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Review Article

The Role of chemokine receptor CXCR4 in breast cancer metastasis

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Abstract: Breast cancer is one of the leading causes of cancer related deaths worldwide. Breast cancer-related mortality is associated with the development of metastatic potential of primary tumor lesions. The chemokine receptor CXCR4 has been found to be a prognostic marker in various types of cancer, including breast cancer. Recent advances in the field of cancer biology has pointed to the critical role that CXCR4 receptor and its ligand CXCL12 play in the metastasis of various types of cancer, including breast cancer. Breast tumors preferentially metastasize to the lung, bones and lymph nodes, all of which represent organs that secrete high levels of CXCL12. CXCL12 acts as a chemoattractant that drives CXCR4-positive primary tumor cells towards secondary metastatic sites leading to the onset of metastatic lesions. Since its discovery in 2001, the CXCR4 field has progressed at a very fast rate and further studies have pointed to the role of CXCR4 in dissemination of tumor cells from primary sites, transendothelial migration of tumor cells as well as the trafficking and homing of cancer stem cells. This review summarizes the information that has been obtained over the years regarding the role of CXCL12-CXCR4 signaling in breast cancer, discusses its potential application to the development of new therapeutic tools for breast cancer control, and elucidates the potential therapeutic challenges which lie ahead and the future directions that this field can take for the improvement of prognosis in breast cancer patients.

Keywords: CXCR4, CXCL12, breast cancer

Introduction

Breast cancer is the leading cause of cancer-related deaths among women. In its early stages, breast cancer is usually not fatal. However, the development of metastatic spread of the disease is responsible for at least 90% of the cancer-associated mortality. The survival rate falls from 90% for localized breast cancer to 20% for metastatic breast cancer. Despite this fact, the precise mechanism leading to metastasis remains the most poorly understood component of cancer pathogenesis. The process of metastasis is known to be the result of several sequential steps - cancer cells from a primary tumor locally invade the surrounding tissue by penetrating the basement membrane (local invasion), enter the microvasculature of the lymph and blood systems (intravasation), survive the circulation as circulating tumor cells (CTCs), exit from the bloodstream (extravasation), survive at distant locations and adapt to the foreign microenvironment, thereby facilitating cell proliferation and the formation of a macroscopic secondary tumor. It is the capacity to metastasize to many anatomical sites that makes the treatment of breast cancer at its advanced stage, a supreme challenge till today. Significantly, certain organs represent common metastatic destinations (for example, the liver, lung, lymph nodes and bone marrow), whereas metastasis to others (including the kidneys, pancreas and skin) is comparatively rare.

Chemokines are 8 to 12 kDa peptides that function as chemoattractant cytokines involved in cell activation, differentiation and trafficking. Chemokines are known to bind to specific G-protein coupled receptors. The chemokine CXCL12 (a.k.a. stromal cell derived factor-1 or SDF-1) binds to its receptor C-X-C chemokine receptor type 4 (CXCR4). Like all chemokine receptors, CXCR4 belongs to the superfamily of seven transmembrane domain heterotrimeric G

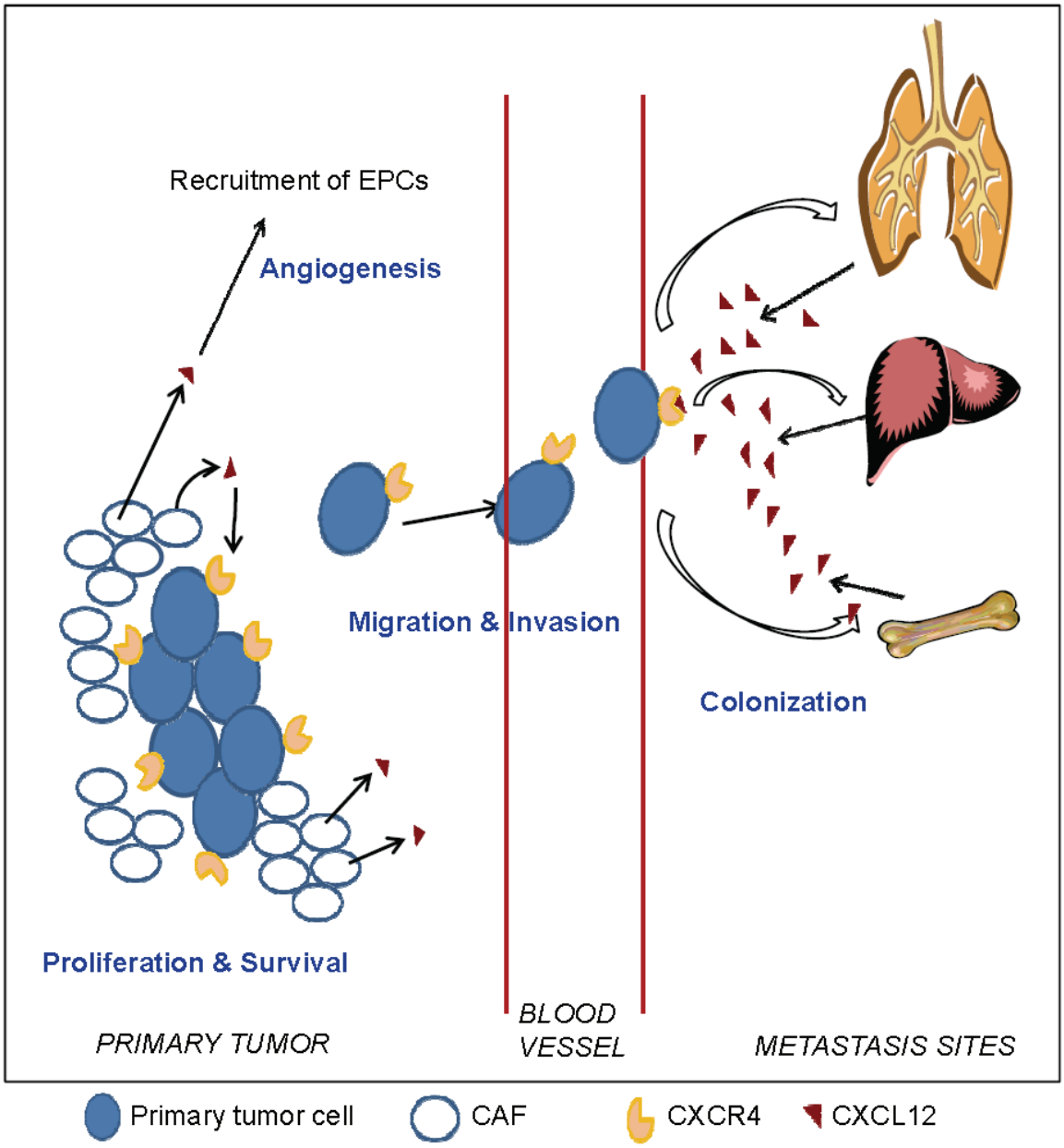

Figure 1. Role of the CXCR4-CXCL12 interaction in primary breast tumor and metastatic sites. The CXCL12 ligand secreted by cancer-associated-fibroblasts (CAFs), or myofibroblasts, in the breast carcinoma, binds to the cognate CXCR4 receptor expressed on the cancer cell surface which causes the activation of downstream intracellular pro-proliferative pathways, thereby enhancing tumor growth directly. CXCL12 also recruits endothelial progenitor cells (EPCs) from bone marrow into the carcinoma to enhance angiogenesis and thus promote tumor growth. The CXCL12-CXCR4 binding leads to migration of CXCR4-positive cancer cells away from the primary tumor in response to a CXCL12 gradient. The CXCL12 ligand acts as a chemoattractant that drives primary tumor cells towards CXCL12-rich secondary metastatic sites leading to the onset of metastatic lesions.

protein-coupled receptors (GPCR). The CXCL12-CXCR4 signaling pathway has been recognized to having a very critical role in the retention and homing of hematopoietic stem cells in the bone marrow microenvironment and lymphocyte trafficking. It has also been implicated in the maintenance of the secondary lymphoid structure. As shown in **Figure 1**, CXCL12 has been found to be expressed constitutively in several organs including the lungs, liver, bone marrow, skeletal muscle, kidney, brain, etc. Recent breakthroughs in cancer biology research and

Table 1. History of development of research on the involvement of CXCR4 receptor in breast cancer metastasis

YEAR	INVESTIGATOR	TITLE OF PUBLICATION	DESCRIPTION
1889	Stephen Paget [4]	The Distribution of Secondary Growths in Cancer of the Breast	This paper first elucidated the "seed versus soil" hypothesis in cancer metastasis progression
2001	Anja Muller and Albert Zlotnik [5]	Involvement of chemokine receptors in breast cancer metastasis	This paper first pointed out the pivotal role of the CXCL12-CXCR4 interaction in pregression of breast cancer metastasis
2003	Schioppa *et al*. [7]	Regulation of the Chemokine Receptor CXCR4 by Hypoxia	This study showed that hypoxic conditions favour the expression of CXCR4 in tumor cells via activation by HIF1alpha
2005	Akira Orimo and Robert Weinberg [8]	Stromal Fibroblasts present in invasive human breast carcinomas promote Tumor Growth and Angiogenesis through elevated SDF-1/CXCL12 secretion	This study identified that CXCR4-CXCL12 binding recruits EPCs to tumor cells, promoting angiogenesis and tumor growth
2005	Kucia *et al.* [9]	Trafficking of Normal Stem Cells and Metastasis of Cancer Stem Cells involve similar mechanisms : Pivotal Role of the SDF-1 CXCR4 axis.	This study first established the role of the CXCR4 in cancer stem cell trafficking and metastasis
2011	Hiroshi Yagi and J. Silvio Gutkind [10]	A synthetic Biology Approach Reveals a CXCR4-G13-Rho Signalling Axis Driving Transendothelial Migration of Metastatic Breast Cancer Cells	This paper elucidated the role of CXCR4 in the initial steps of tumor cell dissemination from primary site and intravasation via trans-endothelial migration

development have proved that CXCR4 plays a pivotal role in breast cancer (and cancer in general), especially in the metastatic spread of the disease [1-3]. Interestingly, the organs which exhibit an enhanced secretion of CXCL12 are also the most common secondary metastatic sites of breast cancer. This observation has further highlighted the important role played by the CXCL12-CXCR4 signaling axis in breast cancer metastasis. This review will highlight the key role of CXCR4 and the regulation of its expression in breast cancer as well as the potential of targeting CXCL12-CXCR4 signaling as a future therapeutic strategy in breast cancer treatment.

Historical review of the research breakthroughs in the field

For a long time it has been recognized that the capacity of cancer cells to metastasize to many secondary sites is the key factor that makes the prognosis of cancer so poor. In case of breast cancer, it has been seen that a few secondary organs such as the bone marrow, lungs, liver, lymph nodes and brain are more favorable as metastatic sites than other organs.

As outlined in **Table 1**, in 1889, Stephen Paget attempted to explain this phenomenon through a 'seed and soil' hypothesis, in which he stated that tumor cells, somehow liberated from the primary tumour, would seek 'nurturing' conditions that resemble their original microenvironment to grow and thereby create secondary metastatic lesions [4]. His hypothesis was proved to be accurate more than a century later in 2001. A breakthrough study published in *Nature*, proved that not only can tumor cells travel around the body, but they do so under the influence of signals that determine their migratory behavior [5]. The study identified the pivotal role played by the CXCL12-CXCR4 signaling axis in metastasis, particularly breast cancer metastasis to the lung. Muller and his colleagues reported that CXCR4 receptors are highly expressed in breast cancers cells, malignant breast tumors and metastasis. The ligand CXCL12 also exhibits peak expression in common secondary metastatic sites. In breast cancer cells the CXCL12-CXCR4 ligand-receptor interaction gives rise to actin polymerization and pseudopodia formation leading to chemotactic migration of the cells ultimately leading to metastasis of the cancer cells to CXCL12-rich environments. Subsequent studies involving animal models have established that interfering with the CXCR4-CXCL12 interaction significantly impairs breast cancer metastasis.

Silencing of CXCR4 receptor was also found to severely block breast cancer metastasis [6]. Since these reports, there have been various studies on CXCR4 and its role in breast cancer metastasis.

In 2003, another breakthrough study identified that the expression of CXCR4 is induced when tumor cells are cultured under hypoxic condition [7]. In 2005, Dr. Weinberg's lab showed that tumor associated macrophages activate a particular type of carcinoma-associated mesenchymal cell, the myofibroblast, that secretes CXCL12 [8]. They established that endothelial progenitor cells (EPCs) also exhibited the CXCR4 receptor. Thus through this recruitment of EPCs to the tumor cells via CXCR4-CXCL12 binding, CXCR4 promotes angiogenesis and tumor growth directly. Interestingly, in 2005 and 2006, various studies were conducted which established an important link between CXCR4 expression and cancer stem cells. Cancer stem cells have been found to express CXCR4, and the CXCR4-CXCL12 axis also directs the trafficking of these cancer stem cells to CXCL12-rich environments like lymph nodes, lungs, etc [9]. Finally, in 2011, a breakthrough study showed that CXCR4 is not only associated with the metastatic spread of breast cancer cells to secondary organs, it also plays a key role in the initial steps of tumor cell intravasation and dissemination from the primary tumor site [10].

The CXCL12-CXCR4 signaling pathway

The binding of CXCL12 to CXCR4 results in divergent downstream signaling pathways that mediate chemotactic invasion, cell survival and regulation of gene transcription. CXCR4 is a G-protein coupled receptor that is associated with an intracellular heterotrimeric G-protein linked to the inner surface of the plasma membrane. The receptor has a seven-transmembrane structure with seven helical regions connected by six extramembrane loops. The G-protein heterotrimer consists of Gα, Gβ and Gγ subunits, which remain associated to the guanine nucleotide GDP at the basal state. On ligand binding, GDP is released and replaced by GTP which leads to the separation of the G subunits into a Gα monomer and a Gβγ dimer. GTP is bound to the Gα monomer. The Gα can exist in four different forms termed as $G\alpha_s$,$G\alpha_i$,$G\alpha_q$ and $G\alpha_{12}$. It was believed that the CXCR4 receptor is primarily a $G\alpha_i$-associated GPCR. However, recent reports have confirmed that CXCR4 may be associated with any of the other Gα subunits as well [11]. The four different Gα subunits each have distinct functions and activate distinct downstream signaling pathways, thereby relaying the GPCR signal to different routes. After the relaying of the signal, GTP is rapidly hydrolyzed to GDP, leading to the reassociation of the CXCR4 receptor complex. On the other hand, Gβγ subunits also activate additional downstream signaling pathways.

The $G\alpha_s$ subunit stimulates adenylyl cyclase to produce cyclic AMP (cAMP) which activates Protein kinase A (PKA) which, in turn activates the transcription factor, Cyclic AMP-responsive element-binding protein or CREB. CREB translocates to the nucleus and regulates the transcription of several genes implicated in cancer progression. For example, the expression of Glutathione S-transferase protein 1 (GSTP1) is positively regulated by CREB [12]. Higher expression of GSTP1 is correlated with higher drug resistance. The $G\alpha_i$ subunit is pertussis-toxin sensitive [13]. Both $G\alpha_i$ and $G\alpha_q$ can activate phospholipase C leading to the production of secondary messengers diacylglycerol (DAG) and inositol triphosphate (IP3) which opens up intracellular calcium stores, thereby facilitating cellular chemotaxis. DAG, in turn, activates the protein kinase C family of enzymes [14]. PKCα is especially involved in regulating tumor cell migration [15, 16]. PKCα also activates Iκβ kinase to phosphorylate Iκβ which leads to the NFκB-mediated regulation of cancer-related genes. Chemotaxis has also been shown to be mediated by the activation of MAPK pathways through the signal relayed by both $G\alpha_i$ and $G\alpha_q$ subunit. Lastly, the $G\alpha_{12}$ subunit activates guanine nucleotide exchange factors (GEF) for the Rho family of GTPases. A major downstream target of Rho is the Rho-dependent coiled-coil kinase (ROCK) which activates non-muscle myosin II, thereby stimulating migratory activity in tumor cells [17]. In parallel, CXCR4-mediated chemotaxis is largely mediated by PI3K which may be activated by both the Gβγ dimer or Gα monomer. PI3K activation results in the phosphorylation of several focal adhesion components like FAK which activates downstream signaling pathways which induce migratory activity in tumor cells.

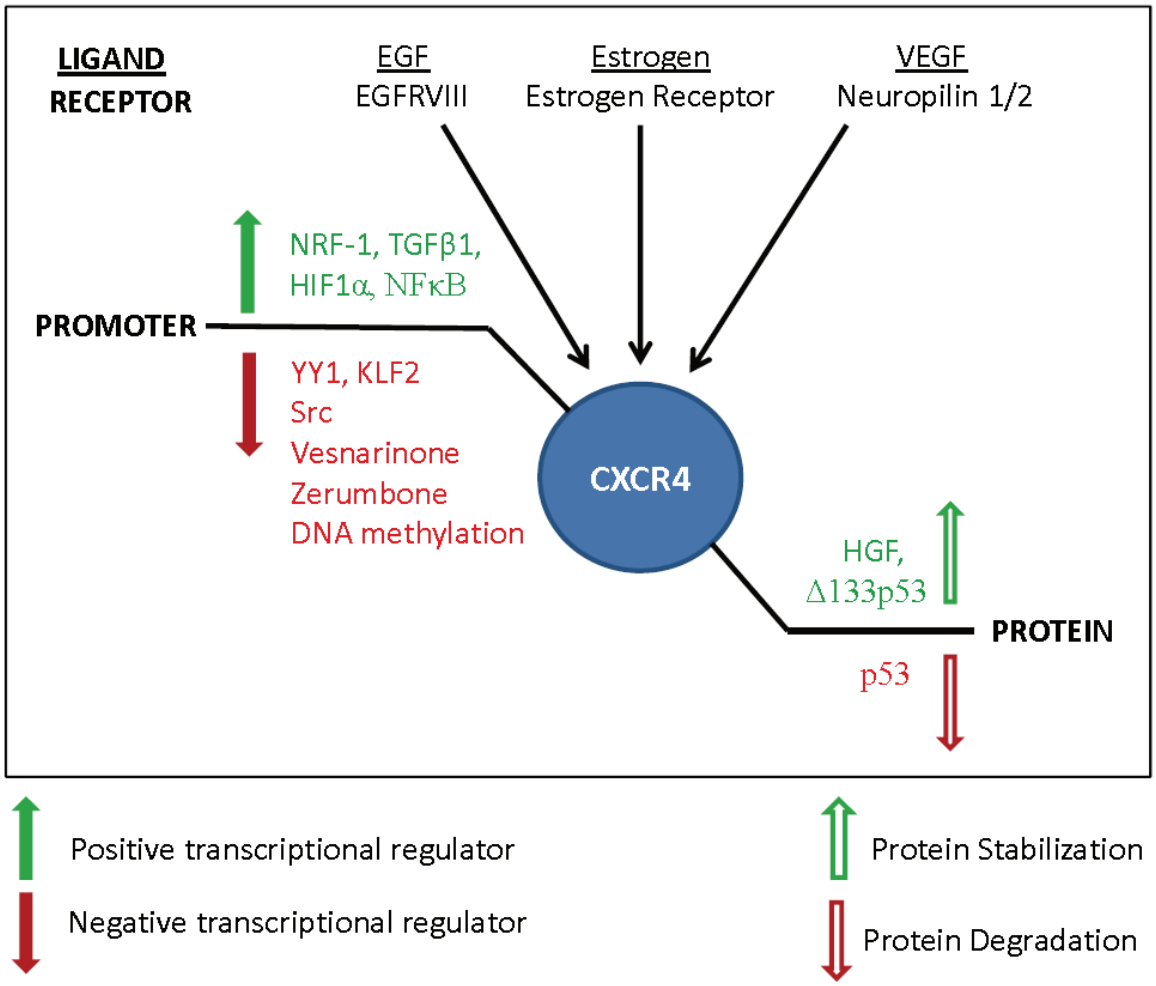

Figure 2. Regulation of CXCR4 expression in breast cancer. Proteins or pathways regulating CXCR4 expression at the transcriptional or post-translational levels are illustrated.

Phosphorylation of the CXCR4 receptor plays a major role in both positive and negative modulation of CXCR4 downstream signaling [18]. Importantly, phosphorylation induced by ligand binding can promote receptor desensitization. Agonist-promoted desensitization is primarily mediated by members of the GPCR kinase (GRK) family which specifically phosphorylate ligand occupied GPCRs [19]. GPCR phosphorylation can promote Gα protein independent downstream signaling, and may also facilitate recruitment of arrestins which target the receptors for clathrin-mediated endocytosis [20]. CXCL12 binding promotes the phosphorylation of seven specific serine residues in the C-terminal tail by GRK2, GRK3 and GRK6 [18]. GRK3- and GRK6- mediated phosphorylation results in the activation of downstream ERK1/2, while calcium mobilization and ERK activation is negatively modulated by GRK2-mediated CXCR4 phosphorylation. Thus, the serine-threonine rich cytoplasmic tail of CXCR4 plays a major role in mediating G protein-independent pathways downstream of the receptor. Furthermore, studies indicate that because of its recently identified role in the activation of G proteins on CXCL12 binding, the cytoplasmic tail may be an essential player in activating G protein-dependent pathways as well [21].

Regulation of CXCR4 expression

CXCR4 expression has been found to be upregulated in primary tumor cells as well as metastatic lesions. The increased levels of CXCR4 expression have prognostic significance suggesting that the acquisition of the novel cancer-promoting effect is, at least in part, due to increased receptor numbers and increased receptor signals. Thus, it is essential to identify

the mechanism that triggers the upregulation of CXCR4 receptor in cancer cells leading to chemotactic migration and ultimately, metastatic spread of the disease.

As outlined in **Figure 2**, specific mechanisms have been identified that regulate CXCR4 expression at transcriptional or post-translational level. A detailed analysis of the promoter region of CXCR4 revealed that it contains a number of predicted consensus sequences. The CXCR4 promoter contains a nuclear receptor-1 (NRF-1) binding site [22]. In human rhabdomyosarcomas, NRF-1 was found to upregulate CXCR4 expression [23]. A negative regulatory element was also identified on the CXCR4 promoter, located at about -300bp upstream of the transcriptional start site [22]. Subsequently in 2005 it was proved that Ying Yang-1 (YY-1) downregulates CXCR4 expression via the identified negative regulatory element on the CXCR4 promoter [24]. This study also demonstrated that the C-terminal Src kinase negatively regulates CXCR4 transcription by reducing the YY1-c-myc association in breast cancer cells.

TGFβ1 upregulates CXCR4 expression in breast cancer cells (MCF-7) at both mRNA and protein level [25]. TGFβ1 is known to play a prominent role in metastasis by enhancing angiogenesis and suppressing immune surveillance at secondary metastatic sites. More research is required to investigate whether TGFβ1 regulates the suppression of immune surveillance via a CXCR4-dependent mechanism during metastatic colonization of cancer cells. Activation of EGFR by EGF under hypoxic conditions was found to cause a dramatic upregulation of CXCR4 expression in non-small cell lung cancer (NSCLC) [26]. Further investigation revealed that the presence of EGF and a low oxygen environment lead to the upregulation of HIF1α expression and subsequently HIF1α-dependent CXCR4 gene transcription. In fact, a constitutively active variant of EGFR, EGFRvIII has been associated with the regulation of CXCR4 expression [27]. EGFRvIII regulates CXCR4 transcription in a hormone-dependent manner by upregulating the CXCR4 transcription regulator HIF1α in breast cancer cells. EGFRvIII has also been shown to inhibit AIP4 and β-arrestin1/2 which are involved in CXCR4 receptor internalization and degradation [28, 29]. Therefore, these studies illustrate two possible mechanisms by which EGFRvIII upregulates CXCR4 expression in breast cancer cells. The promoter of CXCR4 contains several binding sites for NF-kB and it has recently been shown that CXCR4 expression in MDA-MB231 breast carcinoma cells is directly regulated by NF-kB at the transcriptional level [30]. Interestingly, NF-kB is known to be activated in response to hypoxic stress which itself is a factor responsible for the upregulation of CXCR4 expression in breast carcinoma.

The role of the CXCL12-CXCR4 signaling arc in promoting angiogenesis has been of supreme interest to researchers over the years. In 2002, a study indicated that the Vascular endothelial growth factor (VEGF) receptor neuropilin-1 mediated VEGF autocrine pathway induced CXCR4 receptor expression in breast cancer cells, thereby promoting invasion and migration of breast cancer cells towards a gradient of the CXCL12 chemoattractant [31]. Additionally, blocking the neuropilin-2 receptor with neuropilin2-specific antibodies inhibited the expression of CXCR4 and also significantly decreased CXCR4-dependent migration of MDA-MB-231 cells towards the CXCL12 ligand [32]. On the other hand, CXCR4 itself was shown to regulate VEGF promoter activity in MDA-MB-231 cells and blocking the CXCR4-CXCL12 interaction severely impaired VEGF expression levels both at the mRNA and protein level [33]. Expression level of VEGF was found to be reduced by 42% on administering a peptide inhibitor of CXCR4, CTCE-9908 to a transgenic breast cancer mouse model [34]. Collectively, these results indicate that VEGF can enhance CXCR4 expression in breast carcinoma cells via an autocrine pathway, which in turn can enhance the promoter activity of VEGF itself, suggesting that VEGF is produced in a feed-forward mechanism via CXCR4 expression, thus driving enhanced angiogenesis and increased invasion and migration in breast tumor cells.

In 2009, Vesnarinone was recognized as a chemotherapeutic agent that downregulated CXCR4 expression both at the mRNA and protein level [35]. Krüppel-like factor 2 (KLF2) was identified to be a Vesnarinone-responsive protein that binds to the CXCR4 promoter and repressed CXCR4 transcription. Zerumbone, a natural component of subtropical ginger

(*Zingiber zerumbate*) was also identified as a negative regulator of CXCR4 transcription in Her2-overexpressing breast cancer cells [36].

Recent reports have shown that enhanced CXCR4 expression in breast cancer cells is also significantly mediated by post-transcriptional and post-translational modification of the receptor. The estrogen receptor has been shown to play a major role in positively regulating CXCR4 expression by inducing posttranscriptional modification of the receptor, apart from directly inducing CXCR4 transcription in breast cancer cells [37]. Hepatocyte growth factor (HGF) induces functional CXCR4 expression in breast cancer cells through PKCζ and downstream Rac1 activation. HGF treatment induces CXCR4 receptor phosphorylation and can contribute to reduced CXCR4 endocytosis, thereby facilitating chemotactic migration of breast tumor cells [38]. Wild-type p53 has been shown to repress CXCR4 expression, while the cancer-specific isoform of p53, Δ133p53 failed to repress CXCR4 promoter activity [39]. This suggests an alternate mechanism for enhanced CXCR4 in breast cancer through the expression of mutated p53 or isoform expression in breast cancer cells. Furthermore, it has recently been demonstrated that CXCR4 gene expression in primary breast tumors is also regulated by DNA methylation. Loss of DNA methylation in the CXCR4 promoter is associated with aggressive tumor growth, invasion and metastasis in breast cancer patients [40].

Upregulation of CXCR4 under hypoxic conditions

Following the observations made by Muller *et al*, in 2001 which identified the critical role of CXCR4 in breast cancer metastasis, Schioppa *et al*, made the important observation that the expression of CXCR4 is upregulated in tumor cells resulting from a change in the tumor micrenvironment. The tumor cells , when cultured in hypoxic conditions, for example, showed significant overexpression of CXCR4. Furthermore, CXCR4 has been shown to be upregulated by hypoxia-induced factor 1 alpha (HIF-1α)by the same group. This was a very important development in this field since hypoxic conditions are known to promote cancer metastasis. Hypoxia develops when the primary tumor attains a certain volume, whereby it does not have enough blood supply to nourish it due to insufficient angiogenesis [41]. Each tissue has an organized structure, a vascular supply and a variety of nutrients. When cancer cells circumvent this normal organisation they are subject to environmental stress. Such environmental pressures like hypoxia, can select the tumor cells with the capability to grow in spite of such environmental challenges and can also cause them to acquire an aggressive phenotype. Recent studies on the link between hypoxia and CXCR4 expression in breast cancer have provided sufficient evidence that hypoxic condition selects the tumor cells which go on to proliferate and metastasize by activating the expression of CXCR4 in these cells [42]. For example, in non-small cell lung cancer (NSCLC) low oxygen tension lead to enhanced CXCR4 expression via a phosphatidyl-inositol 3 – kinase/PTEN/AKT/mammalian target of rapamycin signal transduction pathway which leads to the activation of HIF-1α and finally to HIF1α-dependent transcription of the CXCR4 gene [26]. The CXCR4 promoter contains a consensus HIF1α binding site which when mutated, leads to inhibited CXCR4 gene expression in the presence of HIF1α. This data suggests that the HIF1α transcription factor regulates the expression of CXCR4. A recent study has also revealed that hypoxia (via HIF-1α) and TGF-β signaling promote bone metastasis of breast cancer cells via a synergistic mechanism [43] Furthermore, under normoxic conditions, the von Hippel-Lindau tumor suppressor protein pVHL, negatively regulates CXCR4 expression because of its ability to target the HIF1α for degradation. However, under hypoxic conditions, the aforementioned process is suppressed, leading to a significant increase in HIF1α-dependent CXCR4 expression in breast carcinoma cells [44]. This suggests a possible mechanism by which cells of a growing tumor are reprogrammed to express the CXCR4 receptor, thereby enhancing the metastatic potential of the tumor cells.

CXCR4 and cancer stem cells

Cancer stem cells are a subpopulation of cells in the tumor that act as tumor propagating cells. Cancer stem cells present a challenge to cancer therapeutics, especially since these cells can resist DNA damage and are, therefore, unresponsive to chemotherapeutic treat-

ment and apoptotic drugs. Cancer stem cells need specific microenvironments to grow and contribute to the growth of both primary and secondary metastatic tumors [45]. Various studies done on brain, prostate as well as breast tumors have underlined the importance of quiescent cancer stem cells in the development of cancer [46, 47]. The CXCL12-CXCR4 signaling axis is known to be involved in normal stem cell homing [48]. Interestingly, cancer stem cells have also been found to express the CXCR4 receptor which suggests that the CXCL12-CXCR4 signaling axis may direct the trafficking of cancer stem cells to secondary organs that express high levels of CXCL12 ligand like lungs, lymph nodes, etc [9] It has been shown in animal models that bone-marrow derived progenitor cells home to specific distant organs and "niche" sites before the formation of metastasis [49]. The ability of cancer stem cells to migrate to distant "niche" sites like the bone marrow-derived progenitor cells and avoid destruction may explain the reason for which in many cases, relapse occurs even after micrometastasis remains dormant for several years after removal of the primary tumor. Due to cancer stem cell mediated micrometastasis, relapse can occur several years after successfully treating the primary tumor. Interestingly, reports suggest that hypoxia promotes the self-renewal capacity of CD133 positive human glial-derived cancer stem cells [50] which leads to the expansion of cells bearing the CXCR4 surface marker. Furthermore, overexpression of NANOG (known to promote cancer stem cell characteristics in tumor cells) in breast (MCF-7) cancer cells leads to significant molecular changes, including the upregulation of the CXCR4 receptor in these cells, accompanied by a significant increase in drug resistance in MCF-7 cells [51]. In pancreatic cancer, two populations of cancer stem cells have been identified: $CD133^+$ cells that maintain primary tumor growth and migratory $CD133^+/CXCR4^+$ cells at the invasive edge of the tumor that promote metastatic growth. Further research may be done to identify whether breast cancer stem cells are present in similar subpopulations in the primary tumor to promote growth and metastatic progression. A recent study has shown that the CXCR4 receptor maintains a cancer progenitor population in tamoxifen-resistant breast cancer cells through aryl hydrocarbon receptor (AhR) signaling [52].

Overall, these studies emphasize the role of CXCR4 in the promotion of malignant cancerous metastatic lesions and highlight the importance of therapeutic intervention to prevent CXCR4 activation.

Role of CXCR4 in initial cancer cell dissemination from primary tumor site

Ever since the role of CXCR4 in breast cancer metastasis was first documented in 2001 [5], this field has moved forward significantly. Many studies have been conducted which confirms conclusively that increased abundance of CXCR4 in breast cancer cells is associated with enhanced metastatic potential. However, whether CXCR4 is required for the initial steps of tumor cell dissemination from the primary site and intravasation had been largely overlooked. In 2011, Yagi *et al* reported that CXCR4 is required for the migration of breast cancer cells from the primary site through the basement membrane. It is also implicated in transendothelial migration via the activation of the small GTPase Rho, through the heterotrimeric G-proteins associated with it. CXCL12 acts through CXCR4 to stimulate migration and intravasation of the breast cancer cells. Interestingly, recent reports suggest that hypoxic conditions induce tumor cell CXCR4 expression along with endothelial CXCL12 expression and stimulate transendothelial migration towards a CXCL12 gradient thereby facilitating the initial steps of metastasis [53]. These studies further highlight the critical role played by CXCR4 receptor in the overall progression and metastasis of breast cancer.

Therapeutic implications

All the work highlighted above point to the immediate need to disrupt the enhanced CXCL12-CXCR4 signaling in cancer cells leading to chemotactic migration and metastasis. In fact, some of the established anti-tumor treatments like DNA damage based chemotherapy promote hypoxic environment, which leads to the upregulation of CXCR4 expression, thereby promoting survival and metastatic invasion of the tumor. The implication of this observation is that certain anti-cancer therapy, while removing the primary tumor, can actually augment the metastatic potential of the surviving tumor cells by further upregulating the expression of CXCR4. This increase in CXCR4

expression may be the cause behind the decrease in overall patient prognosis. Hence, the disruption of CXCR4 or preventing the upregulation of CXCR4 in cancer cells is imperative for effective treatment.

The best studied among the compounds that inhibit CXCR4-CXCL12 interaction is a CXCR4 antagonist AMD3100. Commonly known as Plerixafor, AMD3100 has already been shown to decrease metastatic potential in animal models [54]. The efficacy of AMD3100 and other CXCR4 antagonists and inhibitors in preventing cancer is being clinically tested (ClinicalTrials. Gov identifier: NCT01120457).

Therapeutic challenges and future directions

There are many practical difficulties that are potential barriers to using CXCR4 antagonists to curb breast cancer metastasis in humans. Long-term treatment of such CXCR4 antagonists may be difficult to justify due to their side effects on the immune system. Currently, the application of CXCR4 antagonist therapy for cancer is restricted due to excessive toxicity of global CXCR4 inhibition. It is important to note that CXCR4 antagonists promote the mobilization of hematopoietic stem cells (HSCs) from the bone marrow to the peripheral blood. This effect has significantly hampered the use of CXCR4 blockers and inhibitors (like AMD3100) as adjuvants for breast cancer therapy.

The future goal in this field is to correct abnormal signaling and aberrant upregulation of CXCR4 receptor expression in primary and metastatic breast cancer cells without the inhibition of global CXCR4 signaling. In this regard, it is imperative to identify the mechanism by which CXCR4 expression is induced in primary tumor cells exhibiting metastatic potential. The identification of genes upstream of CXCR4 which induce the expression of CXCR4 in tumor cells specifically may present excellent therapeutic targets for the inhibition of aberrant overexpression of CXCR4 receptor in tumors, thereby leading to attenuated CXCL12-CXCR4 signaling axis, ultimately resulting in the significant decrease of metastatic potential of the tumor cells.

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