phosphorylated on serine and threonine residues in its C-terminal. This is then followed by the activation of some major signalling processes such as phospholipase C (PLC)/ Protein kinase C (PKC)-dependent increase in intracellular calcium level; NF-κB, Ca2+-sensitive protein tyrosine kinase PYK2 and phosphoinositide-3 kinase (PI3K)-Akt pathways; MAPK1/MAPK3 (Mitogen Activated Protein Kinases), JNK (Jun N- terminal Kinases) and PI3K (Phosphoinositide-3 Kinase) activation- dependent processes; Janus kinase signal transducer and activator of transcription (JAK/STAT) pathway; extracellular signal-regulated kinases
1 and 2 (ERK1/2), and Ras/Raf pathways. Other CXCR4 regulatory pathways include Wnt/β-catenin, Sonic hedgehog (SHH)-GLI-NANOG and Notch. These pathways are all involved in cell differentiation, survival, migration, proliferation and chemotaxis. Arrestins play significant role in the regulation of CXCR4/CXCL12 signaling through internalization and desensitization process of CXCR4 **(2).**

CXCR4 and pathogenesis of multiple myeloma

Multiple myeloma (MM) is the second most common form and accounts for 10% of all hematologic malignancies. In spite of significant advances in treatment strategies, MM remains incurable. It is a heterogeneous plasma cell neoplasm characterized by the clonal proliferation and accumulation of malignant plasma cells or excessive production of monoclonal myeloma proteins in the BM compartment and sometimes extra-medullary tissues resulting in osteolytic lesions and thus osteopenia, renal diseases, hypercalcemia and anemia **(3).**

MM is thought to initiate from long lived plasma cells which develop in germinal center of lymphoid tissues. The myeloma plasma cells attain oncogenic potential, home to the BM and due to the support in microenvironment niche, survive for a long time. MM can progress from asymptomatic to symptomatic stages ranging from monoclonal gammopathy of unknown significance (MGUS) to smoldering multiple myeloma (SMM) to MM or plasma cell leukemia **(4).**

The association between CXCR4 and MM has been implicated in different studies. Due to its involvement in normal plasma cell development, CXCR4/CXCL12 axis also plays significant role in MM progression. CXCR4 has role in the expansion and colonization of MM plasma cells in the bone. CXCR4/CXCL12 axis can also regulate homing, adhesion, invasion, migration and mobilization of MM cells out of the BM. It was observed that Persistent chemo-resistant minimal residual disease (MRD) plasma cell clones in MM express high levels of CXCR4, integrins (CD11a/CD11c/CD29/CD49d/CD49e) and adhesion molecules (CD44/CD54), while abrogation of CXCR4/CXCL12 pathway can deregulate BM colonization by hematopoietic cells **(1).**

Elevated CXCR4 expression in MM is induced by different factors present in the malignant cells. For instance, hypoxia, different proinflammatory cytokines like TNF-α, TGF-β, and VEGF were noted to induce CXCR4 expression in MM. It was shown that down-regulation of HIF1α decreased CXCR4 expression and reversed the migration and homing of MM cells into the BM. Some biological roles of CXCR4 in MM is partly played by its interaction with a pleiotropic inflammatory cytokine called macrophage migration inhibitory factor (MIF) which subsequently leads to receptor activation and promotion of chemotaxis **(2).**

CXCR4, carcinogenesis and tumour growth in MM

CXCL12-expressing BM stromal cells (BMSCs) recruit CXCR4- expressing B cells, eosinophils and monocytes that are required for their retention in the BM. It was suggested that CXCR7 itself also has a vital role in cell adhesion, angiogenesis and tumor progression in MM where it indirectly interacts with CXCR4/CXCL12 axis. CXCR7 serves in modulating the function of CXCR4 by forming heterodimeric receptor unit with CXCR4 for

CXCL12 signal transduction as well as recruitment of the tumorigenic monocytes **(5).**

In a similar way as the normal plasma cells, myeloma cells utilizing CXCR4 interact with CXCL12-expressing BMSCs and migrate across the endothelium lining the BM for homing and localization through chemotaxis. Myeloma-BM stroma is rich in integrins like VLA-4 (Very-late-antigen-4, α4β1 integrin, CD49d/CD29) ligands that has significant role in cell adhesion-mediated drug resistance (CAM-DR) **(3).**

When MM cells adhere to BMSCs, CXCL12 up regulates its own secretion, which further up regulates VEGF and IL-6 secretion and thus promote enhanced homing through further expression of integrins. Thus, trans-endothelial migration, homing, adhesion and localization of MM cells in the BM microenvironment to form tumor niche is promoted by the up-regulation of integrins through CXCL12. An increase in intracellular cAMP in association with the activation of protein kinase A (PKA) downregulates CXCL12 mediated α4ß1-dependent cell adhesion and induces apoptosis in MM. It was shown that MM cells express high levels of tumor promoter heparanase enzyme which promotes MM invasion and angiogenesis mediated by VLA-4. Again, MIF has a role in MM cell adhesion to the BMSCs through regulating the expression of adhesion molecules via its receptor CXCR4. MIF mediated B-cell chemotaxis is abrogated when CXCR4 is inhibited. MIF-CXCR4 interaction leads to activation of adhesion molecules in MM cells whereas abrogating MIF made MM cells more chemotherapy-sensitive when co- cultured with BMSCs in vivo. MIF can also bind to CD74 and MM cells were shown to express not only CXCR4 but also CD74. It was evident that MIF-deficient MM cells had aberrant tumor growth **(4).**

Localization and interaction of MM cells in the BM microenvironment leads to the activation of osteoclasts and suppression of osteoblasts which associates with MM progression, metastasis and drug resistance. Hyperactivated Notch signaling is an important mediator of this unbalanced osteoclast and osteoblast activity. It was shown that CXCL12-CXCR4 interaction is pro-osteolytic. Disruption of CXCR4 enhances osteoclast activation and enhances tumor growth in bone. CXCL12 can promote migration of osteoclast precursors and upregulate several pro-osteoclastic genes. Osteoclast precursors express Bruton's tyrosine kinase (BTK), a MM stem cell marker which is involved in the generation of osteoclasts and their migration towards CXCL12. BTK expression is associated with CXCR4 expression in primary myeloma cells. BTK is also involved in myeloma cell homing to the bone. BM-stromal cells secrete different factors along with CXCL12 such as IL6, insulin-like growth factor 1 (IGF1), VEGF, TNFα and osteoprotegerin which are even more upregulated as MM cells localize in the stromal cells. The interaction of MM cells with these factors is associated with osteoclastogenesis. The activity of Osteopontin (OPN), a matrix protein that plays a dual role in MM as a marker for osteoclastic activity and also angiogenesis, has been linked to CXCR4/CXCL12 **(2).**

The NF-κB (RANK)/RANK ligand (RANKL) signaling pathway is another crucial regulatory system of bone remodeling. RANKL is over- expressed in MM cells and the ratio of RANK/osteoprotegerin regulates the level OCL activity. It was suggested that CXCL12 can enhance the pro-resorptive effects of RANKL **(1).**

Previous evidence shows that CXCR4 overexpression is associated with poor disease prognosis. Abrogation of this signaling axis can down regulate BM colonization by hematopoietic cells. Interestingly in a controversial study, it was demonstrated that CXCR4 expression and disease activity is inversely correlated. MM cells express CXCR4 in high levels in the peripheral blood but in low levels in the BM. CXCR4 expression is down-regulated in MM cells from the BM in response to high levels of CXCL12. High CXCL12 in the BM mediates internalization of the CXCR4 receptor from the surface to the intracellular compartment in MM cells. This subcellular location of CXCR4 in MM can activate different downstream signaling pathways, like PI3K and ERK/MAPK pathways **(5).**

It has been proven that even though CXCR4 is a membrane receptor, it can be internalized by CXCL12 from the membrane to the cytoplasm. High CXCR4 expression in the cytoplasm indicates poor prognosis in contrast to a better prognosis evident when CXCR4 is highly expressed in the nucleus. MM plasma cells have the potential for phenotypic plasticity and have different subpopulations existing simultaneously that can affect disease initiation and progression. A proposed model by Shmuel Yaccoby showed that a subpopulation of MM stem-like cells, called quiescent MM stem cells act as tumor-initiators. Quiescent MM stem cells show elevated expression of adhesion molecules and CXCR4, which help in MM cell motility, migration and adherence to BMSCs. However, for proliferative MM stem cells, which is responsible for disease progression and emergence of evolved subclones, the expression of CXCR4 is minimal. This supports the notion that CXCR4 expression is down-regulated in more advanced stage of MM. Since MRD expressed high levels of CXCR4, integrins and adhesion molecules, they supposedly belong to the tumor initiating quiescent stem cells **(3).**

Role of CXCR4 in progression of Myeloma and distant metastasis

In MM, primary tumor without distant metastasis is represented as plasmocytomas. This leads to micrometastasis in MGUS through local invasion, followed by macrometastasis or distant colonization of the small number of carcinogenic MM cells. Some MM features include extramedullary disease (EMD), a metastasis prone phenotype where the tumor cells home to BM niches and can also infiltrate in other organs. Extramedullary spread of MM involves altered expression of different adhesion molecules by MM cells **(4).**

CXCR4 is known as the marker for bone metastatic signature as it is universally up regulated in cancer cells metastasizing in the bone. As CXCL12 is expressed by mesenchymal stromal cells in different organs like liver, lungs and BM, CXCR4 expressing MM plasma cells are recruited to these organs and can metastasize there. The preferential homing of MM cells to the BM requires rolling of the MM cells along the endothelium by binding to selectin and CXCR4/CXCL12. This binding process leads to the activation of MM cells for adhesion and transmigration at a later stage **(1).**

Again, Notch signaling system is known to control the expression and function of CXCR4/CXCL12 axis and therefore, MM metastatic pathway. Increased Notch signaling involves the imbalance in osteoclast and osteoblast activity and is associated with continuous homing and egression of MM cells leading to tumor infiltration in different bone locations **(5).**

For MM, dysregulation of RANK/RANKL/OPG is associated with cancer invasion and metastasis. This signaling also is osteolytic. The role of OPG in bone metastatic process has been known to involve CXCR4/CXCL12 axis. The osteolytic process in MM creates a favorable microenvironment for MM growth, which supports the fertile soil hypothesis by Paget. It also leads to osteoclast activation through the release of different growth factors-further promoting bone resorption by osteoclast activation in a positive feedback loop. MM cells interact with different cellular components, extracellular matrix proteins, cytokines, chemokines, proteolytic enzymes and growth factors in the BM microenvironment which assist in osteoclastogenesis, angiogenesis and MM metastatic process. IL-6, TNFα and NF-κB are significant myeloma- growth promoting lymphokines. IL-6 and TNFα are associated with rapid growth and spread of MM cells to other bones while NF-κB mainly plays growth-inducing and anti-apoptotic roles. CXCR4/CXCL12 axis was shown to induce NF-κB activation in MM and increased secretion of VEGF and IL-6 in BMSCs to promote MM growth, survival and migration **(2).**

Hypoxia is a critical regulator of cancer metastasis. The BM micro- environment is very hypoxic with around 1−2% O2. The hypoxic microenvironment of BM promotes de-adhesion, increased chemotaxis and homing of MM cells to new BM niches through acquisition of EMT phenotype regulated by CXCR4. Pim (Proviral Integrations of Moloney

virus) kinases, which are upregulated in hematological malignancies, are known to have oncogenic potential as they mediate MM cell migration and homing. Hypoxia in BM promotes Pim activity which also correlates with CXCR4 upregulation **(5).**

It was evident that, in addition to CXCR4, CXCR7 might additionally work in the MM metastatic process. Angiogenic monomuclear cells (AMC) have significant role in MM metastasis as they can migrate from BM involving chemotaxis, adhesion and invasion processes. CXCR7 plays an indirect role in MM progression and metastasis. It was confirmed in MM mouse model that CXCR7 is highly expressed on AMCs and enhances metastatic effects complementary to CXCR4. Both in vitro and in vivo studies confirmed that CXCR7 inhibition abrogated trafficking of AMCs and decreased MM progression **(4).**

It was recently shown that protein junctional adhesion molecule-A (JAM-A), a new biomarker in MM, is associated with CXCR4. High JAM-A expression is associated with poor clinical prognosis in MM patients due to its role in invasion and metastasis. Blocking JAM-A activity has shown to impair MM viability, migration, proliferation and spread in MM mice **(3).**

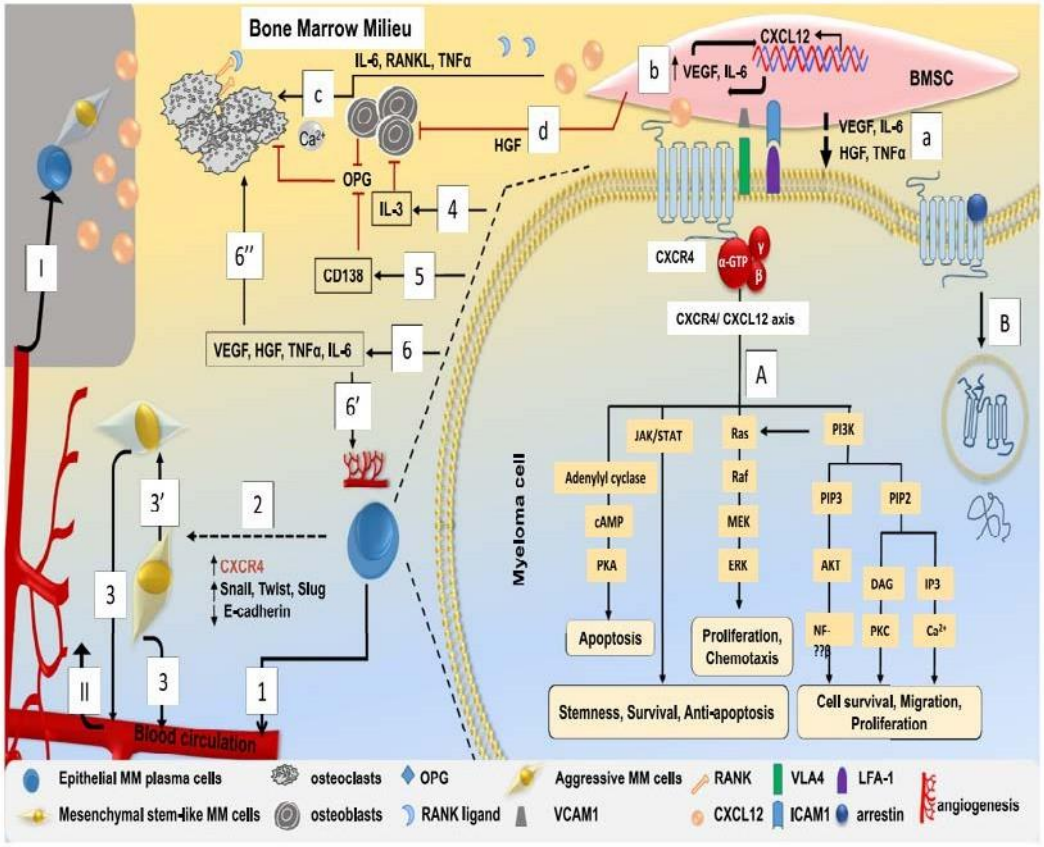

Fig. 1. Role of CXCR4-CXCL12 axis in the possible signalling events in multiple myeloma (MM) (6).

CXCR4/CXCL12-targeted therapy in MM

CXCR4 represents a valuable target for development of novel therapeutics due to its critical role in the crosstalk of MM pathogenesis. The CXCR4 targeting agents include CXCR4 antagonists, small synthetic or natural molecules and peptides. These agents can affect the activity of many genes and proteins via multiple pathways. However, inspite

of being broad spectrum anticancer agents, different clinical trials have shown the efficacy of these agents can be linked to targeting CXCR4/CXCL12 axis. It is often speculated that CXCR4 inhibitors combined with chemotherapy exert additive antitumor effects **(1).**

Plerixafor was the first FDA-approved (2008) selective CXCR4 antagonist which competitively inhibits CXCL12 binding to its receptor. This antagonist inhibited MM cells' migration in vitro and their homing in vivo through interfering with PI3K/AKT and ERK pathways. It also helps with egressing and mobilizing MM cells into the peripheral blood circulation by disrupting their adhesion to the BM microenvironment. It can be used either alone or in combination with granulocyte colony- stimulating factor (G-CSF) to mobilize HSCs to the peripheral blood. This effect was investigated in a phase II clinical trial which showed that Plerixafor combined with G-CSG caused enhanced peripheral blood stem cell mobilization and higher expression of genes associated with superior engraftment than G-CSF alone. It was shown that Plerixafor can induce chemosensitization in MM cells **(5).**

Several studies have shown that it can induce chemosensitivity to the proteasome inhibitor Bortezomib in MM cells. AMD3465, a monocyclam analog of plerixafor, also shows antitumor effect. High- affinity CXCR4 antagonist BKT140 in addition to inducing MM cell apoptosis, was also shown to mobilize hematopoietic stem cells for autologous transplantation when combined with G-CSF. Panobinostat, another FDA approved CXCR4 antagonist was used in several clinical trials for RRMM either alone or in combination with other agents which demonstrated antimyeloma activity (PANORAMA trials). One such phase I/II clinical study for panobinostat in combination with melphalan, showed lack of toxicity effects and low disease progression. Another phase III clinical trial has shown that in RRMM, higher overall survival and progression free survival benefit can be induced with Panobinostat, Bortezomib and Dexamethasone combination **(2).**

Of the anti-CXCR4 antibodies, Ulocuplumab was developed by Kuhne et al. and showed efficacy in inducing apoptosis in vitro and antitumor activity in xenograft models. A phase I clinical trial for MDX- 1338 has been conducted for the treatment of RRMM either alone or in combination with lenalidomide/dexamethasone or bortezomib/dexamethasone. MDX-1338 was shown to affect survival and adhesion of MM cells in a dose-dependent manner along with inhibition of MM cell proliferation in vivo in xenograft mice models. Roccaro et al. tested the effect of MDX-1338 both in vivo and in vitro and identified that it can inhibit MM cell bone-to-bone dissemination by suppressing EMT phenotype **(1).**

Another humanized monoclonal IgG1 anti-CXCR4 antibody F50067 showed MM anti-tumor activity through competing for CXCL12 binding, inhibiting G-protein activation and CXCL12 induced downstream signaling pathways. A phase I clinical trial for F50067 alone and in combination with lenalidomide and low dose dexamethasone (Len- Dex) in RRMM showed promising effects in egression of MM cells to the circulation, though the study was terminated due to hematological toxicity. LY2624587 is another potent anti-CXCR4 antibody which showed dose dependent inhibition in tumor growth in hematological malignancies particularly in human leukemia and lymphoma, showing potentials for its investigation in future MM trials **(4).**

It was recently shown that CXCR4 targeted endo-radiotherapy, which represents an alternative therapeutic mode could be effective in treating MM as it showed to induce better response even in relapsed MM patients. Recent studies further showed CXCR4/CXCL12 axis mediated macrophage polarization phase can be a potential new MM treatment strategy **(3).**

Apart from targeting CXCR4, targeting its ligand CXCL12 has been brought under focus. Olaptesed pegol (Ola-PEG) and Spiegelmer / NOX-A12 (Noxxon Pharma) can specifically bind to CXCL12. In one B- cell line, Ola-PEG inhibited CXCL12 mediated internalization of the CXCR4 receptor and inhibited chemotaxis in a dose-dependent

manner. Ola-PEG also blocked CXCL-12-dependent activation of its second receptor CXCR7 **(5).**

Ola-PEG was shown to be effective than Plerixafor in suppressing tumor growth and metastasis in a xenograft MM model. It also was shown to act synergistically with Bortezomib and associate in MM cell mobilization to the circulation. Also, CTCE-9908 can target CXCL12 and show clinical activity against MM. It was shown that thalidomide, which is used for MM treatment can downregulate both CXCR4 and CXCL12. Tymoquinone and Sorafenib both could also target CXCR4/CXCL12 axis to promote anti-apoptotic effects and blocked chemotaxis of MM cells **(2).**

Other CXCR4/CXCL12 targeting compounds include Carfilzomib, a second-generation proteasome inhibitor which interfere CXCR4/CXCL12 mediated CAM-DR by inhibiting CXCR4 phosphorylation. Interestingly, though carfilzomib can target CXCR4, it was observed that CXCR7 is unaffected by it, warranting a possible independent role of CXCR7 from CXCR4. EPI-X4 is a naturally occurring endogenous CXCR4 antagonist has implications in cancer metastasis. Some synthetic EPI-X4 derivative showed greater potential in blocking CXCR4 signaling than AMD3100, hence its therapeutic potential to treat MM can be evaluated. The other agents which can also target CXCR4/CXCL12 axis in MM include TG-0054, POL6326, BKT- 140, Sorafenib and NOX-A12 **(7-9).**

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