IL-8, a novel messenger to cross-link inflammation and tumor EMT via autocrine and paracrine pathways (Review)

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Abstract. The epithelial-mesenchymal transition (EMT) is a process through which epithelial cells trans-differentiate and acquire an aggressive mesenchymal phenotype. In tumor cells, EMT is a vital step of tumor progression and metastasis. Amid the increasing interest in tumor EMT, only a few studies focused on the soluble mediators secreted by tumor cells passing through this phenotypic switch. In this review, we focus on the essential role of interleukin-8 (IL-8) signaling for the acquisition and maintenance of tumor EMT via direct and indirect mechanisms. Besides the autocrine loop between IL-8 and tumor cells that have gone through EMT, IL-8 could potentiate adjacent epithelial tumor cells into a mesenchymal phenotype via a paracrine mode. Moreover, understanding the role of IL-8 in EMT will provide insight into the pathogenesis of tumor progression and may facilitate the development of an effective strategy for the prevention and treatment of metastatic cancer.

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1. Introduction

Tumor microenvironment, a vastly complicated network composed of various cell populations, soluble factors, signaling molecules and extracellular matrix components, orchestrates the behavior of tumor progression (1). Amid the growing interest in elucidating individual players in the tumor microenvironment, IL-8 appears markedly important and has been presented as one of the prominent promoters of tumor progression. IL-8 is involved in cancer related inflammation. A typical example is that, Epstein-Barr virus (EBV)-associated, undifferentiated type of nasopharyngeal carcinoma (NPC) which is characterized by several inflammation-like features. The inflammation-like microenvironment is crucial for the development of NPC progression. Notably, EBV infection as a critical factor for cancer progression can induce IL-8 secretion. EBV lytic transactivator Zta, which exerting its effect through bingding to Zta-responsive elements, resides in the IL-8 promoter (2).

A pivotal step to establish the progression of a tumor is the obtainment of aggressive characteristics by carcinoma cells. A primary process triggering tumor invasion is EMT through which cancer epithelial cells lose their epithelial properties and trans-differentiate to a migratory mesenchymal phenotype (3). EMT has recently been recognized as a key player contributing to tumor progression and the mechanisms regulating this process have been linked to metastasis and cancer stem cell-like cell formation (4,5). Various signaling events have been proposed to facilitate EMT in a variety of human tumors. However, initiating EMT in a tumor is mainly dependent on multiple soluble mediators in the surrounding microenvironment (6).

In this review, we aim at elucidating the complex interactions of IL-8 induction of EMT through direct and indirect mechanisms. We introduce current research on the cytokines, pro-inflammatory mediators and enzymes secreted by neutrophils and TAMs and the mechanism of their induction of EMT.

Additionally, we address the potential therapeutic implications of IL-8 cancer treatment.

2. EMT of tumor

EMT originally takes place during the process of embryogenesis, but it also occurs in adult tissues going through wound healing and remodeling (7). Moreover, in some certain pathological process it is associated with fibrosis and tumor progression. During the EMT process, epithelial cancer cells evolve to a mesenchymal phenotype, by losing their epithelial characteristics and acquiring a fibroblastoid-like morphology especially at the invasive front. Cells undergoing EMT reduce cell polarity and adhesion, exhibit decreased expression of epithelial surface molecules such as E-cadherin and cytokeratins. In parallel, epithelial tumor cells acquire enhanced presentation of mesenchymal proteins such as vimentin and fibronectin as well as increased cell motility, invasiveness and metastasis (8).

Recent studies have focused on EMT in the tumor biology context, since acquisition of mesenchymal features is linked to an improved invasive capacity, that is, could promote tumor infiltrating growth and metastasis (9). Multiple studies have shown that the involvement of EMT is related to tumor progression in different tumor types (10-12). For instance, in adenoid cystic carcinoma which is characterised by local infiltration and distant metastasis, EMT is considered to promote greatly the high rate of metastasis (13). Consistently, downregulation of epithelial marker E-cadherin and increased expression of the mesenchymal markers N-cadherin and vimentin have noted to positively correlate with the aggressiveness and metastasis of breast cancer (14).

Additionally, several reports have shown that cancer cells undergoing EMT present properties of cancer stem cells (CSC) (5), including chemo- and radio-resistance and the ability to self-renewal. Fan demonstrated that hepatocellular carcinoma cells undergoing EMT acquire enhanced CSC-like traits when co-cultured with TAM. Furthermore, depletion of TGF-β1 blocked acquisition of the CSC-like properties by inhibition of TGF-β1-induced EMT (15). Increasing number of studies have identified distinct signaling pathways regulating this step (16,17).

3. IL-8

IL-8 (alternatively known as CXCL8), a prototype of the cysteine-X-cysteine (CXC) chemokines, was originally discovered as a leukocyte chemoattractant (18) and subsequently found to play multiple roles in cancer development (3). Human genes for IL-8 are located on chromosome 4 between 4q13 and 4q21 (19). IL-8 is mainly secreted from leukocytes and endothelial cells under special conditions such as exposure to IL-1 or TNF-α. Additionally, fibroblasts and malignant tumor cells can also secrete IL-8 as a result of various environmental stress including hypoxia, and chemotherapy agents (20). Since existing in monomer or dimer forms, IL-8 activates and regulates its two cell surface receptors respectively (21).

IL-8 exerts its effect by binding to the IL-8Rs, which are two heterotrimeric G protein-coupled receptors, CXCR1 and CXCR2. The two receptors are primarily presented in neutro-

phils, monocytes as well as endothelial cells. However, they are also found on the surface of tumor cells and tumor-associated stromal cells (22). The two receptors show different binding specificities as a result of differences in their N-terminal domains (23). CXCR1 binds IL-6 and IL-8, while CXCR2 has high binding affinity for IL-1, 2, 3, 5, 6, 7 and 8 (24).

IL-8 has demonstrated to induce angiogenesis (25) and promote the progression of many human cancers including prostate cancer (26), non-small cell lung carcinoma (27), melanoma (28), and ovarian cancer (29). Moreover, studies have shown that IL-8 has prognostic value in many malignant tumors (30,31). Aberrantly elevated serum IL-8 level can even precede diagnosis of lung cancer by several years (32). Tumor-derived IL-8 induces proliferation and migration of tumor cell via its autocrine activity. Simultaneously, IL-8 promots the angiogenic response in endothelial cells and the recruitment of neutrophils to the tumor site via its paracrine activity (33).

It has been reported that IL-8 is regulated by microRNA network at post-transcription level (34) and significant correlation between microRNAs and IL-8 is identified in a variety of studies. miR-302c was found to inhibit IL-8 expression and restrain tumor invasion and metastasis. In parallel, IL-8 signaling also exerts a feedback effect on modulating miR-302c and IL-8 expression (35). Qu *et al* suggested that IL-8 was a direct target of miR-203 and miR-23a. Reduced expression of the two miRNAs promoted nasopharyngeal carcinoma radioresistance through IL-8/AKT signaling and IL-8/Stat3 pathway respectively (36,37). Additionally, hsa-miR-200c-3p directly reduced IL-8 expression in inflamed colon of patients with ulcerative colitis (38). Moreover, IL-8 can be suppressed by diverse miRNAs such as miR-K9, miR-K5, miR-17, miR-484, and miR-148a, through indirect manner (34,39-41). On the other hand, microRNA network has been found to be very important in tumor initiation and progression. Several anti-metastatic miRNAs have been identified in a number of cancers, such as miR-335, miR-126, and let-7 family. In addition to anti-metastatic miRNAs, a number of miRNAs are pro-metastatic such as miR-21, miR-373 and miR-520c (42,43).

4. The role of IL-8 in EMT

Tumor cells passing through EMT have been indentified to secrete more chemokine IL-8 as well as to enhance the expression of its receptors. Tumor-derived IL-8 exerts its effect through an autocrine loop to maintain the mesenchymal traits of tumor cells. Furthermore, IL-8 recruits neutrophils and TAMs to the tumor site via a paracrine fashion. In this review, we highlight the cytokines, pro-inflammatory mediators and enzymes secreted by neutrophils and TAMs as well as the mechanism of their induction of EMT.

i) Autocrine IL-8 loop maintain tumor EMT. It is demonstrated that, once through EMT, tumor cells maintain their mesenchymal state by ongoing autocrine signaling loops (44). IL-8 stimulates tumor EMT by activation of various signaling pathways that finally affect the EMT-related transcription factors (Fig. 1). The transcription factors Slug, Snail, and Twist are known to bind to the E-box regulatory regions to repress the expression of E-cadherin (7). Recently, a T-box transcrip-
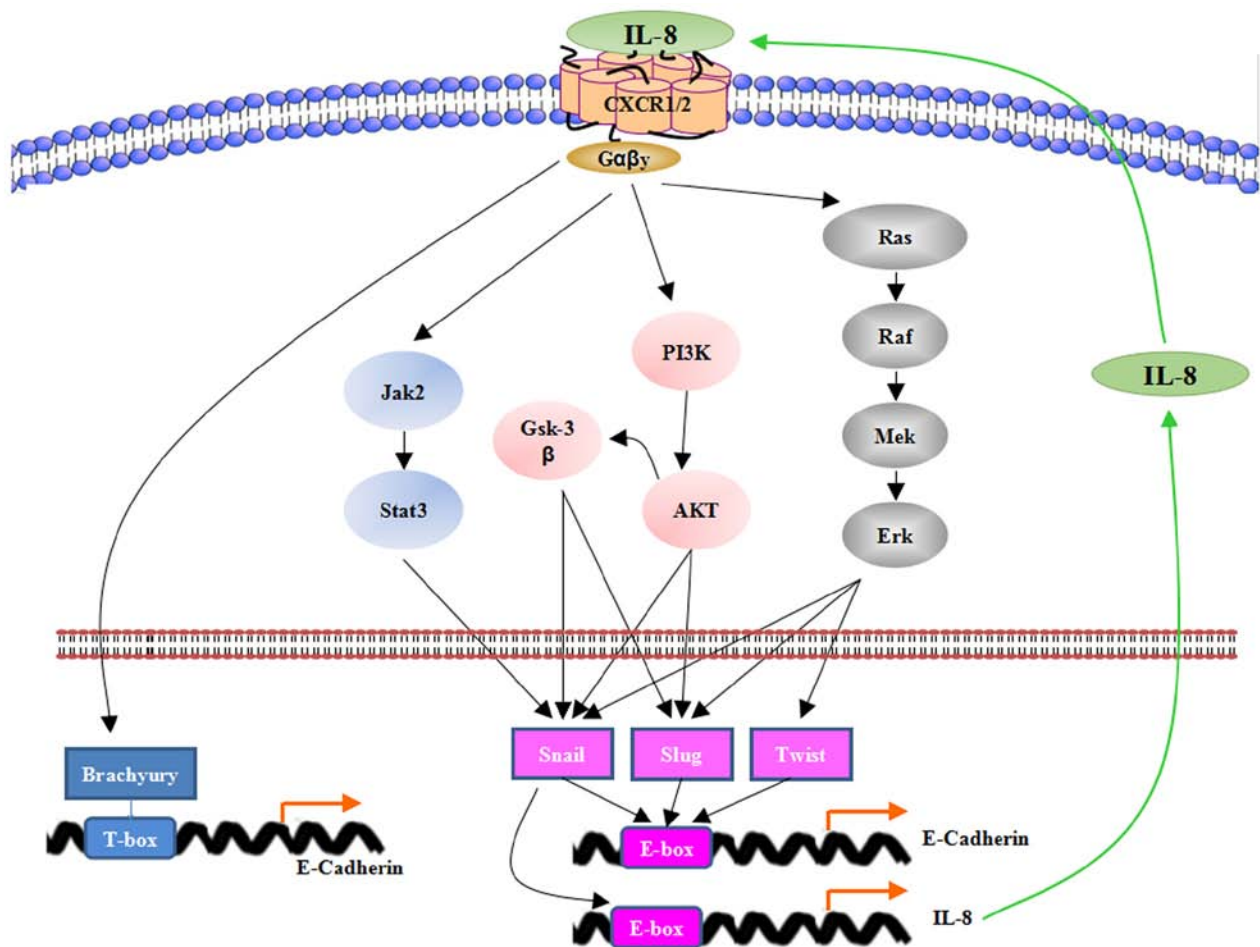
Figure 1. Autocrine IL-8 loop maintain tumor EMT. Tumor-derived IL-8 function in a positive autocrine loop to maintain the mesenchymal traits of tumor cells mainly through AKT, MAPK/ERK and JAK2/STAT3 signaling pathways. Besides, IL-8 can induce the overexpression of T-box transcription factor Brachyury leading to reduced expression of E-cadherin.

tion factor brachyury was identified as a novel trigger of tumor EMT (45). Therefore, the mesenchymal transition occurs. In return, the induction of EMT via Snail upregulation is noted to induce IL-8 secretion. Since Snail could bind to E3/E4 boxes residing in the IL-8 promoter, it directly regulates the expression of IL-8 (46).

IL-8 has been shown to activate AKT signaling in prostate cancer, nasopharyngeal carcinoma (NPC) and thyroid cancer (TC) cell lines (47-49). The serine/threonine kinase AKT, a downstream target of PI3K, phosphorylated glycogen synthase kinase 3β (GSK3β) which induced the phosphorylation and translocation of Snail and Slug (50,51). Thereby, inhibiting GSK-3β activity, AKT activates Snail and Slug indirectly, leading to EMT. In NPC S18 cells, the elevated level of phosphorylated AKT could be suppressed by knocking down IL-8 expression using short-hairpin RNA. Moreover, IL-8-promoted EMT could be inhibited by knocking down AKT expression or applying the PI3K inhibitor LY294002. Besides, suppression of AKT has been shown to revert EMT and stemness responses of TC cells.

MAPK/ERK signaling is accepted as one of the most important regulators in EMT. Via activating small G proteins, IL-8 promotes activation of the MAPK signaling which is characterized by Raf/MEK/ERK cascade (52). ERK trans-

locates to the nucleus and upregulates the activity of several EMT-related transcription factors such as Snail, Slug and Twist. Therefore, the expression of E-cadherin is suppressed (53,54). In addition, IL-8 has been found to induce EMT and promote hepatocellular carcinoma (HCC) cell migration and invasion through JAK2/STAT3/Snail signaling pathway (55).

Additionally to the E-box transcription factors, Brachyury, the T-box transcription factor, has been discovered to promote tumor EMT and cancer cell metastasis in multiple types of human cancer (45). In breast cancer cells, IL-8 induces the overexpression of Brachyury and a mesenchymal-like phenotype. Furthermore, Brachyury displays increased expression of IL-8 and CXCR1/2, which amplified the effect of IL-8 on tumor EMT (56).

ii) Paracrine factors promote tumor EMT. Besides the autocrine loop between IL-8 and tumor cells that have gone through EMT, IL-8 could potentiate adjacent epithelial tumor cells into a mesenchymal phenotype via a paracrine mode (Fig. 2). Tumor cells undergoing EMT exhibit an elevated level of IL-8 as well as CXCR1/2, which amplified the effect of IL-8 on tumor EMT. Besides the effects on tumor cells, IL-8 is identified as an important regulator of neutrophils and TAMs recruited into the tumor microenvironment.
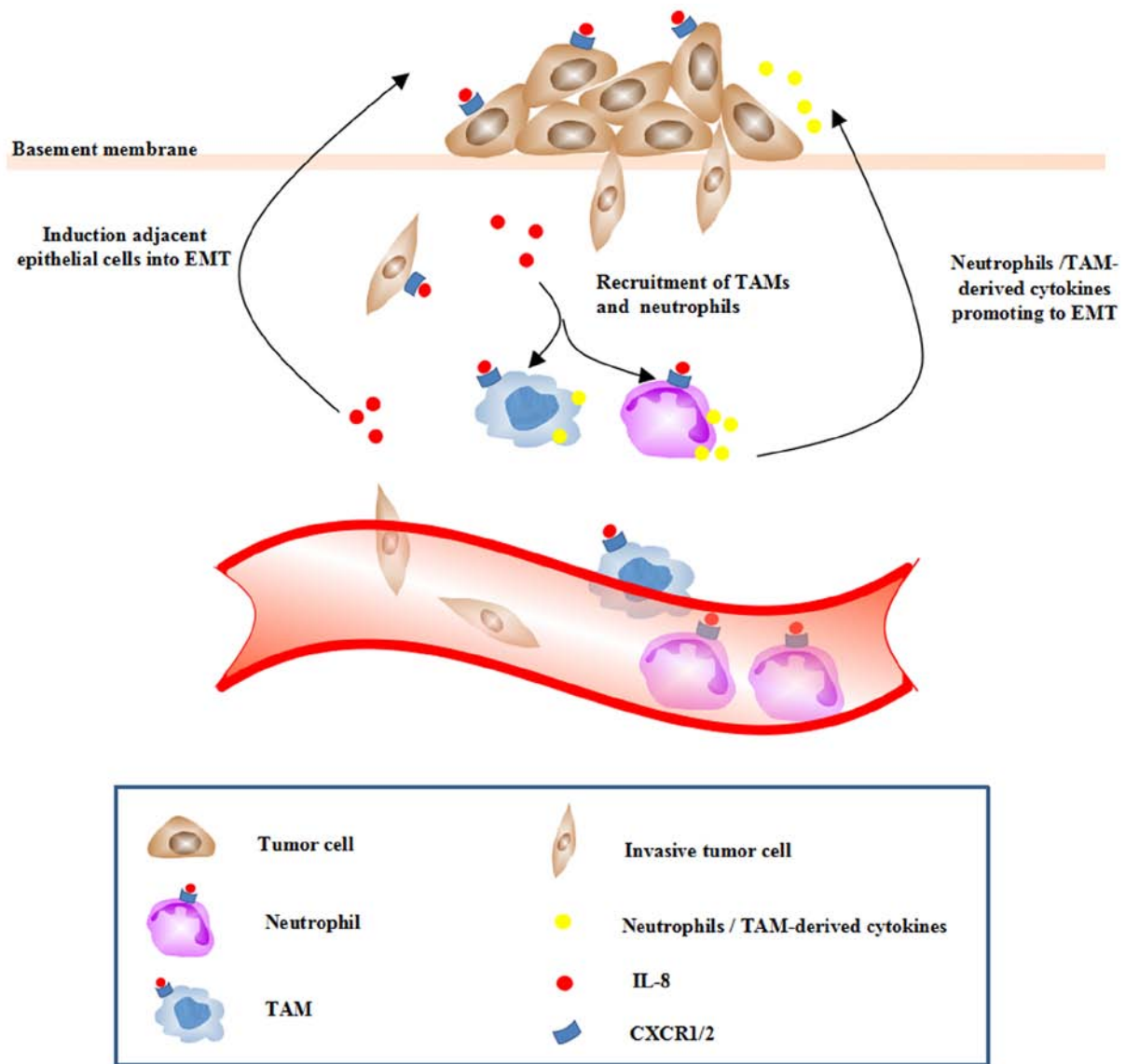
Figure 2. Paracrine IL-8 promotes tumor EMT, IL-8 could potentiate adjacent epithelial tumor cells into EMT by a paracrine mode. IL-8 activates endothelial cells in the tumor vasculature to promote angiogenesis and induces a chemotactic infiltration of neutrophils and TAMs into the tumor site. Neutrophils and TAMs secrete additional growth factors, cytokines, chemokines, enzymes further promoting EMT in the tumor microenvironment.

Macrophages infiltrated in the tumor sites have been shown to induce EMT of HCC cells. TAMs can secrete a vast diversity of cytokines, chemokines and proteases that may influence tumor cells in various ways. Mast cells induced EMT and stem-like traits of TC cells (49). The enhanced intratumoral IL-8 expression could also lead to enhanced recruitment of neutrophils and TAMs, which, in turn, have been found to secrete various cytokines, chemokines, enzymes promoting EMT. Various cytokines (TGF-β1, TNF-α, IL-4, IL-6 and IL-10) secreted by activated macrophages in the cholangiocarcinoma context were shown to induce EMT via elevating the expression of EMT-related genes (57).

Cytokines and chemokines function mainly by binding to certain transmembrane receptors of tumor cells, which are members of a large family of G protein-coupled receptors. In parallel, some enzymes act on the extracellular matrix (ECM), breakdown connective tissue, inhibit E-cadherin synthesis and promote the mesenchymal phenotype in tumor cells.

Growth factors. As one of the most important members of the transforming growth factor family, transforming growth factor-β (TGF-β) is a potential inducer of EMT in cancer cells (58,59). TGF-β mediates EMT via two specific pathways, a Smad-dependent pathway and a Smad-independent pathway (60). After binding to its receptor, TGF-β phosphorylates Smad2 and Smad3, which collaborate with Smad4, and then translocate into the nucleus to regulate the transcription of EMT-associated genes, like Snail (61). Bonde *et al* found that TAMs induced intratumoral epithelial cell EMT via TGF-β/Smad signaling. Data presented in his study identified that macrophage-derived TGF-β led to decreased expression of the epithelial adhesion, increased expression of mesenchymal markers and an aggressive phenotype (62). Additionally, cancer-associated fibroblasts could also promote EMT of breast cancer cells through paracrine TGF-β1 (63).Collective studies have shown TGF-β1 induced EMT mainly through Smad, MAPK, PI3K/AKT and ERK pathway.

Several studies now indicate that EGF activation can break cell adhesion, enhance cell motility and promote tumor EMT (64,65). In EGF-treated cholangiocarcinoma cells, EMT-transcription factors as well as mesenchymal markers were induced. In addition, the EGF-mediated EMT can be suppressed by gefitinib, the inhibitor of EGFR (66).

Cytokines and chemokines. IL-6 has been suggested to induce EMT in breast, colorectal, prostate and lung cancer cells (67-70) via aberrant activation of JAK/STAT3 signaling. Additionally, IL-6 boosted the expression of Snail induced by TGF-β/Smad pathway, contributing greatly to EMT (61,71).

Similarly to IL-6, IL-1β contributes to EMT via different pathways. IL-1β enhanced binding of Zeb1 to the E-box to silence E-cadherin expression (72). IL-1β has also been reported to promote the expression of E-cadherin by upregulaing Snail (73). In addition, cooperated with TGFβ-3, IL-1β activated matrix metalloproteinase (MMP)-1, MMP-3, and MMP-10 gene expression in A549 lung adenocarcinoma cells through MAPK-dependent pathways, and both cytokines stimulated EMT and invasion (74).

Tumor necrosis factor α (TNF-α), which is primarily derived from by macrophages, is one of the critical pro-inflammatory cytokines involved in the tumor microenvironment (75). Several studies suggest that TNF-α induces EMT via NF-κB or AKT/GSK signaling through regulating the expression of Twist and Snail in breast, renal, colon and hypopharyngeal cancer (4,76). Collectively, the evidence indicates that TNF-α may affect the key processes of tumor EMT.

TAM with M2 phenotype could produce a chemokine called chemokine (C-C motif) ligand 18 (CCL18) (77) which exerts its activity mainly by binding to the transmembrane receptor-PYK2 N-terminal domain interacting receptor 1 (Nir1). Nir1 is present in human breast cancer cells (78) and it could induce EMT by stabilising Snail via the PI3K/AKT/GSK3β signaling pathway through binding to CCL18 *in vitro* and *in vivo* (79).

Enzymes. Studies have shown that neutrophil-derived elastase could degrade E-cadherin leading to dyshesion of the pancreatic ductal adenocarcinoma and HCC cells. Furthermore, the EMT transcription factor Twist was upregulated, Zeb1 appeared in the nucleus, β-catenin translocated into the nucleus, and keratins were downregulated (80). In addition, the MMPs that exist in the ECM are associated with various EMT processes. The MMPs have the ability to degrade the functional components of the ECM and contribute to tumor cell migration. Therefore, the mesenchymal transition occurs and each EMT case involves a subset of specific MMPs.

Overexpression of MMP-9 in a prostate cancer model confirmed the association of MMP-9 with tumor invasiveness (81). It has also been found in a gastric carcinoma model that IL-8 upregulates MMP-9 expression and consequently increased neoangiogenesis (82). Besides, TNF-α induces the expression of invasion mediators MMP-7, MMP-9, and the intracellular adhesion molecule-1.

Taken together, various growth factors, cytokines, chemokines as well as enzymes secreted by TAMs and neutrophils can facilitate EMT of tumor cells.

5. IL-8 as target for cancer therapy

As IL-8 is associated with EMT and tumor progression, it is of interest to speculate that therapy targeting IL-8 could improve tumor outcome. Blockade of the IL-8 and its receptors seems a promising therapeutic approach which could reverse the metastatic phenotype of tumor cells undergoing EMT by disturbing the autocrine positive loop between IL-8 and tumor cells. Additionally, it could also reduce the paracrine signals that IL-8 exerted on other non-metastatic tumor cells by lessening recruitment of neutrophils and TAMs. CXCR2 is upregulated in some types of tumors (83-85) and pharmacological inhibition of CXCR1 and CXCR2 represses neutrophil recruitment into A547 lung tumor sites resulting in slower tumor growth (86). Small-molecule antagonists for CXCR2 and CXCR1 have been proposed to inhibit IL-8 functions.

Studies show that CXCR1 blockade by either a CXCR1 specific blocking antibody or repertaxin, a small-molecule CXCR1 inhibitor, selectively depleted human breast cancer stem cells (87). In addition, selectively targeting CXCR2/CXCR1 with orally active small-molecule inhibitors is an effective therapeutic approach for repressing melanoma growth and angiogenesis (88). CXCR2/CXCR1 antagonists may be a useful therapeutic agent in the treatment of many other cancers, such as lung carcinomas.

Small-molecule antagonists for CXCR2 and CXCR1 may represent a promising target for cancer therapy. A better understanding of the function of IL-8 and further knowledge on the interaction between IL-8 and tumor microenvironment may open the way to innovative therapeutic strategies for cancer patients.

6. Conclusion

EMT plays a central role in tumor invasion and metastasis and may be induced by local inflammation. In this review, we focused on IL-8, because it is a major component of the infiltrates present in the tumor microenvironment and plays a vital role in the tumor progression and metastasis. We highlight the cross-link between inflammation and EMT-related tumor development. IL-8 induction of EMT, despite being sophisticated and requiring solid experimental investment, opens new horizons for an efficient tumor therapy.

Acknowledgements

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