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Functions of chemokines in the perineural invasion of tumors (Review)

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Abstract. The perineural invasion (PNI) of malignant tumors is a form of tumor progression in which cancer cells encroach along nerves. PNI hinders curative resection. Residual tumor cells in or around nerves can bring about local recurrence, infiltration and metastasis. This behavior is usually associated with a poor clinical prognosis. Therefore, it is necessary to investigate novel ligand-receptor crosstalk between nerves and tumor cells that promote the process of PNI. Chemokines are regarded as one of pivotal factors involved in the process of PNI. The present review collates information provided by previous studies with regard to the role of chemokines in PNI. The study presents a definition of PNI in cancer, generalizes the biological characteristics and the expression of chemokines and their receptors in cancer types associated with PNI, and discusses the underlying molecular mechanisms of chemokines, the reciprocal interactions between chemokines and other factors in PNI, and the interconnectivity of the microenvironment and chemokines. The aim of the review is to thoroughly illustrate the molecular cues of chemokines in cancer with PNI and to identify novel antitumor targets.

Contents

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1. Introduction

Perineural invasion (PNI) is the existence of cancer cells along the sides of nerves and/or inside the epineural, perineural and endoneural spaces of the neuronal sheath (1,2). Solid tumors disseminate in four well-known ways: Direct invasion of surrounding tissues, lymphatic spread, hematogenous spread and seeding along body cavities, with PNI regarded as the fifth route of cancer spread (1). PNI is considered as a marker of poor prognosis for numerous malignant neoplasms, including head and neck (1,3), pancreatic (4), prostate (5), colorectal (6), gastric (7), salivary (8) and breast (9) cancer. It is closely associated with increased post-operative locoregional recurrence and a decreased survival rate.

PNI was first proposed more than 100 years ago, and a clear shift in the understanding of its pathogenesis has occurred. The traditional theory for the pathogenesis of PNI was that tumor cells spread passively along the connective tissues that covered the nerves or through the perforating vessels of the nerve beams, where there was least resistance (10). However, recently, studies have revealed that cancer cells have an innate ability to actively migrate along nerves in a mechanism called neural tracking, which is supported by various molecules, including nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), neural cell adhesion molecule, matrix metalloproteinases (MMPs) and chemokines, which are secreted by tumor cells and other non-tumor cells in the tumor microenvironment (11). Among these molecules, the role of chemokines and their receptors in the PNI of malignant neoplasms have gained a high degree of attention recently (Fig. 1). The present review will evaluate the biology and the expression of chemokines and their receptors in cancer type associated with PNI, thoroughly discuss the underlying

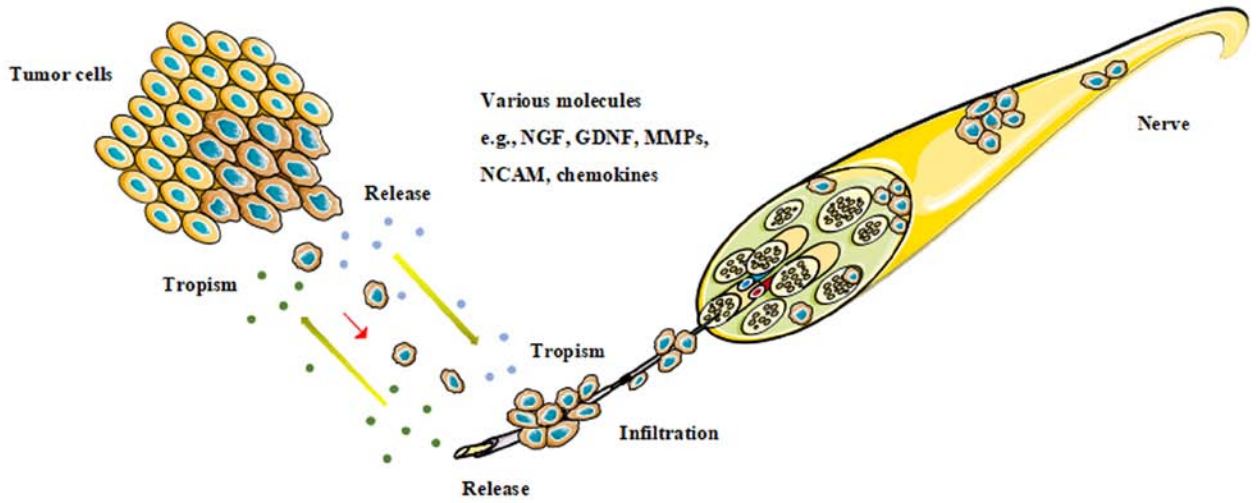

Figure 1. Schematic representation of molecules involved in perineural invasion. Various molecules, including NGF, GDNF, NCAM and chemokines, secreted by tumor cells and nerve cells in the tumor microenvironment, promote tumor cell migration toward nerves and invasion of the nerves. NGF, nerve growth factor; GDNF, glial cell line-derived neurotrophic factor; NCAM, neural cell adhesion molecule; MMP, matrix metalloproteinase.

molecular mechanisms of chemokines in PNI and identify novel antitumor targets.

2. Biology of chemokines and their receptors

Chemokines are a group of small soluble peptides (8-14 kDa) secreted by various cell types, including epithelial, endothelial and immune cells, as well as certain tumor cells (12-14). Approximately 50 chemokines have been detected, and they are divided into four chemokine ligand subtypes, known as the C, CC, CXC, and CX3C subtypes, respectively, according to the number and relative spatial position of their conserved cysteine residues in the amino-terminal region of the peptides (Fig. 2). The majority of chemokines are composed of the CC and CXC subfamilies, including 28 (CCL1-28) and 16 (CXCL1-16) members, respectively. The C and CX3C group are two minor subfamilies, and only 2 members (CL1 and CL2) and 1 member (CX3CL1), respectively, have been described (14-17). Chemokines are pivotal components and orchestrators during the process of immune and inflammatory reactions, acting by controlling the adhesion and cross-endothelial movement of leukocytes, lymphocytes and monocytes from the circulatory system to corresponding inflammatory sites. Chemokines are also involved in other processes, including embryonic growth and development, homeostasis of the central nervous system, wound healing, and the occurrence and development of tumors (18,19).

Chemokines unleash their broad biological functions by combining with their G protein-coupled receptors (GPCRs). These are transmembrane proteins with an extracellular N-terminal domain, which is necessary for specific binding and activation of their corresponding ligands, and an intracellular C-terminus, which is essential for receptor-mediated signal transduction (15,20) (Fig. 3). Chemokine receptors can be divided into C receptor (CR1), CC receptor (CCR1-10), CXC receptor (CXCR1-7) and CX3C receptor (CX3CR) according to the subfamilies of chemokine ligands they bind to. In the immune and inflammatory reactions, several chemokine ligands can interact with a single receptor, and a single chemokine ligand may also activate multiple receptors. Conversely, a homeostatic chemokine ligand has a relatively strict specificity and a single receptor can be activated by only one or two chemokines typically (21,22).

In general, heterotrimeric GPCR is composed of an α subunit and a βγ heterodimer. The βγ subunit acts as a Gα inhibitor by binding to the Gα subunit. Once a chemokine binds to and activates its receptor, conformational changes in the transmembrane region of GPCR will be induced, leading to the transition of the Gα subunit from inactive guanosine diphosphate-bound to active guanosine triphosphate (GTP)-bound and dissociation from the βγ heterodimer (23-25). Thus, the downstream signaling effectors, including G protein-coupled receptor kinases, ion channels, protein kinase C and phospholipase C can be initiated. This further brings about signaling cascades, including the activation of Ras homolog gene family A kinase, extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt) and Janus-activated kinase-2, which can promote gene transcription and evoke various cellular responses, including cell proliferation, reorganization of the actin cytoskeleton, shape change and migration (25-28) (Fig. 3).

3. Chemokines promote the progression of cancer

Studies have demonstrated that chemokines serve an important role in the progression of tumors (29). Chemokines, as autocrine growth factors, can accelerate tumor growth via activation of growth factor receptors. Chemokines also promote the proliferation of tumor cells by making the tumors insensitive to anti-growth signals (30,31).

Chemokines can modulate tumor invasion and metastasis by promoting epithelial mesenchymal transition (EMT), upregulating the expression of proteases and downregulating the expression of E-cadherin and integrin through a series of signaling pathways, including the MAPK/ERK/PI3K/Akt signaling pathways. Furthermore, chemokine receptors expressed by tumor cells can make a response to their corresponding chemokine ligands and migrate directionally

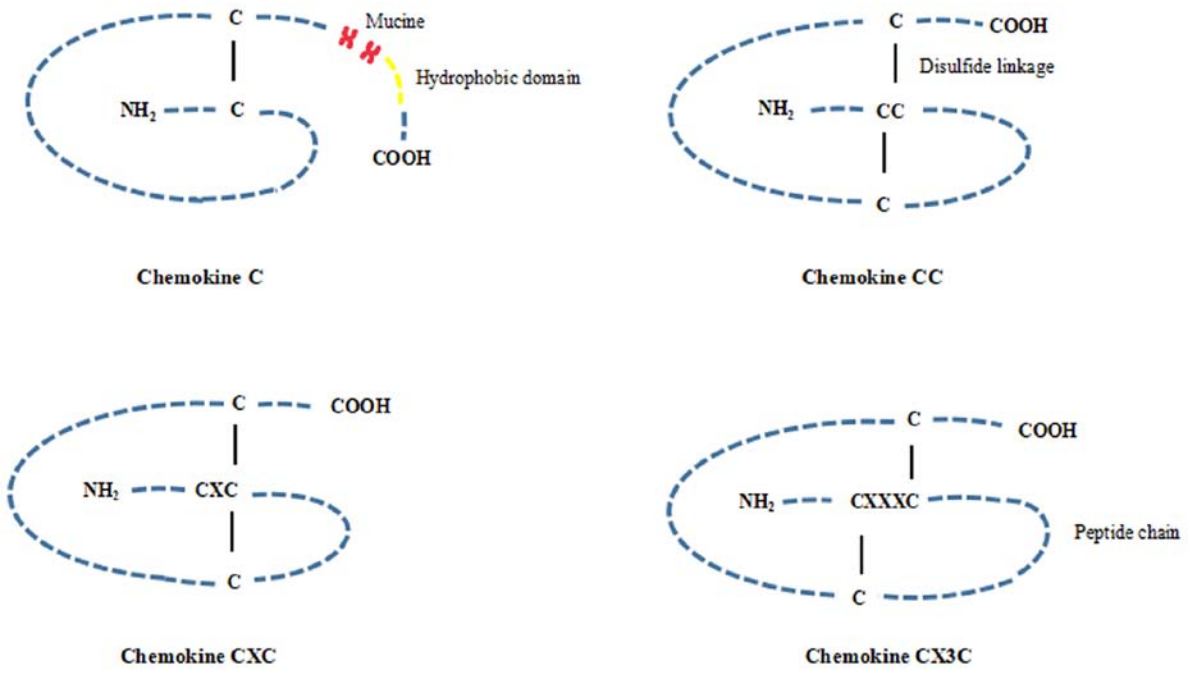

Figure 2. Structure of the four subtypes of chemokine ligand.

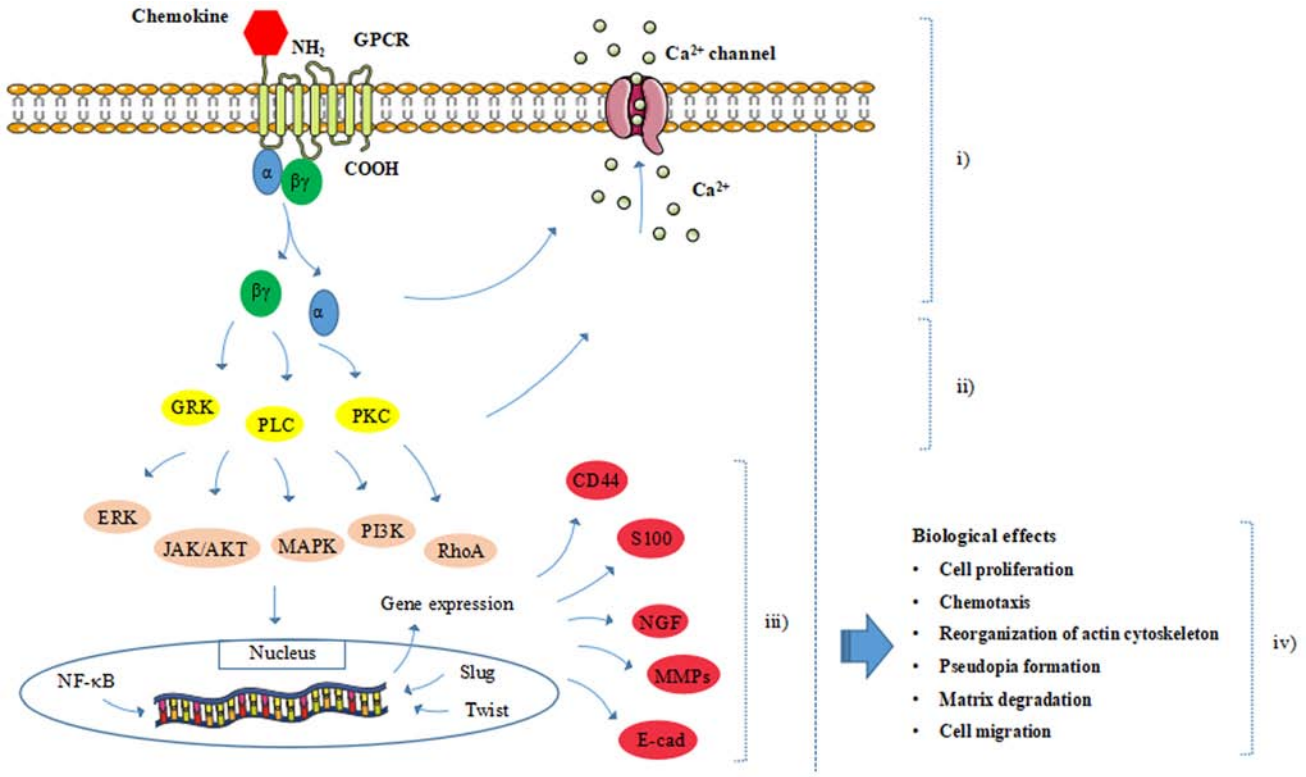

Figure 3. Chemokine-induced signal transduction in cancer with perineural invasion. i) The binding of chemokine ligands to their GPCRs leads to the activation of an α subunit and a βγ heterodimer, and dissociation from GPCR. ii) The downstream signaling effectors, including GRK, ion channels, PKC and PLC, can be initiated. iii) This further leads to signaling cascades, such as the activation of RhoA kinase, ERK, MAPK, PI3K, Akt and JAK-2, which can promote transcription and expression of genes such as CD44, NGF and MMPs. iv) All above evoke broad biological effects, including cell proliferation, chemotaxis, reorganization of actin cytoskeleton, pseudopodia formation, matrix degradation and cell migration. GPCR, G protein-coupled receptors; GRK, GPCR kinases; PKC, protein kinase C; PLC, phospholipase C; RhoA, Ras homolog gene family A; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated proteins kinase; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; JAK-2, Janus-activated kinase-2; CD44, cluster of differentiation 44; NGF, nerve growth factor; MMP, matrix metalloproteinase; E-cad, E-cadherin; NF-κB, nuclear factor-κB.

towards concentration gradients of chemokine ligands to achieve organ-specific metastases (32,33). Another potential mechanism is that the binding of chemokine ligands to their receptors may induce membrane wrinkling and the formation

Table I. Expression of chemokines and their corresponding receptors in cancer with PNI.

Chemokine ligand	Chemokine receptor	Tumors with PNI	(Ref.)
CXCL12	CXCR4	Prostate cancer	(34)
		Pancreatic cancer	(43)
		Breast cancer	(44,45)
		HNSCC	(50)
CX3CL1	CX3CR1	Pancreatic cancer	(54,60)
		Breast cancer	(61)
		Prostate cancer	(62)
		HNSCC	(63)
CCL2	CCR2	Prostate cancer	(11,35)
		Breast cancer	(64)
		Hepatic carcinoma	(65)
CCL5	CCR5	Prostate cancer	(66)
		Lung adenocarcinoma	(67)
		SACC	(36)
CXCL13	CXCR5	Prostate cancer	(68)
		Colorectal	(69)

PNI, perineural invasion; HNSCC, head and neck squamous cell carcinoma; SACC, salivary adenoid cystic carcinoma.

of pseudopodia in tumor cells, which facilitates tumor cells to adhere to and pass through the extracellular matrix (ECM) and basal membrane to achieve invasion and metastasis (34).

4. Role of chemokines in cancer with PNI

Recently, chemokines have been widely investigated in the process of PNI, particularly the CXCR4/CXCL12, CCL2/CCR2, CCL5/CCR5, CXCL13/CXCR5 and CX3CL1/CX3CR1 signaling axes (11,35-37). The significance and associated mechanisms of these chemokine signaling axes in cancer with PNI will now be discussed (Table I) (Fig. 3).

CXCL12/CXCR4 in cancer with PNI. The CXCL12/CXCR4 axis is one of the most widely studied signaling pathways among the chemokine family and their receptors. CXCR4 functions through combining with its specific chemokine ligand, CXCL12, also known as chemokine stromal cell-derived factor 1α (38). This signaling pathway has been found to serve multiple functions, including regulating the proliferation of cells, angiogenesis and EMT (39). This pathway is also regarded as an important candidate in support of the occurrence, invasion and metastasis of tumors (40-42). Furthermore, CXCR4-positive tumor cells can migrate toward distant organs as a response to CXCL12 concentration gradients (38).

A previous study showed that CXCL12 expression in prostate cancer was significantly stronger than that in prostate hyperplasia. CXCR4 was mainly expressed in tumor cells, and CXCL12 was expressed highly in Schwann cells around the tumor. *In vitro*, the invasiveness of tumor cells treated with CXCL12 increased significantly, and this invasiveness could be inhibited by the CXCR4-specific inhibitor AMD3100 (34). Therefore, AMD3100 may be a potential anti-neoplastic agent. In addition, in another PNI-associated tumor, pancreatic cancer, CXCR4 was examined in 6 pancreatic cancer lines, CFPAC-1, Panc-1, AsPC-1, SW1990, MiaPaCa-2 and BxPc-3 cells. Weak expression of CXCL12 in pancreatic tumor cells was detected by ELISA, although it was not detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or western blot assays. RSC96 Schwann cells and newborn rat dorsal root ganglia (DRG) showed that the expression of CXCL12 was strong, as determined by immunofluorescence analysis and ELISA (43). *In vitro* and *in vivo* analyses have revealed that the CXCL12/CXCR4 signaling axis can promote the chemotactic migration of pancreatic cancer cells towards nerve cells, which can be inhibited by blocking the CXCL12/CXCR4 pathway axis using CXCR4 short hairpin RNA and AMD3100.

In addition, Kang *et al* (44) confirmed that CXCL12 was observed in the human breast cancer MDA-MB-435S cell line, but that it was not expressed in the MDA-MB-231 cell line, as determined by RT-PCR. CXCR4 was detected in the MDA-MB-231 and MDA-MB-435S cell lines (45). When silencing CXCL12 in MDA-MB-435s via a ribozyme transgene, the invasion and migration potential of the tumor cells significantly declined. However, transfection of CXCL12 increased the invasion and migration abilities of the MDA-MB-231 cells (45). The CXCL12/CXCR4 axis can promote PNI by increasing Rho GTPase activities, mediating the polymerization of F-actin and stimulating transmembrane mobilization of Ca^{2+} (38,46). The activation of the CXCL12/CXCR4 axis also can induce phosphorylation of the focal adhesion components (e.g., related adhesion focal tyrosine kinase, Paxillin and Crk) and pseudopodia formation, facilitating tumor cell adhesion to the basement membrane components, and promoting invasion and metastasis (45,47). Furthermore, CXCR4 also exhibits high expression in gliomas (48,49) and other cancers of non-neural origin with PNI, including head and neck squamous cell carcinoma (HNSCC) (50). The combination of CXCL12 and its receptor CXCR4 may promote cell proliferation, chemotaxis and invasion by activating multiple signaling pathways, including ERK/MAPK and PI3K/AKT, and leucine-rich repeat-containing protein 4 can block the chemotaxis of tumor cells and PNI induced by the CXCL12/CXCR4 axis via curbing the ERK/MAPK and PI3K/AKT signaling pathways (49,51). CXCR4 may downregulate the expression of p53, p21 and E-cadherin, and upregulate the expression of cluster of differentiation (CD)44, resulting in the increased adhesion of tumor cells and the PNI ability (52).

The aforementioned studies suggest that tumor cells express a high level of CXCR4, that the metastasis-targeted organs and nerves exhibit high expression of CXCL12, and that CXCR4-positive tumor cells are attracted towards target organs expressing CXCL12, thus promoting the PNI of the tumor. However, given the fact that CXCL12 was also detected in prostate and pancreatic cancer cells, an autocrine or paracrine pathway may also participate in the process of PNI, mediated by chemokines and their receptors (34,43).

CX3CL1/CX3CR1 in cancer with PNI. CX3C motif chemokine ligand 1 (CX3CL1), also known as fractalkine or neurotactin,

is the only member of the CX3C subfamily of chemokines and forms a high affinity signaling axis by binding to its chemokine receptor CX3CR1 exclusively (53). CX3CL1 can be abundantly expressed by activated endothelial cells and neurons, and it can exist in either a membrane-anchored adhesion molecule or a soluble chemoattractant form (54,55). Previous studies have shown that the CX3CL1/CX3CR1 axis serves an important role in the nervous system by facilitating the information exchange among neurons, glia and microglia, promoting nerve generation and restricting tissue damage during the inflammatory response. This occurs by promoting the mobilization of intracellular Ca^{2+}, chemotaxis and the inhibition of apoptosis, induced by the Fas signaling pathway or mediated by the activation of LPS (45,56). In addition, in nerve-derived tumors, including glioma and neuroblastoma, CX3CR1 is highly expressed and involved in the adhesion, transendothelial migration and invasion of tumor cells (57-59).

Notably, CX3CR1 has also been detected in certain tumors of non-neural origin, including pancreatic (54,60), breast (61) and prostate (62) cancer, and HNSCC (63), which are widely known to invade and metastasize along peripheral nerves frequently. In a pancreatic cancer study, up to 90% of the surgical specimens were CX3CR1-positive, and 56% exhibited a high intensity score according to immunohistochemical staining (60). CX3CR1 mRNA expression was detected in pancreatic cancer cell lines by RT-PCR, and the intrapancreatic nerves were demonstrated to express CX3CL1, while the pancreatic cancer cells expressed CX3CL1 only weakly (60). *In vitro*, CX3CL1 expression could induce the migration of CX3CR1-positive pancreatic cancer cells in a dose-dependent manner, thus promoting cancer cell adhesion to nerve cells through activation of GPCRs and redistribution of β1 integrins and focal adhesion kinase. *In vivo*, pancreatic cancer cells expressing CX3CR1 were found to infiltrate the peripheral nerves when CX3CR1-positive pancreatic cancer cells were injected into the mice at the middle of the back, while peripheral nerve invasion was not observed in tumors from CX3CR1-negative cells (60).

Andre *et al* (61) found that CX3CR1 was closely associated with the invasion and metastasis of prostate cancer cells to the bone, whose bone marrow endothelial cells and differentiated osteoblasts secreted CX3CL1. Furthermore, in breast cancer, CX3CR1 was determined to forecast metastasis to the brain, where CX3CL1 was expressed abundantly (62). These results suggested that the CX3CX1/CX3CR1 axis may serve a pivotal role in the invasion and metastasis of prostate and breast cancer, although its specific involvement in PNI had not been investigated.

Other chemokine signaling axes associated with PNI. Previous studies demonstrated that 20 out of 21 (95%) specimens with PNI exhibited positive expression of CCR2 in the cytoplasm in 34 prostate cancer cases assessed by immunohistochemistry. Just 3 specimens exhibited positive expression of CCR2 in the 13 cases of prostate cancer without PNI. The *in vitro* co-culture model and the *in vivo* mouse model demonstrated that CCL2 released by DRG facilitated CCR2-expressing prostate cancer migration and PNI (11,35). Western blotting showed that p-MEK1/2 and p-Akt expression was upregulated in prostate cancer cells. The expression of p-Akt could be inhibited totally and the expression of p-MEK1/2 could be decreased by anti-CCL2 antibody. Thus, CCL2 may promote the invasion and PNI of prostate cancer by activating Akt and MAPK pathways through CCL2/CCR2 (35). CCR2 also exhibits high expression in breast and hepatic carcinoma, and it can promote the motility and invasion of cancer cells by binding to its ligand CCL2 through mothers against decapentaplegic homolog3 (Smad3) protein and p42/44 MAPK-dependent mechanisms (64,65). Thus, Akt, MAPK and Smad3 may be novel targets of antitumor therapy.

CCR5, receptor of CCL5, is primarily expressed in prostate cancer and lung adenocarcinoma, and is closely associated with the invasion and metastasis of these tumors (66,67). In another PNI tumor, salivary adenoid cystic carcinoma (SACC), CCR5 and CCL5 expression was detected by flow cytometric analysis and immunofluorescence analysis. Migration and invasion assays showed that the CCL5/CCR5 axis can promote the invasion of nerves in the SACC-83 cell lines by Ca^{2+} elevation and the rearrangement of the actin cytoskeleton (36).

In addition, CXCL13 and CXCR5 expression presents with a significant positive correlation with the occurrence of PNI, particularly in prostate (68) and colorectal (69) cancer, where CXCR5 is overexpressed. CXCL13, the stroma-derived ligand of CXCR5, can stimulate the expression of CXCR5 in prostate and colorectal tumor cells, leading to the phosphorylation of ERK and AKT, and the upregulation of MMPs, thus promoting the PNI of tumor cells (68-71).

5. Reciprocal interaction between other factors and chemokines in PNI

MMPs. MMPs, a group of zinc-dependent endopeptidases that can degrade numerous types of components of the ECM, are believed to promote the invasion and metastasis of tumors (67). Previous studies showed that certain components secreted by peripheral nerves, including NGF and GDNF, or the activation of COX-2 and prostaglandin E2 induced by the combination of tumor necrosis factor-α (TNF-α) and its receptor, can promote tumor cells to secrete MMPs and then facilitate tumor cells to invade nerve tissues in a number of tumors, including cholangiocarcinoma (CCA) and pancreatic cancer (72-74). Additionally, MMP-2 and MMP-9 can be upregulated by extracellular MMP inducer (EMMPRIN, CD147), a transmembrane glycoprotein belonging to the immunoglobulin superfamily, thus achieving PNI in SACC (75). Blocking of EMMPRIN by its antibody or silencing of EMMPRIN expression by RNA interference could effectively inhibit the proliferation and PNI activity of SACC cells, and reduce the secretion of MMP-2 and MMP-9 in SACC-83 cells (76,77).

Importantly, recent studies have shown that MMPs can also respond to chemokines and their corresponding chemokine receptors to promote PNI in certain tumors. The expression and activity of MMP-2 and MMP-9 in human glioblastoma can be markedly upregulated by the CCL12/CXCR4 axis, mediated through activation of the ERK1/2 and Akt signaling pathway (49). In the progression of breast and pancreatic cancer, the autocrine CCL12-CXCR4 signaling pathway can promote PNI by increasing the secretion of and promoting the activation of MMP-2 and MMP-9 (43,45). The CXCL12/CXCR4 and CXCL13/CXCR5 signaling axes have

been demonstrated to induce prostate cancer cells to secrete MMP-2 and MMP-9, which degrades the matrix around the tumor and the nerve tissue, promoting PNI (34,68) (Fig. 3).

NGF. NGF was the first member of the neurotrophic factor family to be identified, and was widely expressed in tumor tissues, and involved in tumorigenesis and tumor growth (78). An increasing number of studies have shown that NGF and its receptor tropomyosin receptor kinase A (TrkA) are overexpressed, and that the combination of NGF and TrkA promotes cancer cell growth, increases invasiveness and metastasis, and eventually causes nerve invasion in a number of human cancer types, including CCA, and pancreatic and prostate cancer (79-81).

It has been suggested that chemokines can increase NGF expression, which further accelerates the process of PNI by receptor binding (34,43). The mechanism of the combination of NGF and TrkA promoting invasive behaviors acts to increase the synthesis and release of MMPs via the activation of the p44/42 MAPK signaling pathway (82,83). The combination of NGF and TrkA can facilitate nerve cellular axon growth in the direction of the tumors by supplying chemical tropisms (79,84). The overexpression of NGF and TrkA also contributes to the PNI of SACC (85). Additionally, NGF may upregulate the expression of S100 and reduce the expression of E-cadherin, leading to decreased adhesion between cancer cells, an increased ability for metastasis and ultimately, nerve tissue invasion in SACC (86,87) (Fig. 3).

Nuclear factor κB (NF-κB). NF-κB belongs to the transcription factors family, and the heterodimeric complex of the p65 and p50 subunits is the predominant form of NF-κB; it is physically confined to the cytoplasm of normal cells and remains inactive through interaction with the NF-κB inhibitory protein (88). Following stimulation by various reagents (e.g., cytokines, viruses, growth factors and DNA damaging agents), a series of signaling events promote the p65/p50 heterodimer to dissociate from the NF-κB inhibitory protein and translocate to the nucleus. This causes the activation of the expression of various genes involved in the prevention of apoptosis (89), and the promotion of the invasion and metastasis of cancer cells (90,91).

NF-κB is also closely associated with the PNI potential of tumors. Huang *et al* (91) reported that NF-κB may function through the induction of CXCR4, which is one of the NF-κB target genes, containing a putative NF-κB binding site (5'-GAGGCATTTCC-3', 230-240bp) in the promoter region (92). In glioma, Esencay *et al* (48) found that the activation of NF-κB could induce CXCR4 expression and promote the migration of tumor cells. CXCR4 silencing could suppress the invasion and adhesion potential of U87 cells and pancreatic cancer cells, and decrease the transcriptional activity of NF-κB promoter (43,52). CCR7 could activate MMP2 and MMP9 through the NF-κB signaling pathway to mediate the migration and invasion of human malignant glioma, and these biological effects could be reversed by treatment with small interfering CCR7 or a CCR7 neutralizing antibody *in vitro* (93). In another PNI tumor, CCA, studies showed that NF-κB signaling could be activated by the CCL5/CCR5 axis through increasing the expression of MMP2 and MMP9, thus contributing to the invasion and metastasis of tumor cells, which could be inhibited by CCL5 neutralizing antibody or CCR5 inhibitor maraviroc (94). Wang *et al* (95) found that CX3CL1/CX3CR1 could promote the invasion of pancreatic cancer through the activation of the NF-κB signaling pathway (Fig. 3).

Slug. Slug (Snail2), a member of the Snail family of zinc-finger transcription factors, is known to serve pivotal roles in the process of cell migration, ranging from the formation of a number of tissues during embryonic development to the acquisition of invasive and metastatic properties in epithelial tumors (96,97). It was reported that the expression of Slug was associated with PNI in different tumors, including human breast cancer (98), pancreatic cancer (99) and SACC (100). Slug mediates the PNI of these tumors mainly by promoting EMT in epithelial cells (100,101). He *et al* (102) found that Slug promoted PNI and the metastatic capacity of SACC via the ERK/MAPK signaling pathways, and MAPK-knockdown reduced the expression of Slug in SACC cells (103).

Studies demonstrated that CCL18 could trigger the invasiveness and metastasis of oral squamous cell carcinoma, and that Slug-knockdown could reverse CCL18-induced EMT (104). The CCL21/CCR7 pathway led to the occurrence of the EMT process by activating the Slug pathway, and it promoted migration and invasion in human chondrosarcoma and lung cancer (105,106). CCL18 promotes the invasion and metastasis of gastric cancer cells by increasing the expression of Slug and promoting EMT (107). The CXCL12/CXCR4 signaling axis has also been shown to be closely correlated with progression and the metastatic characteristics of CCA through Slug-induced EMT (108). These results indicated that Slug-induced EMT is involved in chemokine signaling (Fig. 3); however, whether chemokine signaling axes may promote tumor PNI via Slug-induced EMT remains unclear.

Twist. Twist, a transcription factor, belongs to the family of basic helix-loop-helix proteins and can bind to E-box regions in the promoters of certain genes to activate or inhibit transcription (109,110). Upregulation of Twist was found in certain human tumors, including breast cancer (111), prostate cancer (112) and gastric cancer (113). Twist mainly functions by promoting EMT in malignant tumors (111,114).

Recently, attention has been focused on the association between chemokine signaling axes and Twist in malignant tumors. Chen *et al* (115) reported that the CCL2/CCR2 axis could enhance EMT by upregulating the expression of Twist in non-small cell lung cancer. Low-Marchelli *et al* (116) found that CCL2 recruited macrophages to promote tumor progression by activating Twist signaling. Studies of Yang *et al* and Xu *et al* showed that CXCL12/CXCR4 may upregulate Twist through ERK and PI3K/AKT signaling, leading to the progression of EMT and the invasion and metastasis of tumor cells in human glioblastoma (111,117). Koo *et al* (118) found that CXCL11 could promote the invasive capacity of epithelial ovarian cancer via the enhancement of Twist expression. In addition, the CCR7 pathway upregulated Twist expression via ERK and PI3K/AKT signaling to manage EMT in pancreatic ductal adenocarcinoma (119). All the aforementioned studies showed that chemokines may be involved in tumor PNI through

Twist-induced EMT; however, the concrete mechanisms between Twist and PNI require further investigation (Fig. 3).

6. Interconnections between the microenvironment and chemokines in PNI

The expression and roles of chemokines and their receptors in malignant neoplasms can also be regulated by various microenvironmental factors, including chronic inflammation, hypoxia, hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) (44,48). Biphasic effects exist between chronic inflammation and chemokines. Franciszkiewicz *et al* (120) reported that chronic inflammation, which was partly driven by the chemokine signaling axis, was closely associated with the development of gastrointestinal malignancies (120,121). By contrast, chemokine expression can also be induced by inflammation. For example, CX3CL1 expression was significantly driven by inflammation *in vitro*, and pro-inflammatory TNF-α and interferon-γ could induce a higher expression level of CX3CL1 mRNA (122).

Hypoxia can influence the occurrence and development of tumors through the induction of chemokines and their receptors (123,124). For example, hypoxic conditions within solid tumors may induce CXCL12 and CXCR4 expression via hypoxia inducible factor-1 (HIF-1) and VEGF, contributing to the adhesion, migration and PNI of tumor cells (123). In glioblastoma and breast carcinoma, necrotic foci and the pseudopalisading regions are hypoxic and are the main distribution sites of HIF-1 and VEGF, where overexpression of CXCR4 exists (44,125). Similarly, under hypoxia, the metastasis of breast cancer was promoted by increasing the expression of CCR5 and its ligand CCL5 at the metastasis site via HIF-1 (126). Furthermore, hypoxia induces increased CCR7 expression in lung cancer cells, which further promotes the invasion of tumor cells and lymph node metastasis (127). In prostate cancer and pancreatic ductal adenocarcinoma, cancer invasion and metastasis is closely associated with the CX3CR1 expression of tumor cells, which is induced by hypoxia, and the ligand CX3CL1 is mainly expressed at the site of metastasis (128,129).

HGF, a multifunctional cytokine secreted by fibroblasts and other stromal cells in tumors, exerts multiple functions in tumors, including proliferation, invasion and metastasis (130-133). HGF can upregulate the expression of CXCR4 protein in a number of tumors, including breast carcinoma and glioma, and HGF pre-treatment increases cancer cell migration toward CXCL12 via NF-κB (44,48).

Hence, HIF-1, HGF and VEGF are considered potential targets of anticancer therapies. In fact, some of these inhibitors are in clinical trials as anticancer therapy drugs. For example, farnesyltransferase inhibitors, HGF binding peptide-1 and bevacizumab are treated as tumor therapeutic agents targeting HIF-1, HGF and VEGF, respectively (48,134-136).

7. Conclusions

PNI occurs in a number of human malignant neoplasms and is closely associated with postoperative relapse and a reduced survival rate. Accumulating evidence has indicated that chemokines and their corresponding receptors serve pivotal roles in the process of PNI. Although they are discussed in detail in this review, the precise mechanisms of chemokines in PNI deserve further investigation. With the development of molecular biology and advancements in proteomics technology, an increasing number of pivotal molecular markers associated with chemokines will be found in cancer with PNI. This should be conducive to improving our understanding of the mechanism of PNI and highlight novel chemokine-targeted drugs in the clinic to decrease relapses caused by the PNI of tumors.

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Authors' contributions

MZ and ZZ wrote and edited the manuscript. XG and JW provided scientific revision of the manuscript. YT and XL prepared and reviewed the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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