The CXCR2-SNAIL Axis: Is this a Novel Anti-Tumor Therapeutical Target for Cancer Cells Undergoing Epithelial-Mesenchimal Transition Process?

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ABSTRACT

The epithelial-mesenchymal transition (EMT) plays an important role in the progression of cancer, metastasis and drug resistance. Several factors are known to mediate EMT-driven drug resistance in cancer cells, among them the tumor microenvironment (TME). This phenomenon has gained attention in the field of cancer biology for its potential contribution to the progression of carcinomas. It is also known that tumor cells experiencing EMT increases the secretion of specific factors in the TME, including cytokines, chemokines and growth factors, which can play an important role in tumor progression. The main event in EMT is the repression of E-cadherin driven by transcriptional factors including SNAIL, SLUG and ZEB1. Chemokines function as growth factors, activating, through its receptor CXCR2 and transcription factors such as SNAIL, thus inducing the EMT phenotype, contributing to the progression of the disease. Studies have investigated how the acquisition of mesenchymal characteristics could contribute to the development of a tumor microenvironment, and point to a possible link between the CXCR2 pathway and EMT. This review describes the mechanism by which CXCR2 is involved in EMT through SNAIL, contributing to progression of cancer and summarizes new advances in the research of EMT-associated CXCR2.

Keywords: Tumor Microenvironment; Epithelial-Mesenchymal Transition; CXCR2; SNAIL

INTRODUCTION

Despite significant advances in research to improve early diagnosis and treatment of patients with cancer, chemoresistance remains a determining factor for treatment failure, contributing to metastasis, which is the main cause of mortality from the disease. Metastasis is responsible for 90% of approximately 600,000 cancer related deaths each year in the United States [1,2]. Response to therapy varies between patients. Significant proportion of patients will ultimately develop disease recurrence, associated with chemoresistance. Thus, chemoresistance and the ability of tumor cells to metastasize are very important drivers in disease progression. Therefore, cancer drug resistance will be a key factor to determine the success of the upcoming targeted therapy drugs. Traditionally, the resistant phenotype has been associated with changes in the cell death pathway, increased DNA repair, changes in checkpoints that occur during the cell cycle, changes in the intracellular signaling pathways and epithelial-mesenchymal transition (EMT) have traditionally been implicated in promoting a resistance phenotype [3,4]. However, the tumor microenvironment has received increasing attention, since there is a functional interaction between tumor cells and adjacent cells through secreted factors, such as cytokines and chemokines, which play an essential role in tumor initiation and progression. Chemokines of the CXC family, such as CXCL6, CXCL8 and CXCL2, have already been associated with chemoresistance and metastasis from breast and ovarian cancer
by promoting the migration of myeloid cells to the tumor [5,6]. However, it is known that these chemokines play their role by binding to the CXCR2 receptor, whose expression is related to a metastatic potential, modulation of angiogenesis and resistance in other types of cancer such as melanoma [7], colon [8] and lung [9]. CXCR2 is expressed in a variety of tumor cells, including breast, melanoma, pancreas and ovary, and these cancers have a high capacity to metastasize. CXCR2 appears to be involved in tumorigenesis via several signaling pathways in cancer, such as ERK1/2, PI3K/AKT, MAPK and NF-KB [10-12]. Recent studies have reported the association of CXCR2 in the mesenchymal epithelium transition, where the increased expression of SNAIL, a mesenchymal marker, induces the expression of chemokines such as CXCL1 and CXCL2, and the blocking of this receptor decreases the expression of SNAIL thus decreasing proliferation and consequently metastasis and angiogenesis [6]. EMT is an important factor for cancer progression, better understanding its role and the relationship of the tumor microenvironment in this context is crucial to contribute to the fight against cancer. Thus, this review focuses particularly on the interaction of CXCR2 and its ligands in the phenomenon of EMT and its contributing to cancer progression.

**EPITHELIAL-MESENCHYMAL TRANSITION**

The EMT is a process in which cells lose cell-cell adhesion, polarity and transition to mesenchymal cells with reduced cell interactions and increased migratory capacity. EMT is involved in numerous biological and pathological processes, including embryonic development, wound healing, cancel cell metastasis and drug resistance. [13,14]. EMT is characterized by an increase in the expression of mesenchymal markers such as Vimentin, SNAIL, SLUG, β-catenin and loss of epithelial markers such as E-cadherin [15]. This phenomenon promotes the acquisition of a fibroblastic type morphology by epithelial cells, resulting in greater mobility and invasiveness of tumor cells, greater metastatic propensity and resistance to chemotherapy, radiation and certain therapies directed at small molecules [15,16]. It is also known that tumor cells undergoing EMT increase the secretion of specific factors, including cytokines, chemokines, and growth factors, which can play an important role in tumor progression. The EMT process is correlated with the remodeling of the extracellular matrix, changes in the stroma, angiogenesis, invasion, migration and infiltration of immune cells [17,18]. Furthermore, EMT has been studied extensively in cancer, and is believed to play a crucial role in invasion, metastasis, chemoresistance and processes that are involved in cancer cell aggressiveness.

Several signaling pathways including AKT-mTOR [19], MAPK/ERK [20], are involved in EMT that lead to the activation of EMT transcription factors (EMT-TFs). SNAIL (SNAI1, also known as SNAIL and SNAI2, also known as SLUG) is considered a key transcription factor regulating EMT [18]. These EMT-TFs bind to specific DNA sequences, such as E-box to regulate EMT-target genes. SNAIL is also one of the main transcriptional repressors of E-cadherin, by binding to the proximal region of the E-cadherin promoter, it plays a fundamental role in tumor progression [18,21]. E-cadherin assists in suppressing tumor invasion and its function is necessary for the maintenance of stable adherent junctions and for the polarity of epithelial cells [22]. The altered expression of this epithelial marker is correlated with cell de-differentiation, increased local invasion and metastatic disease in different types of cancer including, breast, gastric and colon [22-24]. EMT-TFs are also known to promote cancer drug resistance. TGF β, a well-studied EMT-related cytokine, has been associated with drug resistance [25]. Overexpression of Twist, another EMT-TF induces EMT and promotes resistance in colorectal cancer cells [26]. Additionally, other EMT-TFs, such as SLUG and ZEB, is reported to be related to drug resistance [27,28].

Tumor microenvironment is also a factor mediating EMT-driven drug resistance. SNAIL induces intratumor traffic of myeloid-derived suppressor cells through the positive regulation of CXCR2 ligands, in addition to inducing other immunosuppressive cells such as T cells [21,30]. SNAIL is largely related to tumor immunity, it positively regulates the expression of chemokines, such as CXCL1 and CXCL2, and it improves the infiltration of suppressor cells into the tumor, via CXCR2 and inhibits anti-tumor immunity whilst promoting progression [6].

**FAMILY OF CXC CHEMOKINES AND CXCR2 RECEPTOR IN THE TUMOR MICROENVIRONMENT**

The various components of the tumor microenvironment actively participate in different tumor processes and influences the initiation of tumors as well as their malignant progression, spread and response to therapies. Both tumor cells and stroma cells release cytokines, and chemokines, creating an inflammatory microenvironment. These molecules assist tumor progression as growth factors, promoting proliferation and angiogenesis, as well as modulators of the immune system which in turn favors tumor growth and blocks cellular mechanisms that help in the identification and destruction of the tumor [31-33]. In particular, the interaction of chemokines and their receptors mediate the traffic of immune cells into the tumor microenvironment, resulting in the recruitment and activation of different immune system cell types, such as macrophages and lymphocytes [32]. The immune cells that make up the tumor microenvironment undergo a process of co-optation by the tumor cells, which contributes to angiogenesis and proliferation. Three stages of this cooptation by immune cells are described as: the recruitment of immune cells via the production of cytokines and chemokines, the interaction of immune cells via the secretion of cytokines, which regulates differentiation in order to promote the tumor progression and the immune response in which cells generate cytokines, growth factors and proliferation-promoting agents [34]. The tumor-infiltrating immune cells determines the balance between antitumor and pro tumor responses [35].

The chemokine receptors and their ligands are primarily responsible for leucocyte migration under conditions of homeostasis, as well as during inflammation and carcinogenesis. These are part of the molecular mechanisms involved in the survival, motlity and invasion of tumor cells. One example is the chemokine receptor CXCR4, which is often overexpressed in malignant cells and by different types of tumors such as...
colorectal cancer, breast, liver and esophageal cancer, its expression in primary tumors correlates with frequent lymphatic metastasis [36-38].

CXCL6 is an inflammatory cytokine commonly produced in response to infection and stress. In cancer, it is one of the most abundant cytokines in the tumor microenvironment, including prostate, ovary, colon and breast cancer. It binds to its receptor CXCR1/2, activating the STAT3, PI3K and Wnt/β-catenin signaling pathway, thus stimulating proliferation, activation of survival pathways, inducing EMT among other characteristics that contribute to tumor progression [39,40].

CXCL8 is a chemokine secreted by macrophages, endothelial and epithelial cells in response to infection or tissue damage, its effect is mediated through its connection with its CXCR2 receptor. The increased expression of the proangiogenic chemokine CXCL8 suggests that it may function as a significant regulatory factor within the tumor microenvironment [41]. This chemokine plays a crucial role in cell proliferation, migration, chemoresistance and survival. Its overexpression has been linked to a variety of tumors such as lung, prostate, ovary, breast and melanoma [42,43]. CXCL2 is an important factor in the chemoresistance and migration of breast cancer. In one study, the authors showed that CXCL2 expression increases as cells acquire a malignant phenotype and in response to Doxorubicin in breast cancer [5].

CXCR2 is a cell surface chemokine receptor coupled to protein G and having 7 transmembrane domains is normally found in neutrophils. It is also expressed in macrophages, endothelial cells, epithelial cells and multiple tumor cells, and can be induced by activated oncogenes. The function of CXCR2 has been primarily studied in leukocytes in association with inflammatory diseases and an immune response [43,44]. The receptors are named according to the type of chemokines that bind to it. The nomenclature refers to the number of cysteines, as well as their position. Being CXC, the two cysteines are separated by an unconserved amino acid. Several chemokines bind to this CXCR2 receptor, such as CXCL1, CXCL2, CXCL6 and CXCL8 [45-47].

Many studies to date have shown that CXCR2 contributes to chronic inflammation and pulmonary pathology, it is the target of studies in angiogenesis, tumorogenesis, chemosensitivity and metastasis in various types of cancer such as melanoma, colon, lung, ovary and prostate cancer [7-8,43]. Evidence through in vivo and in vitro studies indicate that metastatic infiltration of distant organs is mediated by chemokines and their receptors. Blocking this complex (ligand and receptor) reduces the development of metastases in various cancers, such as, colon [48], lung [49], colorectal [50], osteosarcoma [51] and gastric cancer [52].

ROLE OF CXCR2 IN THE EPITHELIAL-MESENCHYMAL TRANSITION VIA EXPRESSION OF SNAIL

The immune system has a fundamental role in preventing the progression of cancer; it is important in the line of defense against tumor development, demonstrating recently, efficiency in immunotherapy for some types of tumors [28]. However, the immune system can also contribute to tumor development through chronic inflammation, suppressing anti-tumor adaptive immune response associated with an increased risk of cancer. An established tumor can also stimulate an inflammatory reaction that leads to the recruitment of a range of cell populations from the immune infiltrate in the tumor microenvironment [16,31]. During tumorogenesis, cancer cells, innate immune cells, such as dendritic cells or tumor-associated macrophages (TAMs), and cancer-associated fibroblasts (CAFs) or endothelial cells, produce a variety of cytokines and chemokines in response to the danger signals originating from the tumor. This prolonged reaction favors tumor cell survival and proliferation, immunosuppression and angiogenesis [28,29,31]. During metastatic progression, tumor cells undergo phenotypic changes, allowing the cells to adapt to the different microenvironment encountered. EMT appears as a major modulator during these phenotypic conversions. Thus, cancer-associated EMT and chronic inflammation involve a series of inflammatory mediators such as soluble factors, oxidative stress, or hypoxia and production of proinflammatory mediators, including cytokine, chemokines, and matrix metalloproteinases, which fuel the tumor [18,25,53].

Chemokines function as growth factors, activating through its receptor, CXCR2, transcription factors such as SNAIL, which induces the EMT phenotype and contributes to the progression of the disease. CXCR2 ligands function as chemokines to attract Myeloid-derived suppressor cell (MDSCs) to the tumor. SNAIL induces infiltration of suppressor cells, accelerating EMT and leading to tumor progression. Blocking this cycle using CXCR2 antagonist, appears to be a promising treatment strategy, as this inhibition suppressed recruitment of MDSCs to the tumor and inhibited progression, slowing down EMT [6,30,44].

Studies have investigated how the acquisition of mesenchymal characteristics could contribute to the development of a tumor microenvironment, and point to a possible link between the CXCR2 pathway and EMT [54,55]. One study showed positive regulation for CXCL8 in cells undergoing EMT, showing higher levels of CXCR1 and CXCR2 receptors, in colorectal carcinoma cells [44]. CXCL8 / CXCR1 blockade has been shown to decrease the migration of mesenchymal tumor cells [42-44]. Data from another study showed that EMT involves the induced expression of CXCL8 and its receptor CXCR1, and that these molecules function, in the chemokinetic and chemotactic migration of cells after mesenchymal epithelium transition [43,44]. The author’s further state that the induction of CXCL8 expression by tumor cells is associated with the transition process of these cancer cells, and other signaling pathways may be involved in the expression of chemokines when the receptor is inhibited, such as PI3k/AKT and MAPK. As an example, a study demonstrated that the interaction of CXCL1/CXCR2 induces activation of PI3K signaling. SNAIL phosphorylation mediated by PI3K, induced by CXCR2, promotes nuclear accumulation of SNAIL and, consequently, its repressive activity in the E-cadherin promoter, thus inducing EMT. Increasing evidence implies the contributions of EMT in the emergence of therapeutic failure and tumor recurrence. Consequently, blocking EMT induced by CXCL1 / CXCR2 successfully prevented post-chemotherapy relapse, showing to be a promising alternative to prevent cancer progression and recurrence [56,57].

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Different studies have shown that cells resistant to different drugs, such as Paclitaxel and Doxorubicin, showed higher expression of EMT markers, such as vimentin and β-catenin, as well as lower expression of E-cadherin, through the overexpression of CXCL8/CXCR2. When this complex is inhibited, the expression of the EMT markers is decreased. The authors believe that targeting CXCR2 signaling may help to overcome resistance to cancer therapy [55,58]. It was further demonstrated that overexpression of SNAIL led to increased tumor growth in vivo, associated with increased angiogenesis and high levels of CXCR2 ligands such as CXCL5 and CXCL8. It was suggested that SNAIL promotes CXCR2-mediated progression and angiogenesis and when blockade of CXCR2 occurs, tumor growth mediated by SNAIL is reduced [59,60]. SNAIL also induces trafficking in myeloid suppressor cells through positive regulation of CXCR2, and an analysis of ovarian cancer data from TCGA indicated that SNAIL is correlated with many chemokines, including CXCR2 ligands [61]. Overexpression of SNAIL has also been shown to induce secretion of CXCL8. Although CXCL8 can be secreted by a variety of stromal cells, EMT pathways induce CXCL8 expression and secretion in tumor cells as shown in colorectal cancer cell lines. In this study, SNAIL has been shown to directly promote IL-8 transcription upon binding [62,63]. Blocking the CXCL8 signaling pathway in solid tumors can favor the clinical outcome, suppressing tumor growth, angiogenesis and EMT promoting activity through the CXCL8/CXCR2 pathway [63].

DISCUSSION

It is important to note that the acquisition of a mesenchymal phenotype by tumor cells is a transient process and may involve some cells at the tumor-stroma interface. However, the epithelial-mesenchymal transition is still controversial, since studies have supported the idea that mesenchymal tumor cells are necessary for the return of the epithelial phenotype, once they have reached the site of metastasis. This transient nature of EMT raises the question of when an inhibition of CXCL8 signaling may be adequate to block or reverse the EMT phenotype. The identification of EMT regulators can lead to combinatorial strategies that can more effectively prevent tumor metastases, and blocking the CXCR2 pathway appears to be an attractive strategy for decreasing tumor-promoting signs [64].

These results support the idea that SNAIL has a role in multiple functions, including the immune system and EMT, since chemokines can intrinsically regulate transcription factors such as SNAIL and SLUG, and these transcription factors control EMT. This leads us to conclude that CXCR2, as well as chemokines, has a fundamental role in resistance, which is directly associated with the epithelial-mesenchymal transition, and can therefore contribute to cancer metastasis.

CXCR2 and its ligands can be a therapeutic target for tumors with high SNAIL expression. Thus, the importance of studying tumor protectors and promoters in the immune system and in the epithelial-mesenchymal transition process arises.

CONCLUSION

CXCR2 is associated with chemoresistance, tumor progression and the induction of EMT through increased SNAIL expression. This review provides insights into the mechanism that might link EMT to tumor immunity, suggesting that this complex may be a therapeutic target to contribute to cancer treatment, decreasing chemoresistance and EMT and consequently decreasing tumor progression.

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