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Cellular and molecular mechanisms of perineural invasion of pancreatic ductal adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignant disease
with a unique tumor microenvironment surrounded by an interlaced network
of cancer and noncancerous cells. Recent works have revealed that the dynamic
interaction between cancer cells and neuronal cells leads to perineural invasion
(PNI), a clinical pathological feature of PDAC. The formation and function of
PNI are dually regulated by molecular (e.g., involving neurotrophins, cytokines,
chemokines, and neurotransmitters), metabolic (e.g., serine metabolism), and
cellular mechanisms (e.g., involving Schwann cells, stromal cells, T cells, and
macrophages). Such integrated mechanisms of PNI not only support tumor
development, growth, invasion, and metastasis but also mediate the formation
of pain, all of which are closely related to poor disease prognosis in PDAC. This
review details the modulation, signaling pathways, detection, and clinical rele-
vance of PNI and highlights the opportunities for further exploration that may
benefit PDAC patients.

KEYWORDS
neurotrophins, pancreatic ductal adenocarcinoma, perineural invasion, schwann cells, tumor
microenvironment

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1 | BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and the fourth-leading cause of cancer death in the United States, with a 5-year overall survival (OS) rate of 10% [1]. Despite advances in medicine, the incidence of PDAC continues to rise, with 60,430 new cases and 48,220 deaths reported in 2020 [1]. It is one of the most aggressive solid malignant tumors, and its poor prognosis is due to many reasons, including but not limited to late diagnosis, early metastasis, and therapy resistance [2]. These challenging clinical features of PDAC are determined by its tumor microenvironment, which has hypoxia and immune escape characteristics and is formed by various cellular and noncellular components [3]. Understanding the composition of the tumor microenvironment and its role in tumor progression is the cornerstone of the development of new anti-PDAC therapies.

The nerves are fibers that receive and send information between tissues, and they are usually parallel to blood vessels throughout the body. As early as the 19th century, researchers have observed the phenomenon of perineural invasion (PNI) of cancer [4]. PNI is usually defined as the appearance of tumor cells along the nerves and/or within the epineural, perineural, and endoneurial places of the neuronal sheath, with cancer cells surrounding at least 33% of the nerves [4, 5]. Recently, there has been an increasing interest in understanding the phenotypes and molecular basis of the interaction between the nerves and cancer cells that drive pancreatic tumorigenesis and treatment resistance. Histopathological studies have found that PNI can occur in over 80% of PDAC tumor tissues and is an early event of tumorigenesis in preclinical and clinical models [6–8], which leads to significant tumor characteristics. In particular, PNI is associated with tumor progression, increased local recurrence, strong pain, and poor prognosis in PDAC patients [4]. Therefore, targeting PNI may provide an alternative strategy to improve the prognosis of PDAC patients.

The nerves and tumor cells can influence each other to provide a suitable microenvironment for tumor survival and growth. On one hand, the infiltrated nerves promote the proliferation of tumor cells and the formation of pancreatic intraepithelial neoplasia in KRAS[G12D]-driven PDAC mouse models by neuronal inflammation [9]. Ablation of the sensory neurons in the pancreas protects the nerves from inflammatory damage, thereby delaying tumor formation in mouse models [9]. These findings establish the potential role of the nerves in shaping an inflammatory tumor microenvironment. On the other hand, cancer cells invading around or inside the nerves may ultimately lead to neurological dysfunction, increased plasticity, or structural destruction [10–13]. Such dynamic communication between the nerves and PDAC cells further facilitates immune escape, tumor growth, and metastasis.

Complementing previous reviews [14–16], in this Review, we aim to provide new insights into the relationship between the nerves and pancreatic cancer. We will not only delineate the key mediators in controlling the interplay between the nerves and cancer cells in the tumor microenvironment but also highlight the detection, function, and significance of PNI in PDAC.

2 | MOLECULAR MEDIATORS OF PNI

2.1 | Neurotrophins

Neurotrophins are a family of proteins that induce the survival, development, and function of neurons. They can also be expressed in PDAC cells and have a direct effect on the interaction between cancer cells and the nerves in the tumor microenvironment through corresponding receptors. Below, we review recent findings that different neurotrophin subfamilies exert various effects in regulating PNI and pancreatic tumor metastasis (Figure 1).

2.1.1 | The nerve growth factor family (NGF)

Neuronal cells and cancer cells can release neurotrophins to promote cell growth, survival, and maintenance [17]. NGF and other known members of the neurotrophin family, such as brain-derived neurotrophic factor, neurotrophin 3, and neurotrophin 4, as well as their sharing receptors, neurotrophic receptor tyrosine kinase 1 (NTRK1), neurotrophic receptor tyrosine kinase 2, and nerve growth factor receptor (NGFR), are expressed in different PDAC cell lines, indicating that PDAC cells and neural cells may influence each other through these neurotrophins. Apart from the research conducted using cell lines, the mRNA levels of genes encoding the neurotrophin family members and NTRK1 in the tumor tissues from PDAC patients have been found to be upregulated, mainly in neuronal cells [18]. Interestingly, NTRK1 and NGFR are used as opposite prognostic biomarkers for PDAC patients. For example, a retrospective study of 56 PDAC patients showed that a high expression of NTRK1 was associated with poor prognosis while the overexpression of NGFR was linked to a relatively long survival [19], suggesting that different neurotrophin signals play a selective role in predicting patient survival.

Pancreatic cancer cells require more nutrients or energy to support their growth and metastasis by altering their metabolism. The reprogramming of energy metabolism is
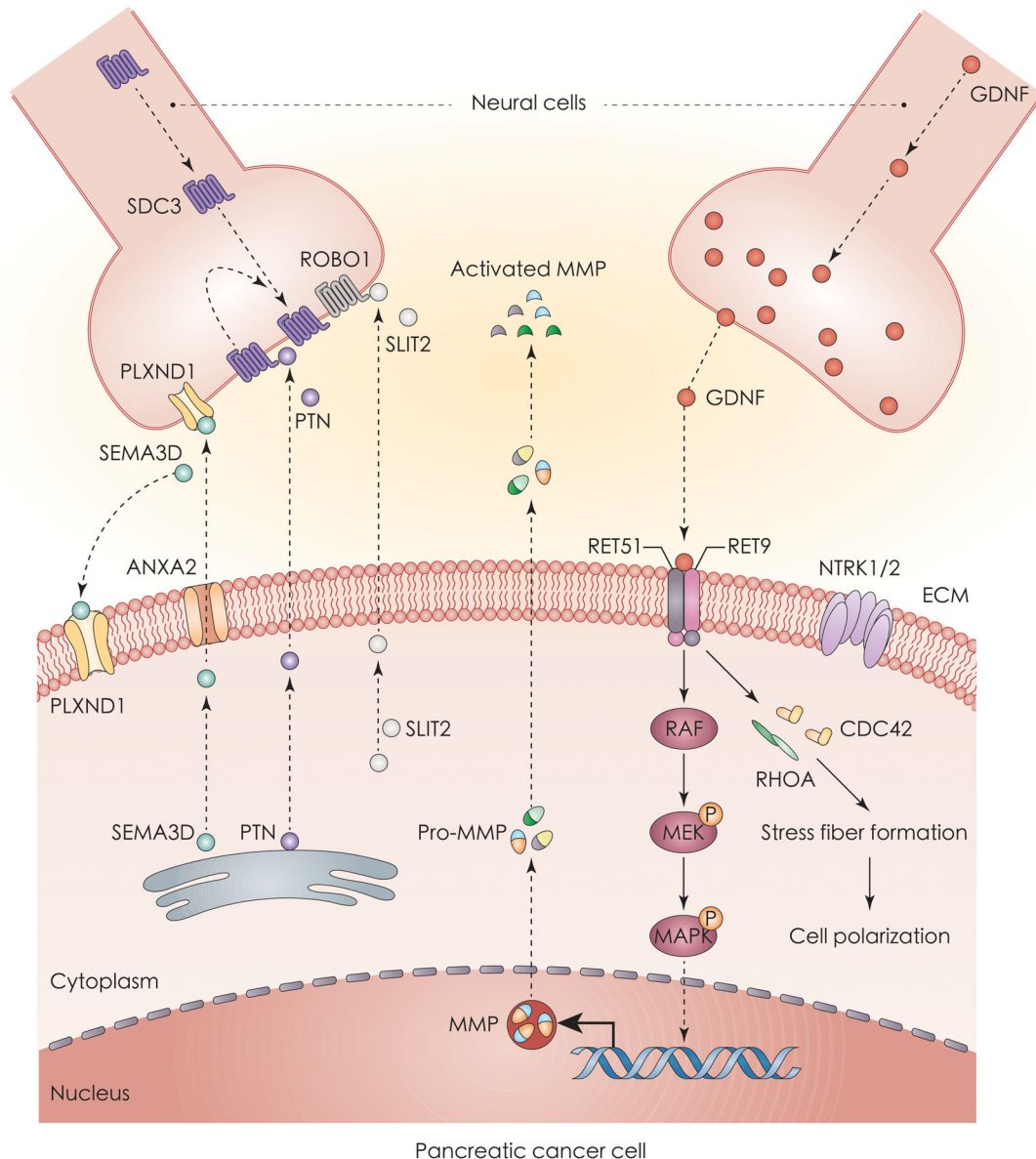
FIGURE 1 Neurotrophins in PNI and PDAC. Neurotrophins are associated with PNI in pancreatic tumorigenesis. A series of interactions between multiple neurotrophin ligands and receptors, such as SEMA3D-PLXND1, ARTN-GFRA3, and PTN-SDC3, promote the movement of cancer cells toward neural cells. The binding of GDNF to RET receptors activates downstream KRAS signaling and upregulates the expression of MMPs. It also accelerates the formation of stress fibers, thus resulting in cellular polarization and metastasis during PNI. Abbreviations: ANXA2, annexin A2; CDC42, cell division cycle 42; ECM, extracellular matrix; GDNF, glial cell-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; MAP2K, mitogen-activated protein kinase kinase; MMP, matrix metallopeptidase; NTRK1, neurotrophic receptor tyrosine kinase 1; NTRK2, neurotrophic receptor tyrosine kinase 2; PDAC, pancreatic ductal adenocarcinoma; PLXND1, plexin D1; PNI, perineural invasion; PTN, pleiotrophin; RAF, Raf-1 proto-oncogene, serine/threonine kinase; RET, ret proto-oncogene; RHOA, Ras homolog family member A; ROBO1, roundabout guidance receptor 1; SDC3, syndecan 3; SEMA3D, semaphorin 3D; SLIT2, slit guidance ligand 2

a dynamic process that can be achieved through multiple mechanisms involving amino acid metabolism, glucose utilization, and autophagic degradation. For example, PDAC cells rely on exogenous serine (a conditionally essential amino acid) in varying degrees for tumor proliferation [20]. Regarding the nervous system, axons and

dorsal root ganglia (DRG) can release serine into the pancreatic tumor microenvironment to provide additional energy support [20]. After serine deprivation, PDAC cells express and release more NGFs by upregulating their mRNA translation [20], thereby enhancing the movement of axons toward the tumor nest. These findings represent

the interaction between cancer and axons during nutrition starvation which promotes PNI formation. Targeting serine supply or NGF secretion may be an attractive strategy to inhibit tumor growth in the pancreatic tumor microenvironment. Anti-NGF treatment significantly reduced the ratio of neurogenic inflammation and PNI in an 8-week KRAS[G12D]-driven PDAC mouse model [21], indicating that NGF is also a driving factor of nerve-related inflammation within the tumor microenvironment.

Not all neurotrophins are highly expressed in PDAC cells. For example, slit-guiding ligand 2 (SLIT2) is a neurotrophic protein related to cell navigation, and its mRNA level was low in PDAC cell lines and patient tumor tissues [22]. Functional studies have shown that the overexpression of SLIT2 in human PDAC cell lines (MiaPACA2 and PANC1) suppressed cell migration and invasion. Conversely, blocking the interaction between SLIT2 and its receptor, roundabout guidance receptor 1 (ROBO1), enhanced the motility and invasiveness of PDAC cells, further supporting the negative role of SLIT2 in cancer cell migration and invasion [22]. In the co-culture system with Schwann cells or a Matrigel/DRG culture system, SLIT2 restricted bidirectional movement between PDAC cells and neural cells [22]. However, another group reported that cancer-associated fibroblasts (CAFs) from PDAC patients expressed higher levels of SLIT2 than those from healthy controls, thereby promoting neurite outgrowth and Schwann cell migration by activating the cadherin 2 pathway [23]. Moreover, the inhibition of SLIT2-ROBO1 signaling hampered this neural remodeling effect [23]. Altogether, these data indicate the possibility of inhibiting PNI by targeting different NGF family members expressed in PDAC cells and other cells of the tumor microenvironment.

2.1.2 | Glial cell-derived neurotrophic factor family ligands and receptors

Glial cell-derived neurotrophic factor (GDNF) family ligands are a series of proteins including GDNF, neurturin, artemin (ARTN), and persephin, which can bind to specific receptors to perform distinct functions [24]. GDNF is an essential protein for the maintenance of neuron growth and is highly expressed in the peripheral and central nervous systems [25]. Compared to patients without PNI (24/40, 60.0%), PDAC patients with PNI had a higher frequency of GDNF expression (16/18, 88.9%), indicating that GDNF is involved in the neural invasion process [26]. During the growth process of neurons, certain neural cells (e.g., Schwann cells and motor neurons) could secrete GDNF into extracellular space [27]. Ret proto-oncogene (RET) is the receptor of GDNF and is selectively expressed

in some PDAC cell lines (e.g., MiaPACA2, ASPC1, SW1990, and CAPAN2) [28]. Compared with RET-negative PDAC cells or GDNF-nonsecreting neuronal cells (such as IMR32 cells), RET^{+} PDAC cells (such as MiaPACA2 and ASPC1) co-culture with GDNF-secreting neuronal cells (such as T98G cells) promoted the migration of tumor cells to neuronal cells [28]. Cell migration mediated by the GDNF-RET axis depends on the activation of KRAS proto-oncogene, GTPase (KRAS), and the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic signaling pathway [29], indicating that classical oncogene signals are involved in promoting the neurotrophin pathway.

Structural studies have documented that RET has two isoforms, namely RET9 and RET51, which are completely different in terms of protein interactions and oncogenic potentials [30]. RET51 plays a leading role in the GDNF-RET-mediated PDAC invasion process [31]. After RET was activated, it not only induced the polarization and invadopodia of cancer cells but also facilitated matrix degradation by producing more matrix metallopeptidase 2 (MMP2), MMP9, and MMP14 [31], thereby accelerating cell invasion. Similarly, the knockdown of GDNF family receptor alpha 1 (GFRA1), the co-receptor of RET, limited the migration of MiaPACA2 cells. Accordingly, the combination of exogenous GFRA1 and GDNF stimulated the RET mitogen-activated protein kinase cascade and induced the invasion of cancer cells [32]. These findings establish a model for neural signals to promote PDAC metastasis, namely the GDNF-GFRA1-RET axis.

ARTN is another member of the GDNF family. PDAC cell lines (ASPC1, BxPC3, CAPAN1, MiaPACA2, PANC1, SU86.86, T3M4, and COLO357) and tissues showed a high expression of ARTN and its receptor GDNF family receptor alpha 3 (GFRA3) [33, 34]. ARTN had the ability to trigger GFRA3-dependent invasion in PDAC cells [33, 34]. Further research is needed to explore the structural basis of the ARTN-GFRA3 pathway in promoting cell invasion. It is also unclear whether neurons are the main source for extracellular ARTN to drive tumor metastasis.

2.1.3 | The midkine (MDK) family

The MDK family includes two members, namely MDK and pleiotrophin (PTN). They share the same receptor, syndecan 3 (SDC3), to promote the outgrowth of neural cells [35]. The level of MDK in pancreatic cancer specimens was higher than that in normal pancreatic tissues [36, 37], which was associated with PNI and poor prognosis [38]. The neurotrophic factor PTN was also highly expressed in PDAC cells and can function as a damage-associated molecular pattern in the tumor microenvironment [39]. Once released by necrotic tumor cells, extracellular PTN

was recognized by the receptor SDC3 on the pancreatic nerves, which leads to nerve proliferation [40]. However, the upregulation of SDC3 in neurons and Schwann cells might cause an accumulation of PTN+ PDAC cells around neuronal cells, thereby aggravating nerve damage [40]. These findings indicate that PTN-SDC3 signaling plays a dual role in regulating neuroplasticity during PNI.

2.1.4 | The axon guidance gene family

Members of the axon guidance gene family, such as semaphorin 3D (SEMA3D), play a role in the formation of neuronal networks. The knockdown of *SEMA3D* or blockade of its receptor plexin D1 (PLXND1) attenuated the invasion of tumor cells (primary murine KPC cells) towards the nerves *in vitro* [41]. Reducing SEMA3D or PLXND1 expression also decreased the nerve density in tumor tissues *in vivo* [41]. Overall, these preclinical and clinical studies indicate that high levels of SEMA3D and PLXND1 may be implicated in PNI, and lay the foundation for further study on the changes of different neurotrophins in the pancreatic tumor microenvironment.

2.2 | Chemokines

The C-X-C motif chemokine ligand and C-X-C motif chemokine receptor chemokine family consists of a series of peptides, which primarily act as a chemoattractant for immune cells and neural cells. In this family of chemokines, the overproduction of some of them is associated with the PNI process (Figure 2). For example, C-X3-C motif chemokine receptor 1 (CX3CR1) was detected in some PDAC cell lines (MiaPACA2, CFPAC, PACA44, T3M4, PANC1, ASPC1, and A8184) and surgical specimens of patients but it was undetectable in normal pancreatic tissues [42]. C-X3-C motif chemokine ligand 1 (CX3CL1) is a specific ligand of CX3CR1 which is expressed by neurons and the nerves. From the histological evaluation, increased CX3CR1 expression was closely associated with tumor PNI in PDAC patients ($P = 0.026$), implying a role of CX3CR1 signaling in the pancreatic tumor microenvironment [42]. Mechanistically, the migration of CX3CR1+ PDAC tumor cells towards recombinant CX3CL1 protein or CX3CL1+ neural cells depended on the activation of integrins and G protein-coupled receptors *in vitro* and *in vivo* [42].

The expression of C-X-C motif chemokine receptor 4 (CXCR4) in PDAC human specimens was also significantly related to PNI ($P = 0.0001$) [43]. C-X-C motif chemokine ligand 12 (CXCL12) is a main ligand of CXCR4. Subsequent co-culture experiments showed that DRG releases CXCL12 in a paracrine fashion, thereby attracting PDAC cells to DRG [43]. *In vivo* evidence demonstrated that the blockade of CXCL12-CXCR4 signaling could remarkably reduce tumor size, nerve injury degree, and PNI level in tumor tissues [43]. Although the exact mechanism of activation of these pathways needs to be further studied, neutralizing antibodies or inhibitors that block CX3CR1 or CXCR4 may be a way to inhibit PDAC by reducing PNI.

Alternatively, hepatocyte growth factor, a multifunctional chemokine expressed by DRG, interacts with MET proto-oncogene, receptor tyrosine protein-coding kinase, which leads to the upregulation of NGF and MMP9 of PDAC cells, thereby promoting PDAC cell migration and invasion through a paracrine manner [44]. The co-culture model showed that hepatocyte growth factor enhances the invasion of tumor cells toward DRG, leading to a subsequent outgrowth of DRG [44]. Since these signaling axes play a vital role in normal health and function, researchers cannot readily assess the balance between physical and pathological functions. In addition to cancer cells and the nerves, chemokines could directly affect the infiltration of various immune cells in the tumor microenvironment [45], highlighting a complex influence of these chemokines in promoting the development and progress of PDAC.

2.3 | Neurotransmitters

Catecholamines are neurotransmitters containing epinephrine, norepinephrine, and dopamine. They play an important role in maintaining different physiological functions and stress responses. In preclinical PDAC models, norepinephrine induced tumor invasion to the nerves by activating the signaling pathway involving adrenoceptor beta 2 (ADRB2), protein kinase CAMP-activated catalytic, and signal transducer and activator of transcription 3 (STAT3) [46]. The STAT3 transcription factor is an important regulator of pancreatic tumorigenesis, and for promoting tumor stem cell proliferation and maintaining an inflammatory tumor microenvironment [47]. However, clinical studies have not confirmed the role of STAT3 in PNI. A study based on the histological results of 79 PDAC patients showed that the phosphorylation of STAT3 has no relationship with PNI [48]. Regardless, the interaction of catecholamines with ADRB2 would promote the PNI of PDAC by stimulating NGF secretion and increasing the nerve density in PDAC tissues [49]. These studies provide new insights into the relationship between neuropsychological stress or sympathetic nerve stress and the progression of PDAC [49]. Despite the need to evaluate potential adverse effects, the inhibition of the catecholamine pathway may be a valuable treatment for advanced PDAC.
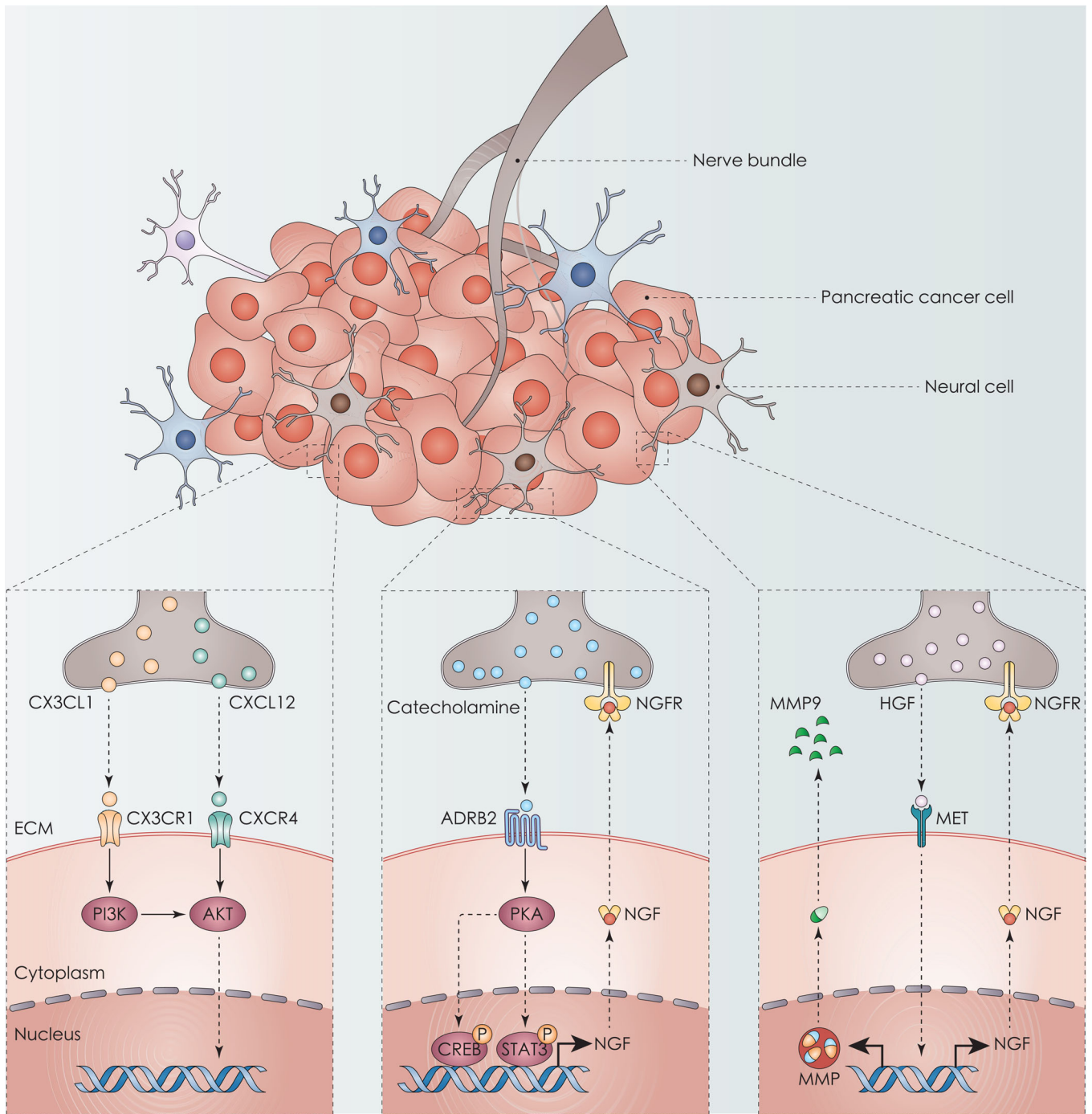
FIGURE 2 Chemokines and neurotransmitters in PNI and PDAC. Neural cells release C-X-C motif chemokine ligands (CX3CL and CXCL12), which can bind to receptors CX3CR1 and CXCR4, respectively, and activate the PI3K-AKT pathway. In addition, neural cells secrete catecholamine and then induce tumor invasion to the nerves through the ADRB2-PKA-STAT3 signaling pathway. Similarly, HGF secreted by DRG binds to MET receptors on PDAC cells in a paracrine manner. The activation of MET leads to the upregulation of NGF and MMP9, which may enhance PNI. Abbreviations: ADRB2, adrenoceptor beta 2; CREB, CREB/ATF BZIP transcription factor; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine receptor 1; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C motif chemokine receptor 4; DRG, dorsal root ganglia; ECM, extracellular matrix; HGF, hepatocyte growth factor; MET, met proto-oncogene, receptor tyrosine kinase; MMP9, matrix metallopeptidase 9; NGF, nerve growth factor; NGFR, nerve growth factor receptor; PDAC, pancreatic ductal adenocarcinoma; PKA, protein kinase CAMP-activated catalytic; PNI, perineural invasion; STAT3, signal transducer and activator of transcription 3

FIGURE 3 Cellular crosstalk in PNI and PDAC. The pancreatic tumor microenvironment is infiltrated by various cells, such as immune cells, neural cells, and CAFs. The crosstalk between these cells promotes tumor proliferation, invasion, and immunosuppression. Cancer cells interact with neural cells through membrane proteins (MAG-MUC1 and NCAM1) or secretory proteins (L1CAM), and then promote the movement of tumor cells toward neural cells. The combination of IL-6 released by cancer cells and IL-6R on Schwann cells 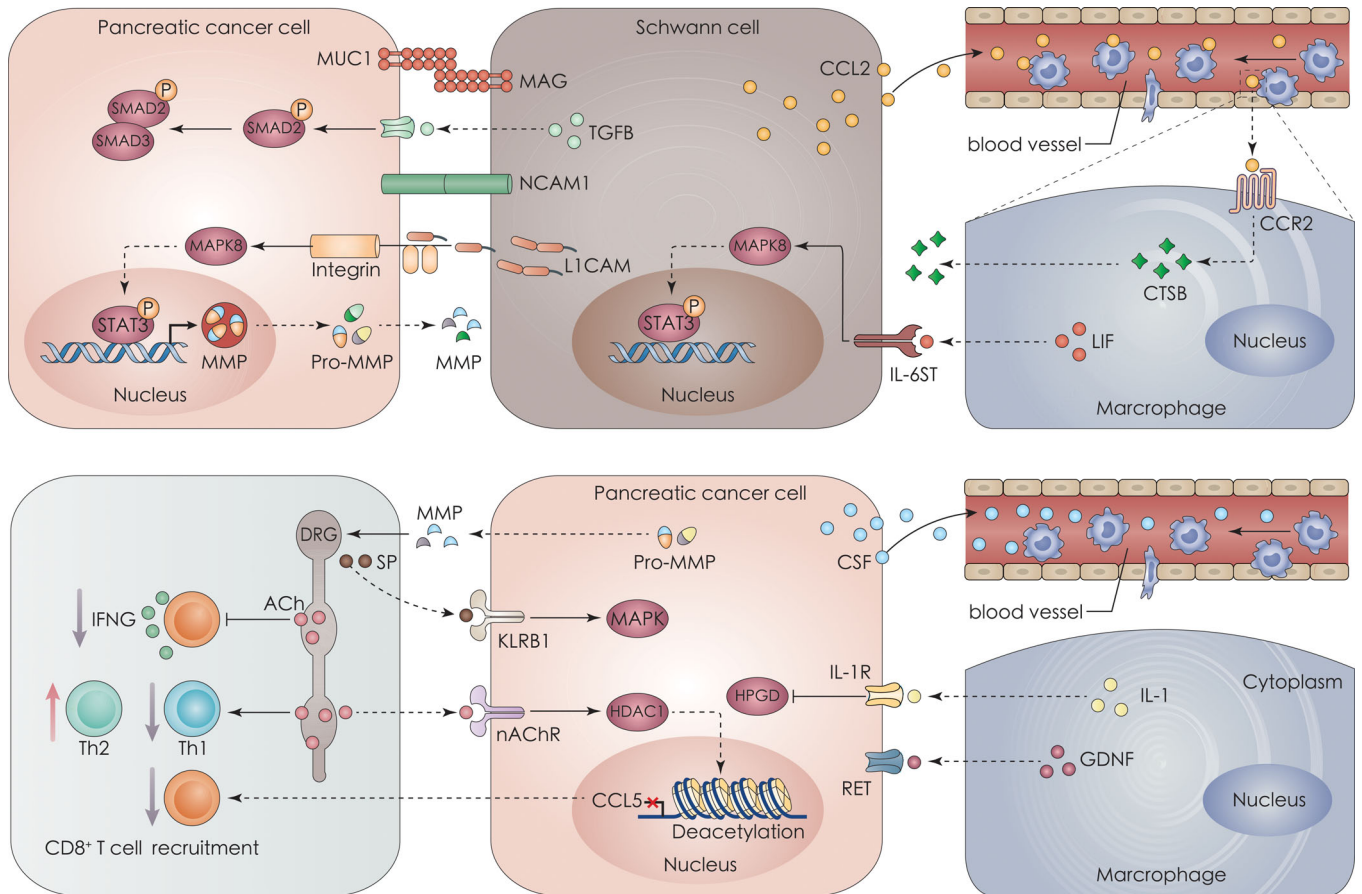is related to less pain in PDAC patients. Both neuronal cells and cancer cells release cytokines, such as CCL2 or CSF1, which attract macrophages from the circulation. Activated macrophages release CTSB to degrade collagen IV (a component of the nerves' perineurium) and IL-1 to downregulate the expression of HPGD. The expression of HPGD is positively correlated with PNI. ACh in DRG can directly inhibit the recruitment, activation, and function of CD8$^+$ T cells, leading to immunosuppression. Abbreviations: ACh, acetylcholine; CAF, cancer-associated fibroblast; CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; CSF, colony-stimulating factor; CTSB, cathepsin B; DRG, dorsal root ganglia; GNDF, glial cell-derived neurotrophic factor; HDAC1, histone deacetylase 1; HPGD, 15-hydroxyprostaglandin dehydrogenase; IFNG, interferon gamma; IL-1, interleukin 1; IL-1R, interleukin 1 receptor; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; IL-6ST, interleukin 6 signal transducer; KLRB1, killer cell lectin-like receptor B1; LICAM, L1 cell adhesion molecule; LIF, LIF interleukin 6 family cytokine; MAG, myelin-associated glycoprotein; MAPK, mitogen-activated protein kinase; MAPK8, mitogen-activated protein kinase 8; MMP, matrix metallopeptidase; MUC1, mucin 1, cell surface-associated; nAChR, nicotinic acetylcholine receptors; NCAM1, neural cell adhesion molecule 1; PDAC, pancreatic ductal adenocarcinoma; RET, ret proto-oncogene; SMAD2, SMAD family member 2; SMAD3, SMAD family member 3; SP, substance P; STAT3, signal transducer and activator of transcription 3; T cell, T lymphocyte; TGFB, transforming growth factor beta; Th1, T helper type 1; Th2, T helper type 2

3 | CELLULAR MEDIATORS OF PNI

As mentioned earlier, the pancreatic tumor microenvironment is composed of various cellular components that form a dynamic network, which is conducive to immune tolerance, metabolic reprogramming, cancer metastasis, and PNI. In this section, we summarize the role of various types of cells in regulating PNI within the tumor microenvironment (Figure 3).

3.1 | Schwann cells

Schwann cells are one of the common cell types in peripheral nerves and play a role in nerve repair and regeneration.

The direct interaction between Schwann cells and tumor cells can be fine-tuned at multiple levels. First, Schwann cells directly interacted with tumor cells through plasma membrane proteins, such as myelin-associated glycoprotein and neural cell adhesion molecule 1 (NCAM1) [50–52]. Second, Mucin 1 further promoted the adhesion of PDAC cells to Schwann cells *in vitro* and the invasion of PDAC cells into sciatic nerves *in vivo* [50, 51]. Third, Schwann cells elicited structural changes in PDAC cells, showing the formation of protrusions to Schwann cells and dispersion among cancer cells [52]. These changes depend on NCAM1 on the Schwann cell membranes, thereby gradually promoting PNI *in vitro* and *in vivo* [52].

Schwann cells can also communicate with tumor cells through secretory proteins, including L1 cell adhesion molecule (L1CAM) and transforming growth factor-beta (TGFB) [53, 54]. Schwann cells express and secrete L1CAM, which displays a powerful chemotaxis to PDAC cells [53]. After tumor cells encounter Schwann cells, L1CAM activated the downstream STAT3 pathway and upregulates the expression of MMP2 and MMP9, ultimately enhancing PNI [53]. In a transgenic mouse model, the administration of anti-L1CAM antibody significantly alleviated PNI [53], confirming that L1CAM is a mediator of PNI. Additionally, TGFB, a multifunctional cytokine belonging to the transforming growth factor superfamily, was produced and released by Schwann cells [54]. The released TGFB augmented the aggressive capacity of PDAC cells, which contributes to PNI progression in preclinical models [54]. The expression and release of TGFB were also increased in PDAC patients [55, 56]. The multiple roles of TGFB in the process of malignant progression may provide abundant opportunities for therapeutic intervention in PDAC.

Apart from the crosstalk between Schwann cells and tumor cells, Schwann cells build a connection with tumor-associated macrophages through secretory proteins [57]. Molecularly, Schwann cells secreted the chemokine C-C motif chemokine ligand 2 (CCL2), which recruits inflammatory macrophages from the circulatory system to the site of PNI [57]. The recruited macrophages were further differentiated into specific macrophages with highly expressed cathepsin B through the CCL2-C-C motif chemokine receptor 2 (CCR2) pathway [57]. Consequently, cathepsin B degraded collagen IV (one of the components in nerve perineurium), thereby aggravating nerve injury and tumor cell invasion [57]. Interleukin-6 (IL-6) family cytokine was produced and released by fibroblasts and mast cells and maintained high levels in the serum or tissues of PDAC patients [58]. After binding to its receptor on the Schwann cells, the IL-6 family cytokine activated the STAT3 pathway to trigger the migration and outgrowth of Schwann cells, thereby driving neuronal plasticity [58].

In particular, granulocyte-macrophage colony-stimulating factor (GM-CSF), a monomeric glycoprotein secreted by immune cells and fibroblasts, acted as a key intercellular cytokine in a pancreatic hypoxic tumor microenvironment during the formation of PNI [59]. The expression of GM-CSF was upregulated by the oxygen-dependent transcriptional activator hypoxia-inducible factor 1 subunit alpha (HIF1A) [60]. Consequently, the activation of the HIF1A-GM-CSF pathway mediated PNI by promoting the migration and accumulation of Schwann cells in tumor tissues, resulting in a poor prognosis [60]. In addition, the upregulation of GM-CSF promoted the immune escape of PDAC, while the suppression of GM-CSF enabled the immune system to clear tumor cells [61]. These findings could help us design effective strategies against PDAC by targeting neuroimmunity.

Altogether, Schwann cells play a crucial role in the process of PNI through extensive communications with various cells in the pancreatic tumor microenvironment. Therefore, a comprehensive understanding of the function of Schwann cells in PNI may open a window for early therapeutic intervention before PNI occurrence.

3.2 | Stromal cells and fibroblasts

Pancreatic stellate cells (PSCs) are the most prominent cell type in the PDAC stroma, accounting for about 50% of PDAC stromal cells [62]. During the co-culture process, PSCs upregulated the production and release of MMP proteins in PDAC (CFPAC1) cells, thereby enhancing tumor invasion ability [63]. The release of the extracellular matrix glycoprotein tenascin C by PSCs also enhanced the interaction between cancer cells and axonal DRG, which may be positively related to PNI, tumor stage, and tumor recurrence [64]. In addition, PDAC cells could release sonic hedgehog signaling molecules to activate hedgehog signaling pathways in PSCs, leading to cancer invasion and nerve dysfunction [65]. These findings indicate that abnormal hedgehog signals are involved in the communication between cancer cells, PSCs, and the nerves in the tumor microenvironment.

CAFs are highly differentiated stromal cells that promote tumor growth, angiogenesis, and matrix remodeling. CAFs express various unique proteins, which are related to the prognosis of PDAC. For example, nectin cell adhesion molecule 1 (NECTIN1)[+] CAFs were an independent prognostic factor in PDAC [66]. The level of NECTIN1 in the CAFs of PDAC patients was positively correlated with advanced tumor stage ($P = 0.016$), PNI ($P = 0.022$), and short OS ($P = 0.003$) [66]. Fibroblast activation protein alpha was a broad marker of CAFs in various cancers and was closely associated with PNI ($P = 0.009$), tumor size

($P < 0.001$), and poor prognosis ($P = 0.0085$) in PDAC patients [67]. Although inhibiting CAFs is a challenge in clinical practice, stromal response still may be a potential target for PDAC treatment.

3.3 | Macrophages

Tumor-associated macrophages (TAMs) produce an immunosuppressive tumor microenvironment in PDAC and participate in every stage of tumor formation and development [68]. By co-staining with macrophage markers (CD68 and CD163) and nervous system marker S100 calcium-binding protein, an immunohistochemical analysis of 59 PDAC patients found that the number and density of TAMs in a PNI-positive group were higher than those in the negative group [69]. Of note, the PNI-negative group may be the case where PNI is present but not detected. Increased TAM invasion in PNI was associated with poor prognosis in PDAC patients [69]. Subsequent mechanistic studies confirmed that IL-1 secreted by CD163+ macrophages inhibited the expression of 15-hydroxyprostaglandin dehydrogenase (HPGD) in PDAC cells [70]. Although the specific mechanism was not yet clear, low levels of HPGD were related to PNI and short survival [70]. The colony-stimulating factor 1 secreted by PDAC cells could recruit and activate macrophages [71]. Activated macrophages produced GDNF, thereby increasing PNI [71]. These findings provide a good example of the molecular communication between cancer cells and macrophages that drives PNI.

Other pathways for TAM-related PNI include CCR2 signaling. In animal models, the invasion of F4/80+ wild-type macrophages in the nerves was much more severe than that of CCR2-deficient macrophages [71]. Activated macrophages stimulated human PDAC cells (PANC1 and MiaPACA2) to secrete MMP1 [72]. Soluble MMP1 further induced DRG to release substance P (SP), which activated killer cell lectin-like receptor B1 (KLRB1) and then induced PNI through the pathway involving SP, KLRB1, and mitogen-activated protein kinase (MAPK) *in vitro* and *in vivo* [72]. To illustrate the complex nature of this process, it is necessary to further identify the feedback mechanism of TAM-associated signal transduction.

3.4 | T cells

T cells are one of the main components of the adaptive immune system and are used to kill infected host cells or cancer cells. The vagus nervous system plays an immunomodulatory role in the pancreatic tumor microenvironment, partly by controlling the infiltration or activation of T cells [73]. The level of acetylcholine (ACh), a neurotransmitter produced by the vagus nerve, was elevated in the PNI-detected samples from PDAC patients [73]. Elevated Ach can impair T cell recruitment and subsequent T cell-mediated anti-tumor immunity [73]. Mechanically, once captured by PDAC cells, ACh suppressed the expression of CCL5 in PDAC via histone deacetylase 1 (HDAC1)-mediated histone deacetylation [73]. Conversely, low levels of CCL5 failed to recruit CD8+ T cells to the tumor site, leading to an immunosuppressive tumor microenvironment [73]. ACh also inhibited interferon-gamma (IFNG/IFNγ) produced by CD8+ T cells, thereby favoring differentiation in T helper type 2 cells over T helper type 1 cells [73]. Therefore, in the orthotopic PDAC mouse model, it was not surprising that disrupting the vagus PNI process through vagotomy could inhibit tumor growth and prolong survival [73]. However, these findings were challenged by another study using a spontaneous model which showed that subdiaphragmatic vagotomy could accelerate KRAS^{G12D}-driven PDAC tumorigenesis in mice [74]. In contrast, complementary treatment with muscarinic agonists was shown to reverse this tumor-promoting phenotype (such as increased cancer stem cells and tumor metastasis) [74]. These different animal model studies together suggest a dual role of cholinergic nervous system invasion in PDAC, coupling the modulation of T cell recruitment and activation.

4 | AUTOPHAGY AND PNI

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved degradation mechanism in which cytosol components are engulfed by autophagosome and then delivered to the lysosome for degradation [75]. Dysregulated autophagy plays an essential role across all stages of PDAC, including the formation of PNI [76]. The expression of the autophagosome marker microtubule-associated protein 1 light chain 3 alpha/beta (MAP1LC3A/B) was positively correlated with PNI ($P < 0.05$), and high MAP1LC3A/B expression was an independent risk factor for PNI and poor prognosis of PDAC ($P < 0.05$) [77]. Although the mechanism by which autophagy in PDAC cells contributes to PNI is still poorly understood, ubiquitin C (one of the sources of ubiquitin) has been shown to act as a bridge between PNI and autophagy [78]. Another area worth exploring in the future is whether PNI can be used as an evaluation index for the clinical effect of the antimalarial agent hydroxychloroquine, a potent inhibitor of autophagy used in clinical trials in PDAC patients [79–82]. It is also interesting to determine whether blocking PNI formation will impair the autophagic process.

5 | BIOMARKERS OF PNI

5.1 | Protein expression and secretion

Several studies have identified upregulation of a panel of proteins in the tumor samples from PDAC patients with PNI (Table 1). These proteins include CD74 (a regulator of antigen presentation by mediating the assembly and subcellular trafficking of the major histocompatibility complex class II [MHC-II] complex) [83–85], cadherin 1, and other membrane proteins [86], which were closely related to the survival rate of PDAC patients. However, an increase in cyclin D1, a member of the G1 cyclin family, was associated with PNI, but not prognosis [87], suggesting that PNI regulators are not always parallel to the course of PDAC. Compared with the PNI-undetected group, a lower expression of proteins, such as kinesin family member 14 and Fas cell surface death receptor [88, 89], were also detected in the PNI detected group. However, the function of these proteins and their effect on prognosis are unclear. Apart from protein expression, the serum levels of collagen type VI alpha 3 chain and baculoviral IAP repeat-containing 5 were positively correlated with PNI [90, 91], providing a convenient way to monitor the occurrence of nerve damage in PDAC.

In addition to researches involving traditional immunohistochemical staining, high-throughput analysis has been applied to identify novel regulators of PNI. Through proteomic analysis of the nerves within PDAC tissues, compared with normal nerves, investigators found that invading the nerves had an increased VGF nerve growth factor inducible (VGF) level [12]. VGF is a secreted protein and neuropeptide precursor, which may play a role in maintaining energy homeostasis and neurite expansion [12]. By using a combination of proteomics and transcriptomics analysis, researchers showed that synuclein gamma (SNCG) had higher expression levels in a PNI group than that in a non-PNI group [92]. Importantly, the overexpression of SNCG was an independent predictor of the OS of PDAC patients, whereas suppressing SNCG expression leaded to a significant decrease in the formation of PNI in preclinical animal models [92]. Since SNCG was overexpressed in various invasive and metastatic cancers [93, 94], it will be interesting to further evaluate its role in tumor metastasis caused by PNI.

5.2 | Gene and noncoding RNA abnormalities

Gene alternations in human PDAC samples are also linked to PNI (Table 2). For example, Ras homolog family member C was abundantly expressed in PNI tissues and was related to poor disease prognosis [95]. The number of noncoding RNAs (such as microRNAs, piRNAs, and lncRNAs) within the human genome is unknown; however, recent transcriptomic studies suggest that some of them were significantly downregulated or upregulated during PNI in PDAC. For instance, the downregulation of *MIR429* in tumor tissues inhibited neurotrophin 3 expression, thereby relieving PNI [96]. LncRNA, AFAP1 antisense RNA 1 was abundantly expressed in PDAC tissues and is positively associated with PNI [97]. However, the effect and mechanism of lncRNAs in PNI and PDAC have not been extensively characterized. An open question is how many transposable elements are reused within lncRNAs for PNI.

6 | CLINICAL SIGNIFICANCE OF PNI

6.1 | Overall survival prediction

A number of retrospective studies have shown that the postoperative survival rate of PDAC patients was negatively correlated with nerve infiltration [10, 98-101] (Table 3). One recent multicenter retrospective study enrolling 778 PDAC patients who received surgical treatment between 2009 and 2014 showed that the rate of PNI was 87% among these patients (T0/1, 61.6%; T2, 86.7%; T3/4, 90.1%; N0, 72.5%; and N1, 93.0%) [102]. PNI was an independent risk factor for disease-free survival (DFS) and/or OS ($P < 0.0001$) [103], and could serve as a prognostic indicator [99, 104, 105]. Being PNI-free was independently protective for long-term survival (longer than 10 years) in PDAC patients [106]. Hence, the existence of PNI is one of the critical references to guide personalized antineoplastic therapeutic protocols [107].

The depth of nerve infiltration has also been shown to influence patients' survival time. Patients with PNI had the shortest survival period ($P = 0.034$) [10]. For patients with tumor cells infiltrated beyond the axon of the nerves, DFS and OS were 13.4 months and 28.1 months, respectively [108]. In patients whose disease did not reach the axon, the DFS and OS were 32.9 months and 45.7 months, correspondingly [108]. Interestingly, a retrospective study of 59 PDAC patients showed that there was no parasympathetic nerve in normal pancreatic tissues, but parasympathetic nerves were abundant in tumor samples (50.8%) [109]. These changes in parasympathetic nerves acted as independent risk factors for poor prognosis [109]. Likewise, meta-analysis data showed that PNI was obviously associated with DFS reduction (hazard ratio = 2.53; $P = 0.0001$) [110]. Not only DFS and OS but also the rate of peritoneal metastases (a sign of recurrence) have been shown to highly correlate with PNI [110]. These clinical studies support the oncogenic effects of PNI in the initiation and

TABLE 1 Protein expression and secretion associated with PNI in PDAC

Protein	Sample sources	Testing method(s)	Expression	Association with PNI	*P* value	Reference(s)
BIRC5	Serum from 80 PDAC patients and 80 healthy controls	ELISA	Upregulated	Positive	< 0.01	[91]
CADM4	Tissues from 258 patients	IHC	Downregulated	Negative	0.001	[126]
CCND1	Tissues from 59 patients	IHC	Upregulated	Positive	NA	[87]
CDH1	Tissues from 46 patients	IHC	Upregulated	Positive	NA	[86]
CD74	Tissues from 67 patients	IHC	Upregulated	Positive	NA	[83]
CD74	Tissues from 68 patients	IHC	Upregulated	Positive	0.006	[84, 85]
COL6A3	Serum from 44 PDAC patients, 46 benign lesion patients, and 30 age-matched healthy volunteers	ELISA; RT-PCR	Upregulated	Positive	0.0337	[90]
CTHRC1	Tissues from 40 patients	IHC; qRT-PCR	Upregulated	Positive	0.025	[127]
CXCR4	Tissues from 51 patients	IHC	Upregulated	Positive	0.042	[128]
FAS	Tissues from 162 patients	IHC	Upregulated	Negative	< 0.05	[89]
HPGD	Tissues from 127 patients	IHC; RT-PCR; Western blotting	Downregulated	Negative	0.013	[70]
IL-13RA2	Tissues from 236 patients	IHC; In situ hybridization	Upregulated	Positive	< 0.001	[129]
LRP1	Tissues from 478 patients	IHC; RT-PCR; Western blotting	Upregulated	Positive	0.001	[130]
L1CAM	Tissue microarray containing 94 cases	IHC	Upregulated	Positive	0.001	[131]
MAP1LC3A/B	Tissues from 109 patients	IHC	Upregulated	Positive	< 0.05	[77]
MDK	Tissues from 42 patients	IHC	Upregulated	Positive	0.018	[38]
MDK	Tissues from 114 patients	IHC; Western blotting	Upregulated	Positive	0.03	[132]
MYBL2	Tissues from 93 patients	IHC	Upregulated	Positive	0.013	[133]
MYC	Tissues from 162 patients	IHC	Upregulated	Positive	< 0.0001	[89]
PODXL	Tissues from 168 patients	IHC	Upregulated	Positive	0.005	[134]
POP1	Tissues from 67 patients	IHC	Upregulated	Negative	< 0.05	[135]
PTN	Tissues from 38 patients	IHC	Upregulated	Positive	0.016	[39]
PTN	Orthotopic tumor mouse model	IHC	Upregulated	Positive	0.019	[136]
SDC2	Tissues from 42 patients; SUB8B8, T3M4, and PANC1 cells	Western blotting; qRT-PCR	Upregulated	Positive	NA	[137]
SDC3	Orthotopic tumor mouse model	IHC	Upregulated	Positive	0.032	[136]
SNCG	Tissues from 62 patients; PNI and orthotopic tumor mouse model	Proteomics; IHC	Upregulated	Positive	0.009	[92]
TIMP2	Tissues from 51 patients	IHC	Upregulated	Positive	0.042	[128]
VGF	Five matched PNI and non-PNI human cancer samples	Proteomics; IHC; Western blotting	Upregulated	Positive	0.0086	[12]
WASL	Tissues from 86 patients	IHC	Upregulated	Positive	0.038	[138]

Abbreviations: BIRC5, baculoviral IAP repeat containing 5; CADM4, cell adhesion molecule 4; CCND1, cyclin D1; CDH1, cadherin 1; COL6A3, collagen type VI alpha 3 chain; CTHRC1, collagen triple helix repeat containing 1; CXCR4, C-X-C motif chemokine receptor 4; ELISA, enzyme-linked immunosorbent assay; FAS, FAS cell surface death receptor; HPGD, 15-hydroxyprostaglandin dehydrogenase; IHC, immunohistochemistry; IL-13RA2, interleukin 13 receptor subunit alpha 2; LRP1, LDL receptor related protein 1; L1CAM, L1 cell adhesion molecule; MAP1LC3A/B, microtubule associated protein 1 light chain 3 alpha/beta; MDK, midkine; MYBL2, MYB proto-oncogene like 2; MYC, MYC proto-oncogene, BHLH transcription factor; PODXL, podocalyxin like; POP1, homolog, ribonuclease P/MRP subunit; PTN, pleiotrophin; QRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SDC2, syndecan 2; SDC3, syndecan 3; SNCG, synuclein gamma; TIMP2, TIMP metallopeptidase inhibitor 2; VGF, VGF, nerve growth factor inducible; WASL, WASP like action nucleation promoting factor; NA, not available.

TABLE 2 Gene and noncoding RNA abnormalities associated with PNI in PDAC

Gene	Sample sources	Testing method(s)	Expression	Association with PNI	P value	Reference(s)
AFAP1-AS1	Tissues from 90 patients	RT-PCR	Upregulated	Positive	0.006	[97]
ARHGDIB	PANC1, COLO357, and T3M4 cells	51K human cDNA chips	Upregulated	Positive	NA	[88]
KIF14	PANC1, COLO357, and T3M4 cells	51K human cDNA chips	Downregulated	Negative	0.05	[88]
MIR429	Tissues from 22 PDAC patients, 11 healthy controls	RT-PCR	Downregulated	Negative	NA	[139]
RHOC	Tissues from 33 patients	RT-PCR	Upregulated	Positive	NA	[95]
TMEM238L	Tissues from 22 PDAC patients, 11 healthy controls	RT-PCR	Upregulated	Positive	0.003	[139]

AFAP1-AS1, AFAP1 antisense RNA 1; ARHGDIB, Rho GDP dissociation inhibitor beta; KIF14, kinesin family member 14; MIR429, microRNA 429; RHOC, Ras homolog family member C; RT-PCR, reverse transcription polymerase chain reaction; TMEM238L, transmembrane protein 238 like; NA, not available.

TABLE 3 Retrospective database analysis for PNI in PDAC

Year	No. of patients	Analysis model(s)	Association with PNI	Prognosis significance	P value/OR	Reference(s)
1996	113 (69.9%)	Kaplan-Meier analysis; Cox proportional hazards analysis	Negative	Not an independent risk factor	0.01	[98]
2002	24 (70.8%)	Kaplan-Meier analysis; Cox proportional hazards analysis	Negative	NA	NA	[99]
2006	56 (NA)	Kaplan-Meier analysis; Cox proportional hazards analysis	Negative	Prognostic parameters	< 0.01	[19]
2007	33 (57.6%)	Kaplan-Meier analysis	Negative	NA	0.023	[100]
2007	75 (65.3%)	Kaplan-Meier analysis; Cox proportional hazards analysis	Negative	Prognostic parameters	0.034	[10]
2010	96 (53.1%)	Log-rank test; Cox multi-regression analysis	Negative	Prognostic parameters	0.0001	[101]
2011	95 (88.0%)	Kaplan-Meier analysis; univariate and multivariate Cox regression analysis	Negative	NA	0.02	[140]
2012	212 (58%)	Kaplan-Meier analysis; Cox regression analysis; Cox proportional hazards models	Negative	NA	0.03	[108]
2015	209 (94%)	Kaplan-Meier analysis; Cox proportional hazards analysis	Negative	Prognostic parameters	0.0001	[104]
2016	173 (60.7%)	Multivariate Logistic Regression	Negative	Absence of PNI is independently associated with increased odds of long-term survival	0.036	[106]
2016	59 (50.8%)	Kaplan-Meier analysis; multivariate Cox regression analysis	Negative	Prognostic parameters	0.0009	[109]
2017	3538 (76.2-97.8%)	Meta-analysis	Negative	Prognostic parameters	< 0.00001	[141]
2019	17,313 (NA)	Meta-analysis	Negative	Risk factors for recurrence	OR: 5·19, 2·79 to 9·64	[110]
2020	778 (87%)	Kaplan-Meier analysis; multivariable Cox proportional hazards models	Negative	Prognostic parameters	0.01	[102]
2020	400 (87.7%)	Kaplan-Meier analysis; univariate and multivariate Cox regression analysis	Negative	Prognostic parameters	0.05	[103]

development of PDAC. Whether PNI can be used as an indicator of early diagnosis may depend on the development of more sensitive image detection technology.

6.2 | Pain occurrence

In the late stage of disease, PDAC patients usually suffer severe pain due to tumor progression [16]. Considered as a negative prognostic factor for survival [111], severe pain not only greatly influences the quality of life for such patients but also may increase their risk of drug abuse. The invasion of tumor cells destroys the normal structure of the neural sheath and produces various molecules, contributing to neuropathic and inflammatory pain [6]. As mentioned earlier, GDNF contributed to the PNI process in PDAC patients [26]. Surprisingly, a rich expression of GDNF was closely related to the degree of back pain before surgery ($P = 0.045$) or 12 months after PDAC resection ($P = 0.028$) [26]. The accumulation of mast cells around the intrapancreatic nerves was also correlated with neuropathic pain [112]. Mechanically, NGF released by cancer cells stimulated the sensitive sensory nerves by interacting with transient receptor potential cation channel subfamily V member 1 (TRPV1), resulting in severe pain in PDAC patients [113, 114]. ARTN and GDNF secreted by tumor cells might upregulate TRPV1 expression, leading to more pain sensitivity [115].

In addition, hypoxic cancer cells secreted IL-6 into the tumor microenvironment, leading to the growth and activation of Schwann cells [116]. The activated Schwann cells suppressed the signal transmission of spinal astroglia and microglia, which may be associated with the relief of pain during tumor initiation in KRAS^{G12D}-driven PDAC mouse models [116]. Subsequent studies addressed the specific regulatory role of PNI in cancer-associated pain through several animal models. For example, a PDAC orthotopic K8484 mouse model showed the main characteristics of human PDAC, including the structural remodeling of histopathology and nerve fibers [117]. *In vivo* methods to quantify pain or hypersensitivity are important to understanding the molecular mechanism of pain and its impact on the process of PDAC [117]. Of note, PHA-848125, a dual inhibitor of cyclin-dependent kinase and NTRK1, has been tested in preclinical trials to suppress PNI and alleviated pain generation in human PDAC cancer cells (BXPC3, MiaPACA2, and CAPAN1) in a xenograft mouse model [118].

6.3 | Hyperglycemia management

Diabetes or hyperglycemia can lead to nerve damage. Several studies have investigated the clinical significance of poor blood glucose control and PNI in PDAC [119, 120]. First, through multivariate analysis, diabetes was proved to be an independent predictor of survival after resection in PDAC patients [121]. Second, pathological analysis confirmed the correlation between diabetes and PNI ($P = 0.026$) [121]. By comparing the tumor specimens of 61 PDAC patients with healthy controls, the frequency and severity of PNI in a hyperglycemia group were higher and heavier [119]. Third, both *in vivo* and *in vitro* experiments have shown that hyperglycemia can enhance the proliferation, NGF expression, neurotropism, and invasiveness of pancreatic cancer cells, thereby destroying the structure of the nerves [19, 120, 122]. These impaired nerves provide an avenue for tumor metastasis. Therefore, controlling blood glucose levels is very important for PDAC patients.

6.4 | Treatment relevance

Traditionally, for PDAC patients who received neoadjuvant therapy, a PNI rate of 58% was reported, which was lower than that in those who did not receive neoadjuvant therapy (80%) ($P = 0.002$) [108]. Radiotherapy might also contribute to limiting PNI, because 4 Gy irradiation could remarkably reduce GDNF release in DRG, thereby inhibiting the invasion capacity of MiaPACA2 cells [123]. *In vivo*, 8 Gy radiotherapy was associated with a decrease in the secretion of GDNF from the sciatic nerves, which inhibited the frequency of PNI and protected sciatic nerve functions from destruction by cancer cells [123]. Sustained low-dose irradiation from iodine-125 seeds inhibited tumor growth and PNI formation in preclinical models [124]. Iodine-125 seeds planted in the local sites of tumors even relieved pain in PDAC patients [124]. It has also been reported that telomerase-specific oncolytic adenoviruses might have therapeutic potential for PDAC patients [125]. Moreover, these viruses repressed the migration and invasion of pancreatic cell lines in a DRG co-culture system [125], indicating their potential role in preventing PNI. Overall, comprehensive multidisciplinary clinical trials are urgently needed to optimize the treatment of PDAC patients.

7 | CONCLUSION AND PERSPECTIVES

In the past decade, we have witnessed progress in understanding the components and functions of the pancreatic tumor microenvironment, including the nerves. The nerves are involved in various tumor biological processes associated with the progression and recurrence of PDAC. In particular, PNI plays a complicated and unfavorable role in the prognosis of human PDAC, coupling various

molecular, metabolic, and cellular components of the tumor microenvironment. However, preclinical animal studies have some contradictory or uncertain results on the effects of neurotransmitters or the vagus nerve on PDAC, which has led to concerns about the plasticity of PNI mechanisms and strategies to block the nervous system.

Some issues deserve further study. First, neurotransmitters are essential for many human physiological functions. Targeting neurotransmitters by neutralizing antibodies or compounds may cause undesirable or even toxic side effects. Second, the pancreatic tumor microenvironment is complex, composed of different systems and cell types. Although many studies have focused on the communication between tumors and the nervous system, research on the connection between the nervous system and other systems (e.g., immunity and microorganism) or conditions (e.g., hypoxia and oxidative stress) needs to be expanded. Third, PDAC relies on autophagy for the survival of cancer cells. How autophagy regulates the PNI process by degrading specific proteins remains an open question. Fourth, the neuronal and molecular machinery underlying pain transitions deserve further comprehensive study, as this may help early diagnosis or improve the quality of life of patients with advanced cancer. Collectively, identifying specific molecular targets or exploring new ways to inhibit the progression of PNI and other neurological abnormalities may have a potential impact on PDAC treatment.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS
All authors wrote, revised, and approved the manuscript.

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