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Influence of IL-8 on the epithelial–mesenchymal transition and the tumor microenvironment

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Abstract

The phenomenon of epithelial–mesenchymal transition (EMT) has gained attention in the field of cancer biology for its potential contribution to the progression of carcinomas. Tumor EMT is a phenotypic switch that promotes the acquisition of a fibroblastoid-like morphology by epithelial tumor cells, resulting in enhanced tumor cell motility and invasiveness, increased metastatic propensity and resistance to chemotherapy, radiation and certain small-molecule-targeted therapies. Tumor cells undergoing EMT are also known to increase the secretion of specific factors, including cytokines, chemokines and growth factors, which could play an important role in tumor progression. This review summarizes the current knowledge on the secretory properties of epithelial tumor cells that have undergone an EMT, with an emphasis on the potential role of the IL-8–IL-8 receptor axis on the induction and/or maintenance of tumor EMT and its ability to remodel the tumor microenvironment.

Keywords

brachyury; epithelial–mesenchymal transition; IL-8; metastasis; tumor microenvironment

The epithelial–mesenchymal transition (EMT) is a physiological process during embryogenesis that appears to be reinstated in adult tissues undergoing wound healing and tissue regeneration, or under certain pathological conditions such as fibrosis and cancer [1–3]. Tumor EMT involves a phenotypic switch that promotes the acquisition of a fibroblastoid-like morphology by epithelial tumor cells, reduces cell polarity and cell-to-cell contacts, and decreases the expression of epithelial markers, including E-cadherin and cytokeratins. Concomitantly, epithelial tumor cells undergoing EMT gain expression of mesenchymal-associated proteins, such as fibronectin and vimentin, and have enhanced cell motility, invasiveness and metastatic propensity *in vivo* [4,5]. Tumor EMT has also been shown to contribute to the acquisition of tumor resistance to chemotherapy, radiation [6–8] and certain small-molecule-targeted therapies [9], thus representing a major mechanism contributing to the progression of carcinomas.

Although evidence for the involvement of EMT in the progression of human carcinomas *in vivo* is limited so far, it has been observed that the loss of epithelial E-cadherin associates

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with tumor progression and metastasis in various tumor types [10–13]. In breast cancer, for example, reduction of the epithelial markers E-cadherin and cytokeratins, and upregulation of the mesenchymal markers vimentin and N-cadherin, have been shown to positively correlate with an aggressive tumor phenotype and a high rate of metastasis [14]. In recent reports, several groups have also demonstrated that induction of EMT in epithelial tumor cells results in the acquisition of features of tumor-initiating cells, also designated as cancer stem cells (CSCs) [2,15], including the ability to self-renew *in vitro* and *in vivo*, and enhanced resistance to cell death induced by various cytotoxic agents. This association between EMT and the acquisition of features similar to CSCs was also shown to take place *in vivo*. Residual breast tumor cell populations surviving following conventional treatments had an increased number of cells with markers of CSCs ($CD44^+$, $CD24^-$ or $CD24^{low}$) and mesenchymal features [16].

A significant area of research concerning the role of EMT in tumor progression has focused on the elucidation of the signaling events that trigger this phenotypic switch in carcinoma cells. Intrinsic signaling events, such as those seen with oncogenic *Ras*, have been shown to contribute to EMT in certain types of human carcinomas [4,17]; however, triggering tumor EMT is most often dependent on a variety of external signals provided by the tumor microenvironment, in the form of soluble growth factors, cytokines or components of the extracellular matrix (ECM) [18]. Well-established signals include those initiated by TGF-β, FGF, EGF and HGF, all of which have been shown to promote EMT in various tumor cell models [19,20]. The various cellular components of the tumor stroma, including cancer-associated fibroblasts, endothelial cells and immune cells, are potential sources of soluble factors capable of inducing EMT in neighboring cancer cells. Conversely, cancer cells can themselves produce cytokines, growth factors and other soluble mediators that may reprogram the surrounding stroma to promote tumor growth and dissemination. Furthermore, the soluble factors produced by tumor cells may act to induce and/or maintain EMT in neighboring tumor cells [21,22].

Amid the growing interest in understanding the role of EMT in tumor progression, only a few studies have focused on analyzing the pattern of secreted factors (the secretome) of tumor cells undergoing this phenotypic switch. For example, a proteomic analysis of the secretome of Madin–Darby canine kidney cells demonstrated that epithelial cells undergoing Ras-mediated EMT have the potential to actively remodel the extracellular compartment by reducing the expression of basement membrane constituents (collagen type IV and laminins) and augmenting the secretion of ECM constituents (osteonectin and collagen type I) and the extracellular proteases, including kallikreins and matrix metalloproteinases (MMPs) [23]. The identification of an ‘EMT-associated tumor secretome’ may not only lead to the identification of molecular markers of tumor EMT, but also to the identification of novel targets for interventions against tumor progression. This review summarizes the current knowledge on the secretory properties of mesenchymal-like tumor cells, with an emphasis on the potential role of the IL-8–IL-8 receptor (IL-8R) axis on the induction and/or maintenance of tumor EMT and in the remodeling of the tumor microenvironment.

Autocrine cytokine loops maintain tumor EMT

Several studies now indicate that, once EMT is initiated, the permanence of tumor cells in a mesenchymal status post-EMT is dependent on the existence of autocrine cytokine and/or growth factor loops that were initially responsible for the induction of EMT in the same cells (TABLE 1) [24,25]. TGF-β is a multifunctional cytokine regarded as being a tumor suppressor in normal epithelial cells and during early tumor growth, while acting as a potent tumor-promoting factor in late-stage tumors [26]. In late-stage or metastatic tumors, TGF-β promotes angiogenesis, recruits various cell types to the site of the tumor, including

fibroblasts and immune cells, suppresses a functional anti-tumor immune response and induces tumor cell migration, invasion and EMT [27,28]. In multiple tumor cell models, it has also been demonstrated that, once tumor cells have undergone EMT, secretion of TGF-β is markedly upregulated [24,29,30]. Once secreted, TGF-β has the ability to function in an autocrine fashion, contributing to the maintenance of the mesenchymal and stem cell phenotypes of carcinomas that have passed through EMT [24,26]. This has been shown, for example, with Madin–Darby canine kidney cells, which were induced into EMT by the addition of TGF-β. This resulted in the establishment of an autocrine TGF-β signaling loop that was essential for sustaining the expression of the EMT regulator ZEB [25].

Similar results were observed with the secretion of the cytokine IL-6 [31] or VEGF [32] by tumor cells undergoing EMT. IL-6 is a pleiotropic cytokine involved in the differentiation and growth of hematopoietic stem cells, T cells and B cells, as well as the differentiation of other cell types that express IL-6R (CD126) and the coreceptor gp130 (CD130), including endothelial cells, osteoblasts and hepatocytes [33]. IL-6 is a well-known regulator of immune responses and a major contributor to the pathogenesis of various autoimmune and inflammatory diseases [34], and it has also been implicated as a potent growth factor for certain tumor cell types, including breast cancer cells [35]. Recent reports also demonstrate a role for IL-6 in tumor EMT [31]. MCF7 cells overexpressing Twist, for example, have been shown to upregulate the secretion of IL-6 and, at the same time, to activate STAT3, indicating the existence of a positive feedback loop involving autocrine-mediated IL-6 signaling events [31].

Tumor cells undergoing EMT are also known to secrete MMPs, which can degrade the structural components of the ECM to facilitate tumor cell migration, while simultaneously activating or inactivating growth factors or protease inhibitors, respectively [36]. The upregulation of MMPs has been associated with a variety of EMT processes, each case involving a subset of specific MMPs. For example, it has been demonstrated that the transcription factor SNAIL promotes EMT and simultaneously mobilizes the membrane-anchored MMPs, MT1-MMP and MT2-MMP, to allow breast cancer cells to transmigrate basement membrane barriers, initiate angiogenesis and intravasate into the circulation [37]. Interestingly, MMPs can also induce tumor EMT; treatment with MMP-3, for example, was shown to induce an epithelial–mesenchymal switch in mouse mammary epithelial cells [38,39]. Similarly, upregulation of MMP-28 expression in lung carcinoma A549 cells was shown to induce a TGF-β-dependent EMT by activating latent TGF-β complexes and ultimately inducing an EMT [40]. Altogether, these results indicate the importance of autocrine loops in maintaining the mesenchymal features of tumor cells that have undergone EMT.

IL-8 as an inducer of tumor EMT

Brachyury, tumor EMT & tumor EMT-secreted factors

The transcriptional control of EMT is dependent on the expression of a few transcriptional regulators, defined as ‘EMT transcription factors’, which are ultimately responsible for the regulation of the epithelial and mesenchymal genes modulated during the course of an EMT. While these transcription factors are normally involved in the control of EMT that takes place during embryogenesis, they are re-expressed by epithelial tumor cells undergoing this phenotypic switch. Examples of well-characterized EMT transcription factors include the zinc- finger proteins SNAIL, SLUG, ZEB1 and ZEB2 [41–44], and the helix-loop-helix transcription factor TWIST [45,46]. Recently, the authors’ group identified brachyury, a T-box transcription factor involved in the formation of the mesoderm during embryonic development [47], and a novel driver of EMT in human carcinomas [5]. The overexpression of brachyury in epithelial carcinoma cell lines has been shown to induce changes

characteristic of an EMT, including increased tumor cell motility and invasiveness [5], while its inhibition in tumor cells having a more mesenchymal phenotype resulted in changes characteristic of a mesenchymal–epithelial transition, with loss of expression of mesenchymal markers, gain of epithelial markers, and concurrent loss of tumor cell motility and invasiveness. *In vivo*, it has been demonstrated that brachyury is required for the dissemination of lung carcinoma cells, as brachyury-silenced H460 cells showed a diminished ability to form spontaneous lung metastases in nude mice [5]. The potential role of brachyury in human cancer progression was also suggested by the observation that brachyury mRNA expression is predominant among late-stage lung tumor tissues, with a lower expression among stage I lung tumors [5].

Fernando *et al.* [48] recently demonstrated that the transition of human tumor cells from an epithelial to a mesenchymal-like phenotype, as a result of brachyury overexpression, is associated with the secretion of multiple cytokines, chemokines and growth factors that have previously been reported to promote tumor growth, motility, invasion and vascularization in various types of carcinomas [49–52]. Breast and pancreatic cancer cells were forced into EMT by overexpression of brachyury and culture supernatants evaluated for the expression of multiple cytokines, chemo kines and growth factors. Induction of tumor EMT was shown to enhance the secretion of a subset of inflammatory cytokines, various chemokines and angiogenic factors, which included IL-6 and IL-8, CXCL1, RANTES, OPG, VEGF, angiogenin and PLGF.

IL-8–IL-8R loops involved in EMT & tumor stemness

The chemokine IL-8, initially identified as a neutrophil-activating and chemotactic factor [53], plays multiple roles as a proinflammatory cytokine by mediating the activation and chemotaxis of various immune cell types, and can lead to chronic inflammatory conditions if aberrantly expressed [54]. Two major sources of this chemokine are monocytes and endothelial cells, which secrete IL-8 in response to various stimuli, such as exposure to IL-1 or TNF-α. In addition, other cell types, including fibroblasts, keratinocytes [55] and tumor cells, can secrete IL-8, particularly under stress conditions such as hypoxia or exposure to chemotherapy agents [56,57]. Once secreted, the activity of IL-8 depends on its binding to the IL-8Rs, IL-8R A (CXCR1) and IL-8RB (CXCR2), which are mainly expressed on neutrophils, monocytes, endothelial cells and some cancer cells [57,58]. In the context of a tumor, IL-8 is known to participate in cancer progression by promoting the angiogenic response of endothelial cells, the recruitment of neutrophils to the site of the tumor, and the proliferation, survival and migration of tumor cells [59,60]. It has been demonstrated that many types of human carcinomas, including breast, colon, cervical, gastric, lung and ovarian cancers, among others, express high levels of IL-8 relative to normal tissues [59]. In addition, multiple clinical studies in melanoma, as well as breast, ovarian, prostate and colon cancer have shown a direct correlation between serum IL-8 levels and disease progression [57]. In recent years, it has also been demonstrated that a link exists between IL-8, tumor EMT and tumor stemness. In colorectal cancer cell lines, for example, the induction of EMT via incubation with TGF-β [61] or via SNAIL overexpression [62] has been shown to induce the secretion of IL-8. Tumorspheres expanded from colorectal tumor tissues and cell lines were shown to contain cells with many features of CSCs, including chemo- and radio-resistance, expression of EMT markers and overexpression of the EMT regulator SNAIL. It was demonstrated that SNAIL directly activates the expression of IL-8 by binding to E3/E4 E-boxes located in the IL-8 promoter [62]. Blocking experiments showed that IL-8 expression is critical for SNAIL-induced EMT and tumor stemness, as blockade of IL-8 signaling resulted in decreased expression of the stem cell-associated genes *SOX2*, *OCT4* and *NANOG*, and decreased formation of tumorspheres by SNAIL-overexpressing colorectal cancer cells [62].

The existence of an autocrine positive loop between IL-8 and the EMT transcription factor brachyury in breast and lung cancer cell lines has also been demonstrated [48]. Upregulation of IL-8 and the IL-8Rs IL-8RA and IL-8RB was observed in epithelial tumor cells undergoing brachyury-mediated EMT. Antibody blockade of IL-8Rs markedly reduced fibronectin expression and invasiveness of brachyury-overexpressing breast cancer cells, providing evidence that the IL-8–IL-8R axis is essential for the maintenance of their mesenchymal, invasive phenotype (FIGURE 1) [48]. The IL-8–IL-8RA axis has also been shown to play a critical role in breast CSCs. Populations of CSCs, characterized by elevated activity of aldehyde dehydrogenase, have been shown to express elevated levels of IL-8RA and, in turn, the addition of purified IL-8 to epithelial breast cancer cells has been shown to increase the percentage of aldehyde dehydrogenase-positive cells, as well as to enhance the migration and invasiveness of CSCs *in vitro* [63]. Blockade of IL-8R activity via neutralizing antibodies or by utilizing the small-molecule inhibitor of IL-8R, repertaxin, decreased the breast CSC population both *in vitro* and *in vivo* [64], reinforcing the importance of the IL-8–IL-8R axis in breast CSCs. Additional studies conducted with primary colorectal cancer cells transfected with the stem cell-associated transcription factor *OCT4* also showed that CSCs secrete higher levels of IL-8 and that neutralizing antibodies against IL-8 are able to inhibit tumorsphere formation along with the expression of the CSC markers CD133, CD44, SOX2, SNAIL and ABCG2, and decrease resistance to treatment with 5-fluorouracil [65]. Altogether, these studies emphasize the importance of IL-8 in carcinoma progression as an essential factor for the induction and maintenance of the mesenchymal and stem-like phenotype of aggressive, metastatic carcinoma cells.

An interesting observation in the studies conducted by Fernando *et al.* was that culture supernatants from brachyury-overexpressing mesenchymal tumor cells were able to induce other epithelial cancer cells, including breast MCF7 and T47D luminal cancer cells, to undergo an EMT characterized by enhanced expression of brachyury, SNAIL and SLUG [48]. IL-8R-blocking studies showed that this EMT-inducing effect was at least in part due to the EMT-inducing activity of the chemokine IL-8. The role of IL-8 in tumor EMT was also demonstrated by directly exposing breast epithelial tumor cells to purified, recombinant human IL-8 *in vitro*, a treatment that significantly reduced the expression of epithelial E-cadherin and increased fibronectin expression in MCF7 and T47D luminal breast cancer cells [48].

Similar to IL-8, the addition of purified, recombinant IL-6 was sufficient to promote an EMT phenotype in breast cancer cells, resulting in increased tumor invasiveness *in vitro* and a high rate of tumor cell proliferation *in vivo* [31]. These results suggest that soluble factors secreted by tumor cells undergoing EMT could function in a paracrine mode to induce adjacent epithelial tumor cells to undergo this phenotypic switch (TABLE 1).

Potential effects of IL-8 on the tumor microenvironment

As depicted in FIGURE 1, IL-8 released by tumor cells undergoing EMT could play several roles in the context of tumor progression by: maintaining the mesenchymal, invasive phenotype of tumor cells that have undergone EMT via an autocrine loop; exerting a paracrine effect on adjacent epithelial tumor cells in order to induce EMT; enhancing angiogenesis and potentially attracting immune cells to the site of the tumor, thus creating an inflammatory environment that could further favor tumor dissemination; and metastasis.

Among the cellular targets of IL-8 are the endothelial cells, which can be induced to proliferate and migrate in response to IL-8 signaling, therefore resulting in neovascularization [66]. In a study with non-small-cell lung carcinoma cells forced into EMT via SNAIL overexpression, the enhanced secretion of the chemokine IL-8 led to

enhanced angiogenesis and tumor growth *in vivo* [67]. The SNAIL-mediated increase in tumor burden was efficiently abrogated with anti-IL-8RB neutralizing antibodies, a result that indicated the fundamental role of IL-8 in SNAIL-mediated tumor progression [67]. These results also demonstrated that tumor cells undergoing EMT have the potential to directly affect their surrounding stroma via the secretion of soluble factors. A similar example was reported with MCF7 breast cancer cells undergoing EMT via Twist overexpression, which grew as highly vascularized tumors *in vivo* as a result of their increased secretion of the angiogenic factor VEGF [68].

In addition to its effects on endothelial cells, IL-8 is also known to be a strong chemotactic factor for neutrophils [59]. The enhanced secretion of IL-8 by tumor cells undergoing EMT could also lead to enhanced recruitment of neutrophils, which, in turn, have been shown to exert various protumorigenic and prometastatic functions. For example, in a study conducted with a K-RAS-mutated lung adenocarcinoma model, enhanced secretion of the chemokines MIP-2 and CXCL1, murine homologs of IL-8, was linked to the recruitment of neutrophils to the site of the tumor [69]. Tumor-associated neutrophils, in turn, have been shown to assist in tumor progression by different means. For example, tumor-associated neutrophils have been shown to directly facilitate the escape of melanoma tumor cells from the circulation into lung tissues via ICAM-1-mediated binding of tumor cells to the surface of neutrophils [70]. Moreover, tumor progression can be assisted by tumor-associated neutrophils via the secretion of multiple proteinases, including MMPs, which could remodel the ECM and favor tumor migration [71]. Although several studies have shown enhanced secretion of IL-8 in the context of tumor EMT, so far there have been no studies specifically investigating the leukocytic infiltrates of tumors characterized by EMT in contrast to those with a fully epithelial phenotype.

Tumor cells undergoing EMT are not the only source of IL-8 in the microenvironment of a progressive tumor. As previously mentioned, various cellular components of the tumor stroma, including fibroblasts, endothelial cells and immune cells, can secrete IL-8 in response to various stress factors. Of particular interest are reports demonstrating that fibroblasts undergoing chemotherapy- or radiation-induced DNA damage and senescence, but not nonsenescent fibroblasts, are able to induce EMT and invasiveness in epithelial cancer cells. It was shown that this effect was mediated via the secretion of biologically active proteins by senescent fibroblasts, designated as the senescence-associated secretory phenotype, and, in particular, via secretion of IL-6 and IL-8 [49]. These observations indicate that the tumor stroma has the potential to promote tumor progression via secretion of multiple factors involved in intercellular signaling, acting in a paracrine fashion on the epithelial tumor compartment. As a result, IL-8 released by the stroma could directly influence tumor cell proliferation [72], migration, invasion and EMT [48,61,73], and, as more recently shown, could help tumor cells to evade stress-induced apoptosis [74].

Conclusion

Multiple studies have now demonstrated that tumor cells undergoing EMT acquire the capacity to secrete a milieu of cytokines, chemokines and growth factors that could potentiate tumor dissemination by modulating the tumor microenvironment. Although most of these studies have been conducted with tumor cell lines *in vitro*, the findings may have implications for cancer therapy. Pharmacological inhibition of these cytokine regulatory loops appears to be a rational approach for improving interventions against tumor progression. For example, blockade of the IL-8 signaling loop in solid tumors might favor clinical outcome by suppressing tumor growth, angiogenesis and the EMT-promoting activity of the IL-8–IL-8R axis. It is important to point out that the acquisition of a mesenchymal-like phenotype by carcinoma cells is postulated to be a transient and, perhaps,

focalized process that may involve a few cells at the tumor–stroma interface. However, the transient character of EMT is still a topic of debate as some studies have supported the idea that mesenchymal-like tumor cells are required to switch back to their epithelial phenotype once they have reached the site of metastasis [75,76], while others have shown that EMT-associated molecules, such as Twist, remain upregulated at the site of metastatic prostate cancer lesions [46]. This potentially transient nature of EMT raises the question of when an intervention against IL-8 signaling could be adequate to block or reverse the appearance of tumor EMT. In this regard, the expression of IL-8 is known to be elevated in tumors compared with normal tissues, but few data are available on the expression of IL-8 in relation to tumor stage, or its levels in primary versus metastatic lesions. To better understand what type of tumors and which stages would benefit the most from anti-IL-8 interventions, further studies are needed to comparatively characterize IL-8 expression and features of EMT at various stages of human tumor development. The identification of additional regulators of EMT and, in particular, their validation as modulators of EMT *in vivo* may ultimately lead to combinatorial strategies that could more efficiently prevent tumor metastasis.

Future perspective

Owing to the relevant role of EMT in the progression of carcinomas, future antitumor interventions that specifically target tumor cells undergoing EMT could be envisioned to interfere with metastatic disease. The elimination of tumor cells that exhibit a mesenchymal phenotype could potentially be achieved by blocking the signaling pathways that trigger and/or maintain tumor EMT. In particular, blockade of the IL-8–IL-8R axis appears to be an attractive strategy to disrupt the autocrine positive feedback loop between EMT and IL-8 while simultaneously decreasing the paracrine signals that mesenchymal tumor cells could exert on their surrounding environment. Secretion of IL-8 is also a feature of the tumor stroma, and blockade of IL-8 signal ing could be fundamental in lessening the tumor-promoting signals originated on stromal fibroblasts, monocytes, neutrophils and endothelial cells in response to stressful environments, including hypoxia, acidosis or genotoxic damage. Supporting this strategy, several preclinical studies have already demonstrated the ability of neutralizing antibodies to the IL-8Rs, a humanized antibody against IL-8 (ABX-IL-8) and the small-molecule inhibitor repertaxin to inhibit angiogenesis, tumor growth and metastasis in xenograft tumor models [64,77,78].

A similar strategy to inhibit EMT via blockade of the signaling pathways that trigger this phenotypic conversion is exemplified in a recent report by Reka *et al.* [79], in which PPAR-γ synthetic ligands were used for inhibition of TGF-β-induced EMT in lung cancer cells. Activation of PPAR-γ was achieved by utilizing thiozolidinediones, compounds commonly utilized as insulin-sensitizing agents for the treatment of Type 2 diabetes, which inhibited TGF-β-induced EMT features, including migration, invasion and secretion of MMPs, and decreased the development of experimental lung metastasis *in vivo*.

An alternative strategy to directly eradicate tumor cells undergoing EMT is the use of cancer vaccine approaches that target essential regulators of the process such as the EMT transcription factors. In this context, the T-box transcription factor brachyury fulfills two major requirements for a molecule to be used as a target for vaccine approaches: brachyury is highly tumor-specific, being expressed in various human carcinomas but absent in most human normal adult tissues [5,80]; and brachyury-specific cytotoxic T lymphocytes can be expanded from the blood of cancer patients against an epitope of the brachyury protein [80]. Moreover, brachyury-specific T cells can lyze tumor cells that express the brachyury protein, indicating that a vaccine approach is a viable option for the generation of a long-lasting immune response against this EMT regulator. Based on these observations, a

brachyury- based cancer vaccine is currently undergoing Phase I clinical evaluation in patients with carcinomas. As multiple preclinical and clinical studies are demonstrating the feasibility and benefit of employing combinatorial therapies for the treatment of cancer, it can be anticipated that the elimination of metastatic tumor cells via targeting of EMT may rely on the combination of the approaches described above.

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Executive summary

Autocrine cytokine loops maintain tumor epithelial–mesenchymal transition

- Tumor cells undergoing epithelial–mesenchymal transition (EMT) can profoundly modulate their microenvironment via enhanced secretion of multiple soluble factors, including IL-8, IL-6, TGF-β, VEGF and matrix metalloproteinases, among others.
- Once EMT has been initiated, tumor cells can sustain their mesenchymal phenotype by establishing autocrine loops that involve tumor-secreted cytokines and/or growth factors and concurrent upregulation of their cognate receptors.

IL-8 as an inducer of tumor EMT

- Brachyury, tumor EMT and EMT-secreted factors:
  - Brachyury, a highly tumor-specific T-box transcription factor, is a regulator of EMT in human carcinomas;
  - Brachyury-induced tumor EMT is characterized by enhanced IL-8–IL-8 receptor (IL-8R) signaling, which is essential for the maintenance of the mesenchymal phenotype of brachyury-overexpressing human tumor cells.
- IL-8–IL-8R loops involved in EMT and tumor stemness:
  - IL-8–IL-8R loops maintain the mesenchymal phenotype of tumor cells undergoing EMT;
  - Blockade of IL-8–IL-8R signaling decreases expression of stem cell-associated markers and tumorsphere formation of cancer stem cells;
  - IL-8 induces EMT in epithelial tumor cells.

Potential effects of IL-8 on the tumor microenvironment

- IL-8 induces angiogenesis and recruitment of neutrophils to the site of the tumor.
- Stroma-derived IL-8 can induce EMT in epithelial tumor cells.

Future perspective

- Pharmacological inhibition of cytokine regulatory loops may improve anticancer interventions by reversing the metastatic phenotype of tumor cells and reducing paracrine signals on the tumor microenvironment.
- Blockade of IL-8 signaling in solid tumors might counter tumor progression by decreasing angiogenesis, tumor growth and the EMT-promoting activity of the IL-8–IL-8R axis.
- A cancer vaccine against the EMT regulator brachyury is currently undergoing Phase I clinical evaluation for the treatment of human carcinomas.

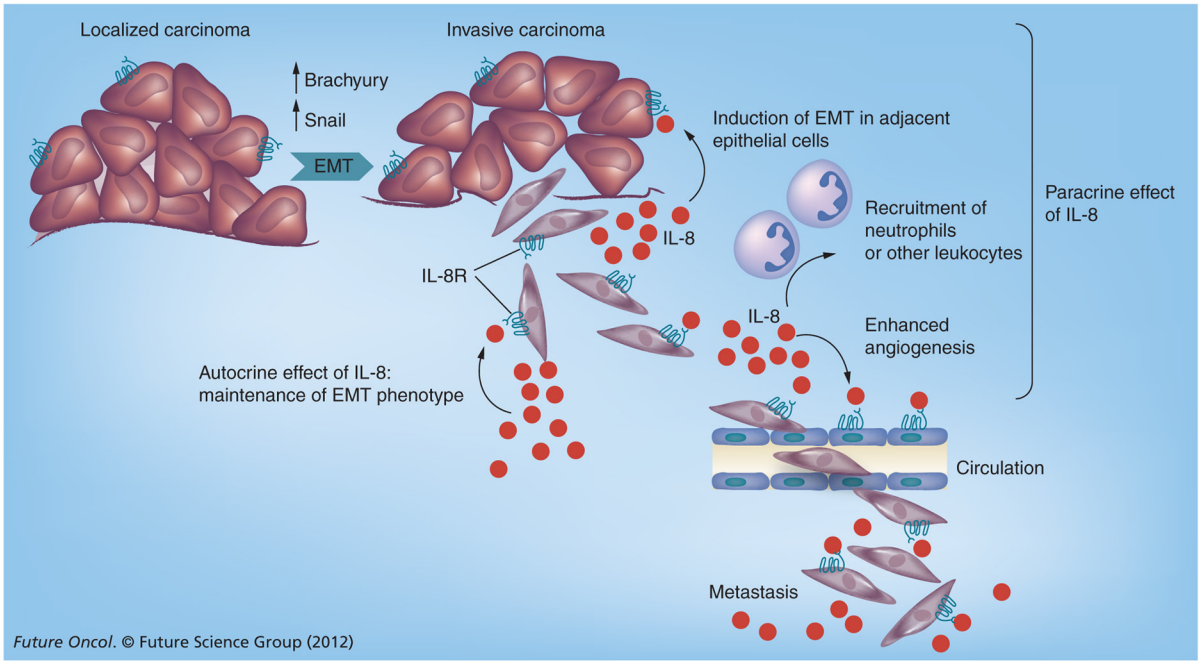

Figure 1. Potential role of the IL-8–IL-8 receptor axis along tumor progression

Tumor cells undergoing EMT have been shown to enhance the secretion of the chemokine IL-8 as well as to upregulate the expression of IL-8R. Secreted IL-8 could function in an autocrine loop to maintain the mesenchymal status of tumor cells that have passed through EMT, as well as in a paracrine fashion to induce adjacent epithelial tumor cells into EMT. The paracrine role of EMT may also involve its activity on endothelial cells to induce angiogenesis, as well as the recruitment of neutrophils and other leukocytes to the site of the tumor.

EMT: Epithelial–mesenchymal transition; IL-8R: IL-8 receptor.

Table 1

Cytokine loops that initiate/maintain tumor epithelial–mesenchymal transition.

EMT-secreted factors	Function during tumor EMT
IL-8	Induction of EMT
	Induction of tumor stemness
	Maintenance of EMT by an autocrine IL-8 loop
	Angiogenesis
	Recruitment of TANs
IL-6	Induction of EMT
	Maintenance of EMT by an autocrine IL-6 loop
VEGF	Angiogenesis
	Induction of EMT in epithelial tumor cells
TGF-β	Induction of EMT
	Maintenance of EMT by an autocrine TGF-β loop
	Impairment of antitumor immune responses
MMPs	Degradation of ECM
	Activation of growth factors
	Inactivation of protease inhibitors
	Induction of EMT

ECM: Extracellular matrix; EMT: Epithelial–mesenchymal transition; MMP: Matrix metalloproteinase; TAN: Tumor-associated neutrophil.