Perineural invasion and associated pain in pancreatic cancer

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Abstract | Perineural invasion (PNI) is a prominent characteristic of pancreatic cancer. PNI is a process whereby cancer cells invade the surrounding nerves, thus providing an alternative route for metastatic spread and pain generation. PNI is thought to be an indicator of aggressive tumour behaviour and has been shown to correlate with poor prognosis of patients with pancreatic cancer. Recent studies demonstrated that some signalling molecules and pathways that are involved in PNI are also involved in pain generation. Targeting these signalling pathways has shown some promise in alleviating pain and reducing PNI, which could potentially improve treatment outcomes for patients with pancreatic cancer.

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It has long been known that, in addition to being found in vascular and lymphatic systems, cancer cells occur in neuronal spaces, which serve as an alternative route for dissemination. This neuro-invasive ability of cancer cells was first described as the lateral growth of cancer cells along nerve fibres surrounding the cancer (reviewed in REFS 1–3). The main reason for the migration of cancer cells along surrounding nerves was originally thought to be because this represented 'a path of least resistance'[2,4]. However, other reports have argued against this interpretation (FIG. 1a). For example, Bockman *et al.*[5] described the presence of cancer cells within the endoneurial spaces associated with Schwann cells, in addition to the presence of cancer cells in the perineurial space. Also, more recently, Leibig *et al.*[2] defined perineural invasion (PNI) as the presence of cancer cells along nerves and/or within the epineurial, perineurial and endoneurial spaces of the neuronal sheath, including cases in which the cells circumscribed at least 33% of the nerve. Thus, PNI, which was previously described as the spread of cancer cells in the perineural spaces of the nerves, can also be described as neural invasion (NI) based on the presence of cancer cells in all three spaces of the nerve sheath (reviewed in REF. 3). Consequently, some researchers have used the term 'neural invasion' instead of 'perineural invasion'[6,7]. Why cancer cells invade nerves is as yet not fully known, but it has been postulated that once cancer cells have invaded the outer layers of the neuronal sheath, they become part of an elite and favourable environment[2,5,8]. Our current knowledge of cancer cells that are well within the inner layers of the nerve sheath adds credence to the possibility that the movement of cancer cells involves highly coordinated signalling events between the cancer cells and peripheral nerves (FIG. 1b). Several signalling molecules — including neurotrophins, chemokines and cell-surface ligands and receptors — have been implicated in the process of PNI[2,6,9]. In many cases, PNI is accompanied by pain, and many of the molecules involved in PNI are also implicated in pain generation[2,6,9,10]. In recent years, substantial efforts have been made to develop targeted therapeutics for cancer-associated pain. A recent article by Demir *et al.*[3] provided an excellent review of the history and present status of PNI research in pancreatic cancer, particularly regarding the *in vitro* and *in vivo* models. Here we review the recent advances in our understanding of the molecular mechanisms of PNI and associated pain in pancreatic cancer, and the therapeutic strategies that are currently being pursued.

PNI in pancreatic ductal adenocarcinomas

Since the first reports in head and neck cancer (reviewed in REFS 1,2), PNI has been reported in a variety of cancers including pancreatic[6,11–13], prostate[14–16], colorectal[17–19], and others[20–27](reviewed in REFS 2,9). Although different cancers have varied incidences of PNI, PNI-positive cases often correlate with poor prognosis and decreased survival[9,18,28] (TABLE 1).

Pancreatic ductal adenocarcinomas (PDAs) have some of the highest incidences of PNI (80–100%) among cancers[6,11–13] (BOX 1). The reasons for the preponderance of PNI in pancreatic cancer are not clearly understood, but can be partially explained by the strong neurotropic effects of PDA cells and the close proximity of the pancreas to several neural plexuses[13,29,30] (FIG. 1). The infiltration and growth of cancer cells within these surrounding

At a glance

- Perineural invasion (PNI) is the process through which cancer cells invade the perineural spaces of surrounding nerves and is not simply the movement of cancer cells along a path of low resistance, as was previously thought. PNI is a directed process that involves many signalling molecules from various signalling pathways; these signalling molecules are produced by both the cancer cells and the nerves. Once the cancer cells have invaded the nerves, they are able to thrive within the neuronal spaces. This constitutes a means for the cancer cells to spread to distant locations.
- The incidence of PNI is particularly high in pancreatic cancer. Although the exact cause for this increased affinity is as yet unclear, the strong neurotropic effects of pancreatic cancer cells are thought to contribute to this phenomenon. Additionally, reciprocal signalling between the pancreatic cancer cells and the surrounding nerves leads to neurogenesis, as well as the increased growth of pancreatic cancer cells.
- PNI also contributes to the generation of the pain that is experienced by pancreatic cancer patients, and many of the signalling molecules that are involved in PNI are also known to be involved in pain signalling. Thus, we hypothesize that agents targeting these signalling pathways may have the potential to prevent PNI and may help to alleviate pain in patients with pancreatic cancer.

nerves is an indicator of aggressive behaviour of the tumour and is associated with pain and poor prognosis in patients with pancreatic cancer, although conflicting evidence has been published[28,31–36]. The abundance of innervations from these neural plexuses into the pancreas generates a readily available interface for the interaction of cancer cells with nerves. Although this, to some extent, explains the high prevalence of PNI in pancreatic cancer, the molecular mechanisms underlying PNI are still being unravelled. Furthermore, although cancer cells have a propensity to travel towards the nerves, the nerves also preferentially grow towards the tumour in response to signalling factors that are secreted by the cancer cells[15,37].

Molecular mechanisms

There has been a paradigm shift in the approach to understanding PNI, as research has progressed into identifying the molecular mechanisms that drive PNI. Current insights suggest that concerted, reciprocal signalling between cancer cells and nerves is essential for PNI. Bockman *et al.*[5] showed that increased expression of transforming growth factor-α (TGFα) in nerves surrounding the pancreas and the expression of epidermal growth factor receptor (EGFR) on the pancreatic cancer cells correspond to an increased affinity of nerves for pancreatic cancer cells and vice versa. This early study paved the way for the exploration of the roles of other nerve-associated growth factors in PNI. Subsequent studies have identified a multitude of molecules — including neurotrophins and their receptors[7,38–40], proteinases[41,42], cytokines[5], chemokines[43] and cell-surface markers[44,45] — for which expression was altered in cancer cells and/or nerves, thus suggesting a potential role for these molecules in PNI (FIG. 2; TABLE 2).

Neurotrophins and their receptors in PNI. Neurotrophins are signalling factors that are secreted by neuronal cells and cancer cells, and are known to have a role in the survival, growth and differentiation of these cells[46,47]. The nerve growth factor (NGF) family is a well characterized family of neurotrophins that consists of four members: NGF, brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NTF3) and NTF4 (also known as NTF5)[48]. The activation of signalling cascades that are crucial for the survival of neuronal cells and cancer cells depends on the type of receptor that the neurotrophins bind to. Different receptors can activate different signalling pathways that control cell growth, neuritogenesis, the survival and differentiation of both neuronal and cancer cells, and the death of neuronal cells[48,49]. The NGF family signals by binding to two different types of receptors: a high-affinity and a low-affinity receptor. Each neurotrophin binds to a specific high-affinity receptor, which belongs to the tropomyosin-receptor kinase (TRK) family, and a low-affinity receptor, p75 neurotrophin receptor ($p75^{NTR}$), which belongs to the tumour necrosis factor (TNF) superfamily[49,50]. NGF binds to TRKA; BDNF and NTF4 bind to TRKB; and NTF3 binds to TRKC, all of which induce pro-survival signals[48,49,51]. In the presence of $p75^{NTR}$, the binding of NGF to TRKA is increased, as is pro-survival signalling through TRKA. Interestingly, some studies have shown that binding of neurotrophins to $p75^{NTR}$ alone can result in apoptosis and other forms of cell death[48–54].

Increased levels of NGF and its receptors, TRKA and $p75^{NTR}$, have been reported in pancreatic cancer cells and the surrounding nerves[40,46,47]. Zhu *et al.*[40] showed that NGF was strongly expressed in pancreatic cancer cells compared with normal exocrine pancreas, whereas TRKA was intensely expressed in the perineurium of pancreatic nerves. Furthermore, the levels of NGF and TRKA in pancreatic tumour tissues correlated significantly with the frequency of PNI. In agreement, experiments using dorsal root ganglia (DRGs) and pancreatic cancer cell co-culture models have shown that pancreatic cancer cells migrate towards the neurites that extend from the DRGs, and that the DRGs also exhibit an increased propensity to extend neurites towards the pancreatic cancer cells[15,37,55–57]. These studies indicate that a mutual tropism exists between the nerves and pancreatic cancer cells as a result of paracrine signalling by NGF, which is secreted by the cancer cells and activates TRKA that is expressed in the surrounding nerves[6,40,58–60]. However, as TRKA is also expressed on pancreatic cancer cells, NGF may exert its stimulatory effects on growth and invasion of cancer cells in an autocrine manner[59,61,62] (FIG. 2). Additionally, the increased expression of NGF in pancreatic cells, and of TRKA in the surrounding nerves, has been reported in tissue samples of chronic pancreatitis, which further suggests the involvement of the NGF–TRKA pathway in pain generation[38,39,63].

Although the upregulation of TRKA in the perineurium of the surrounding nerves has been positively correlated with poor prognosis and an increased invasive propensity of PDA cells, there have been conflicting reports about the prognostic value of the $p75^{NTR}$ receptor in PNI[64,65]. Using reverse-transcription PCR (RT-PCR) to measure mRNA expression in primary pancreatic tumours from 56 patients, Dang *et al.*[64] showed that $p75^{NTR}$ expression was inversely associated with PNI

Perineurium
The connective tissue sheath that surrounds bundles of nerves known as fascicles.

Dorsal root ganglia
A mass of sensory afferent nerve cell bodies that is located on the dorsal root of the spinal cord, one on each side for each spinal nerve.

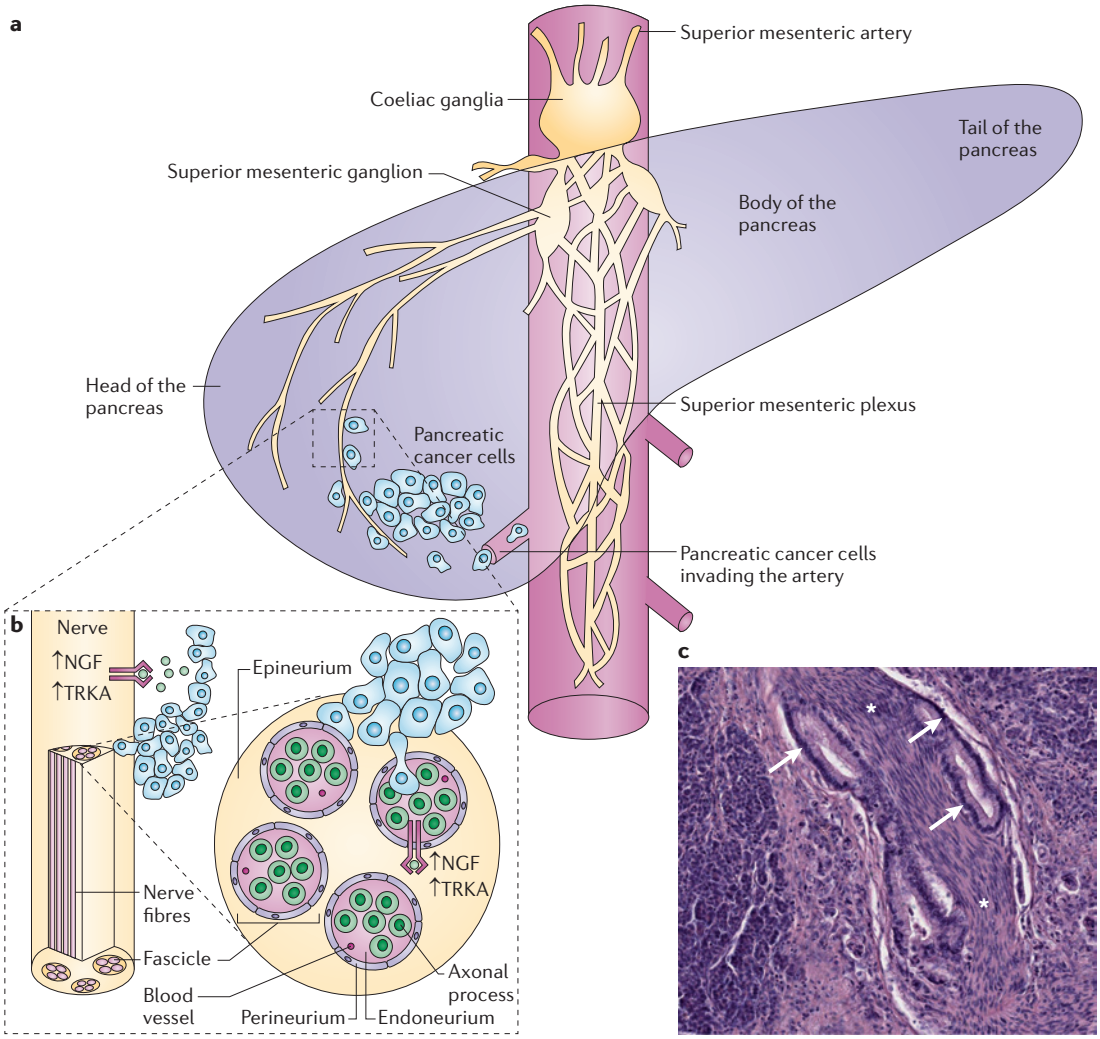

Figure 1 | **Perineural invasion in pancreatic cancer. a** | The head of the pancreas is innervated by the coeliac plexus, which is comprised of the right and left coeliac ganglia (labelled as coeliac ganglia in the figure) and the superior mesenteric ganglion. These nerve plexuses surround the superior mesenteric artery and lie between the pancreas and the artery. The pancreaticus capitalis I plexus (arising from the coeliac ganglia) and the pancreaticus capitalis II plexus (arising from the superior mesenteric ganglion) are shown as yellow branches on the left of the figure and are considered to be the main routes for invasion by pancreatic cancer cells that are located in the head of the pancreas. The major routes for the neural spread of cancer cells from the body and tail of the pancreas are through innervations from the coeliac plexus and the splenic plexus (not shown)[13]. **b** | Peripheral nerves are composed of three layers: the endoneurium, the perineurium and the epineurium. The innermost layer of the nerve consists of the nerve fibre, which is composed of an axonal process (shown in dark green) surrounded by Schwann cells (shown in light green) that are associated with the myelin sheath. Several such nerve fibres are enclosed by the endoneurium (shown in pink), which is a fragile flowing matrix. The endoneurium has a rich vasculature that is distinct from that of the nerve fibres, thereby keeping the integrity of the endoneurial component intact. A group of these nerve fibres is further encased by the perineurium (shown in purple), forming a structure known as a fascicle. The perineurium is a layer of endothelial cells that are tightly arranged in concentric circles. Additionally, the tight junctions on the basal laminae of these endothelial cells are joined using a dovetailed arrangement. This creates strong contacts among the cells and forms an effective barrier between the nerve fascicles and the epineurium, thereby protecting the nerve fibres. Finally, several fascicles bundled together constitute a nerve, which is then enclosed by the outermost layer, the epineurium. The epineurium is composed of elastin and collagen fibres that extend along the length of the nerve[3,30]. Upwards-directed arrows indicate an increase in protein expression levels in the nerves. **c** | A haematoxylin and eosin (H and E)-stained tissue section of a pancreatic ductal adenocarcinoma sample showing perineural invasion, where the nerve denoted by the white asterisks is being invaded by the pancreatic cancer cells denoted by the white arrows. NGF, nerve growth factor; TRKA, tropomyosin-receptor kinase A.

and pain. Furthermore, the positive effects of $p75^{NTR}$ expression on overall prognosis depended on the ratio of expression levels of TRKA versus $p75^{NTR}$ within the pancreatic cancer cells. These authors hypothesized that novel therapeutics designed to increase the activity of $p75^{NTR}$ would prevent PNI and aid in the treatment of PNI and pancreatic cancer[64]. However, in another report, Wang *et al.*[65] indicated that the $p75^{NTR}$ that

Table 1 | **Cancer types in which perineural invasion has been reported**

Cancer type	Incidence of perineural invasion (%)	Refs
Pancreatic cancer	Up to 100	6,11–13
Head and neck cancers	Up to 80	2,9,21,23
Squamous cell carcinoma	2.5–14	9,21,24
Basal cell carcinoma	0.2–10	21
Prostate cancer	75–80	2,9,14–16
Colorectal cancer	9–33	2,17–19
Breast cancer	3–38	2,9,20
Biliary tract cancer	~80	9
Stomach cancer	50–60	2,9,22,25
Cholangiocarcinoma	75–85	2,9,26,27

is expressed on pancreatic cancer cells has a role in chemoattraction. Using immunohistochemistry, they observed a positive correlation between $p75^{NTR}$ expression and PNI in pancreatic cancer tissues. Furthermore, the migration of pancreatic cancer cells in response to a chemoattractant (in this case, conditioned media from U87 neuroglioma cells) increased dramatically when the cells were transfected with $p75^{NTR}$, indicating that $p75^{NTR}$ is involved in the migration of pancreatic cancer cells and so might also be involved in PNI[65].

BDNF expression was also reported to be increased in pancreatic cancer cells. High levels of BDNF increase the proliferative behaviour and invasiveness of cancer cells, which may contribute to PNI[2,39,46]. Similarly, the BDNF receptor, TRKB, is overexpressed in metastatic human PDA cells, and this increase is associated with PNI[66] (FIG. 2). Although increased levels of NTF3 and its receptor TRKC have been reported in pancreatic cancer cells, their involvement in PNI is as yet unclear[2,39,46,66], as is that of NTF4 (REF. 66).

The glial cell line-derived neurotrophic factor (GDNF) family of growth factors is also involved in PNI in pancreatic cancer[6,38]. The GDNF family consists of four members: GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN)[67]. The GDNF neurotrophins signal by binding to their corresponding selective GDNF receptor-α (GFRα) family member: GDNF, NRTN, ARTN and PSPN bind to GFRα1, GFRα2, GFRα3 and GFRα4, respectively[67]. This results in the formation of a complex that contains a ligand-bound GFRα receptor and the rearranged during transfection (RET) receptor tyrosine kinase, which was originally discovered as a proto-oncogene[68]. This GDNF–GFRα–RET complex activates signalling pathways that control cell growth, differentiation, neurite outgrowth and survival[67]. Increased expression of GDNF and GFRα1–RET is seen in pancreatic cancer cells, and high levels of GDNF secreted by neuronal cells can increase the invasiveness of pancreatic cancer cells[38,69] (FIG. 2). Further involvement of GDNF in PNI was shown by a decrease in the invasiveness of pancreatic cancer cells in response to DRGs from mice lacking GDNF[38]. ARTN also increases pancreatic cancer cell invasion. Increased expression levels of ARTN, its receptor GFRα3 and RET in pancreatic cancer cells and surrounding nerves correlates with an increased invasiveness of pancreatic cancer cells and PNI[3,7,29] (FIG. 2). NRTN and its receptor GFRα2 are weakly expressed in pancreatic cancer cells; however, RET in this context has been shown to be highly expressed[69]. Even though NRTN can influence the invasiveness of pancreatic cancer cells in an *in vitro* assay, a link to PNI has not been confirmed[69]. Similarly, although weak expression of PSPN has been reported in pancreatic cancer cells, its involvement in PNI is yet to be determined[69].

Box 1 | **Pancreatic ductal adenocarcinoma and PNI in the clinic**

Approximately 95% of pancreatic cancer cases are pancreatic ductal adenocarcinoma (PDA)[150]. With a median survival of less than 6 months, PDA is a devastating disease. Even with advances in surgery and chemotherapy, patients with PDA have a 5 year survival rate of <6%[142–144]. Standard of care for early stage (Stage I and Stage II) pancreatic cancer consists of surgical resection followed by radiation and/or chemotherapy using gemcitabine alone or in combination with other agents[142,150–157]. Despite an improvement in the rate of successful surgical resections, the outlook for patients with pancreatic cancer remains bleak: not all diagnosed cases of pancreatic cancer are amenable to surgical resection, and in those cases in which resection results in microscopically negative margins, cancer relapse is common[9]. PDA has one of the highest incidences of perineural invasion (PNI) among cancer types, and the presence of PNI in pancreatic cancers is seen by many as being a harbinger of poor prognosis, although the evidence for this is not clear-cut[28,31–36]. In a systematic review of 13 studies detailing outcomes for patients with pancreatic cancer and PNI, Garcea *et al.*[33] found that 11 out of the 13 studies recorded poorer survival for patients with PNI, seven of which achieved statistical significance ($P < 0.05$). Furthermore, a cross-study analysis showed that the median survival of patients with PNI was significantly shorter than that of those without PNI ($P < 0.001$)[33]. Conversely, two recent studies found that although patients without PNI survived longer, the difference was not statistically significant[158,159]. Additionally, pancreatic tumour biopsies have not routinely been histologically examined for the presence of PNI. However, the latest pathological examination protocol for exocrine pancreatic cancer, published by the American College of Pathologists, requires the determination of PNI status[160]. In many instances PNI is under-reported, mainly owing to the presence of alternative trustworthy prognostic factors and the lack of techniques to clearly distinguish between the nerves and the surrounding tissues. Detection of PNI in nearly 75% of Stage I pancreatic cancers suggests that PNI is an event that occurs early in the progression of the cancer[2,3,11–13]. Furthermore, the possible role of PNI in local cancer relapse after surgical resection, as well as PNI being a route for further spread of the cancer and pain generation, makes PNI an important factor when considering possible therapeutic strategies for patients[12,145,146]. Therefore, developing better techniques to distinguish between the nerves and the surrounding cancer tissues will help to readily and accurately identify PNI, which in turn could help to improve diagnosis and treatment selection[9].

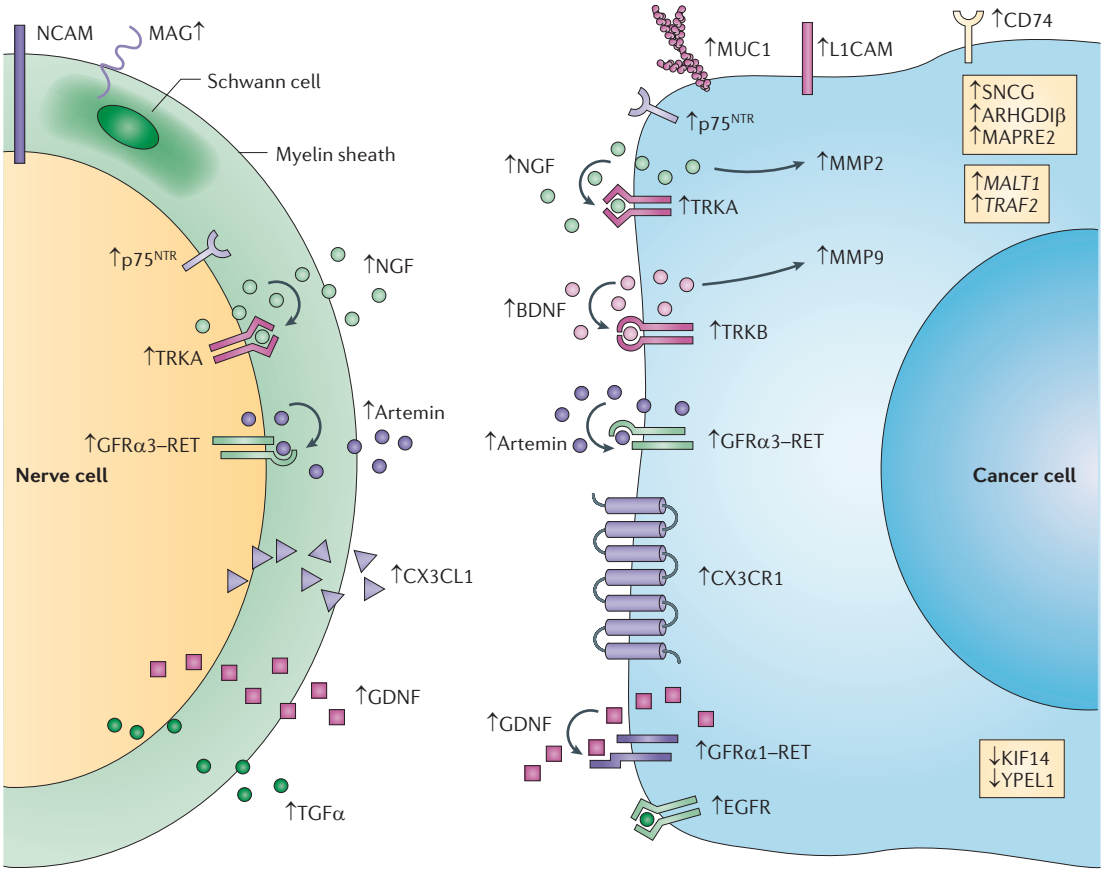

Figure 2 | **Signalling molecules involved in the process of perineural invasion in pancreatic cancer.** Perineural invasion (PNI) is a multifactorial process that involves various signalling molecules from different signalling pathways. The interactions between molecules that are expressed on the cancer cells and the peripheral nerves have an important role in PNI. These signalling molecules include secreted neurotrophins (such as nerve growth factor (NGF), which binds to tropomyosin-receptor kinase A (TRKA); and brain-derived neurotrophic factor (BDNF), which binds to TRKB). p75 neurotrophin receptor ($p75^{NTR}$) is an additional, low-affinity neurotrophin receptor. Other secreted factors are from the glial cell line-derived neurotrophic factor (GDNF) family: GDNF forms a complex with GDNF receptor-α1 (GFRα1) and the rearranged during transfection (RET) receptor tyrosine kinase, whereas the GDNF family member artemin forms a complex with GFRα3–RET. Furthermore, chemokines and their receptors (such as the CX3CL1–CX3CR1 interaction) and other cell-surface molecules — such as mucin 1 (MUC1), myelin-associated glycoprotein (MAG), neural cell adhesion molecule (NCAM), L1 cell adhesion molecule (L1CAM) and CD74 — have also been shown to be relevant for PNI. Additionally, differentially expressed genes and proteins contribute to the invasiveness of pancreatic cancer cells and to PNI. These include the genes mucosa-associated lymphoid tissue lymphoma translocation gene 1 (*MALT1*) and tumour necrosis factor receptor-associated factor 2 (*TRAF2*), and the proteins synuclein-γ (SNCG), RHO–GDP dissociation inhibitor-β (ARHGDIβ), microtubule-associated protein RP/EB family member 2 (MAPRE2), yippee-like 1 (YPEL1) and kinesin family member 14 (KIF14). This figure shows the major molecules and their interactions that are known to be involved in the process of PNI in pancreatic cancer. Upwards-directed arrows indicate an increase (and downwards-directed arrows indicate a decrease) in expression of that gene or protein in PNI, based on literature review. Semi-circular arrows represent an autocrine effect. EGFR, epidermal growth factor receptor; MMP, matrix metalloproteinase; TGFα, transforming growth factor-α.

Chemokines in PNI. The ability of chemokines and their receptors to influence the invasiveness of cancer cells has long been known, and the expression of various chemokine receptors in pancreatic cancer cells adds credence to this theory[70–72]. A possible role for the chemokine receptor CX3CR1 and its ligand CX3CL1 (also known as fractalkine) in PNI of pancreatic cancer cells was suggested by Marchesi *et al.*[43,72]. Immunohistochemical evaluation showed that high expression levels of CX 3CR1 were associated with an increased incidence of PNI in pancreatic cancer. In response to CX3CL1, the migration of CX3CR1-transfected pancreatic cancer cells was increased, and CX3CR1-expressing tumours transplanted into mice exhibited a greater perineural invasive ability[43]. Furthermore, expression of CX3CL1 in the nerves affirms the idea that PNI is a multifactorial process with the mutual involvement of pancreatic cancer cells and surrounding nerves[72,73]. CX3CL1–CX3CR1 signalling also affects cell adhesion, which might also have an effect on PNI. All of these data indicate that CX3CL1–CX3CR1 signalling could be a promising target for the development of novel therapeutics to reduce PNI[72] (FIG. 2).

Table 2 | **Molecular factors involved in perineural invasion and pain generation in pancreatic cancer**

Class of molecules	Molecules	Expression levels	Role in PNI	Refs
Cytokines and their receptors	TGFα	Increased in peripheral nerves	Growth signalling increases the affinity of PDA cells for pancreatic nerves and vice versa	5
	EGFR	Increased in PDA cells		
NGF family and their receptors	NGF	Increased in PDA cells	Autocrine signalling increases the growth and invasiveness of PDA cells. Paracrine signalling is involved in PNI and increases the affinity of PDA cells for pancreatic nerves and vice versa	6,38,40, 46,47, 59–61
	TRKA	Increased in PDA cells and peripheral nerves		
	$p75^{NTR}$	Increased in PDA cells and peripheral nerves	Unclear role in PNI. Expression is inversely correlated to PNI and prognosis in some reports yet positively correlated to PNI in other reports when it functions as a chemoattractant	40, 46,47, 64,65
	BDNF	Increased in PDA cells	Increases the proliferation and invasiveness of PDA cells	39,46
	TRKB	Increased in metastatic PDA cells	Increased levels linked to metastasis and PNI	66
	NTF3	Increased in PDA cells	Role in PNI unclear	39,46,66
	NTF4	Expressed in pancreatic ductal cells and expressed weakly in PDA cells	Role in PNI unclear	66
	TRKC	Increased in metastatic PDA cells	Role in PNI unclear	39,46,66
GDNF family and their receptors	GDNF	Increased in PDA cells and peripheral nerves	Reciprocal signalling increases the invasiveness of PDA cells towards pancreatic nerves	38,69
	GFRα1–RET	Increased in PDA cells	Involved in PNI through the GDNF signalling pathway	38,69
	Artemin	Increased in PDA cells and peripheral nerves	Increases the invasiveness of PDA cells towards pancreatic nerves	7,29
	GFRα3–RET	Increased in PDA cells	Involved in cell invasion and may have a role in PNI	7,29
	NRTN	Weak in PDA cells	Influences PDA cell invasion but a role in PNI is undetermined	69
	GFRα2	Weak in PDA cells	Undetermined role in PNI	69
	PSPN	Weak in PDA cells	Undetermined role in PNI	69
Chemokines and their receptors	CX3CL1	In peripheral nerves	Signalling increases PNI and invasiveness of PDA cells. Additional roles in cell adhesion	70–72
	CX3CR1	Increased in PDA cells		
	SEMA3A	Increased in PDA cells	Role in PNI unclear	74
	PLXNA1			
	NRP1			

In addition, expression levels of the axonal-guidance molecule semaphorin 3A (SEMA3A) and two of its receptors — plexin A1 (PLXNA1) and neuropilin 1 (NRP1) — were shown to be increased in pancreatic cancer tissues and correlated with decreased patient survival and increased invasive and metastatic potential[74]. However, despite 92% of the patients presenting with PNI, no significant correlation between PNI status and SEMA3A expression was observed[74].

Matrix metalloproteinases in PNI. Successful migration and invasion of surrounding tissues is thought to require the degradation of the surrounding extracellular matrix (ECM), a process that is controlled by proteolytic enzymes, such as matrix metalloproteinases (MMPs), several members of which have been implicated in pancreatic cancer[75,76]. The 72 kDa MMP2 (also known as gelatinase A) and the 92 kDa MMP9 (also known as gelatinase B) are both type IV collagenases, whose overexpression in pancreatic cancer influences metastasis and contributes to poor prognosis[77,78]. Increased expression and activity of MMP9 in response to GDNF in pancreatic cancer cells is thought to contribute to the increased invasiveness of pancreatic cancer cells[41,69]. Similarly, expression and activity of MMP2 is stimulated by NGF–TRKA signalling in pancreatic cancer cells and contributes to their increased invasiveness[42]. Given that both GDNF and NGF are expressed in pancreatic cancer cells as well as the surrounding nerves and are known to be involved in PNI, it seems logical to assume that MMPs are also involved in PNI in pancreatic cancer[2,41,42,79] (FIG. 2).

Differentially expressed genes that contribute to PNI. In order to better understand the molecular processes and signalling pathways that are activated in the cancer cells that migrate and invade the surrounding nerves, global expression profiling was used to investigate differences in gene expression between nerve-invading and non-invading cancer cells. In a study by Koide *et al.*[80], gene expression profiles were compared between pancreatic cancer cell lines with a high versus low frequency of PNI following subcutaneous implantation into non-obese diabetic–severe combined immunodeficient (NOD–SCID) mice. Expression of CD74, a type II transmembrane glycoprotein, was increased in pancreatic cancer cell

Table 2 (cont.) | **Molecular factors involved in perineural invasion and pain generation in pancreatic cancer**

Class of molecules	Molecules	Expression levels	Role in PNI	Refs
MMPs	MMP2	Increased in PDA cells in response to NGF signalling	NGF–TRKA signalling increases the invasiveness of PDA cells	42,75,78
	MMP9	Increased in PDA cells in response to GDNF signalling	Signalling through GDNF increases the invasiveness of PDA cells	41,69, 75,77
Differentially expressed genes and proteins	CD74	Increased in highly invasive PDA cells	Roles in the evasion of immune responses and in the invasiveness of PDA cells	80–83
	MALT1	Increased in PDA cells	Pro-survival genes that contribute to a decrease in apoptosis and an increase in neuro-invasiveness of PDA	39
	TRAF2			
	ARHGDIβ	Increased in neuro-invasive PDA cells	Increased expression correlated with invasiveness and PNI	83, 86–88
	KIF14	Downregulated in neuro-invasive PDA cells	Decreased expression correlated with invasiveness and PNI	83–85
	MAPRE2	Increased in neuro-invasive PDA cells	Increased expression correlated with invasiveness and PNI	83
	YPEL1	Deregulated in PDA cells	May have a role in PNI	83
	Synuclein-γ	Increased in PDA cells	Increased expression correlated with PNI	89–91
Other cell-surface molecules and receptors	MUC1	Increased and aberrantly glycosylated in PDA cells	Signalling results in increased proliferation and contributes to PNI and metastatic behaviour	92
	MAG	Increased in Schwann cells and peripheral nerves		
	NCAM	Polysialic acid-linked NCAM in peripheral nerves	Inhibits cell–cell adhesion, increases metastatic and invasive behaviour and may be implicated in PNI	93–95
	L1CAM	Increased in pancreatic cancer	Positively correlated to PNI and poor patient prognosis	96

ARHGDIβ, RHO–GDP dissociation inhibitor-β; BDNF, brain-derived neurotrophic factor; EGFR, epidermal growth factor receptor; GDNF, glial cell line-derived neurotrophic factor; GFRα, GDNF receptor-α; KIF14, kinesin family member 14; L1CAM, L1 cell adhesion molecule; MAG, myelin-associated glycoprotein; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; MAPRE2, microtubule-associated protein RP/EB family member 2; MMP, matrix metalloproteinase; MUC1, mucin 1; NCAM, neural cell adhesion molecule; NGF, nerve growth factor; NRP1, neuropilin 1; NRTN, neurturin; NTF, neurotrophin; $p75^{NTR}$, p75 neurotrophin receptor; PDA, pancreatic ductal adenocarcinoma; PLXNA1, plexin A1; PNI, perineural invasion; PSPN, persephin; RET, rearranged during transfection; SEMA3A, semaphorin 3A; TGFα, transforming growth factor-α; TRAF2, tumour necrosis factor receptor-associated factor 2; TRK, tropomyosin-receptor kinase; YPEL1, yippee-like 1.

lines with a high frequency of PNI, as opposed to those with a lower frequency of PNI[80]. CD74 is the γ-chain of the human leukocyte antigen DR (HLA-DR), a major histocompatibility complex class II (MHC II) receptor[80–82]. CD74 is overexpressed in PDAs and associates with the α- and β-chains of HLA-DR. This association leads to suppression of the host immune response that is directed against cancer cells[81]. Additionally, a role for CD74 as a receptor mediating survival signals has been suggested[80–82]. Thus, not only is the increased expression of CD74 associated with increased cancer invasion and PNI, it may also allow cancer cells to escape the immune response (FIG. 2).

Using the DRG co-culture model, Dai *et al.*[37] demonstrated that MiaPaCa2 pancreatic cancer cells selectively migrate towards nerves and have an increased proliferation rate as indicated by the proliferation marker Ki-67. Gene-expression profiling showed an increased expression of two pro-survival genes, mucosa-associated lymphoid tissue lymphoma translocation gene 1 (*MALT1*) and tumour necrosis factor receptor-associated factor 2 (*TRAF2*) in co-cultured MiaPaCa2 cells, compared with the MiaPaCa2-only control[37]. Thus, increased expression of pro-survival genes, increased proliferation and decreased apoptosis seem to contribute to the neuro-invasive ability of pancreatic cancer cells[37] (FIG. 2).

Abiatari *et al.*[83] used an *ex vivo* model of PNI, in which rat vagal nerves were placed in specialized nerve-invasion chambers with pancreatic cancer cell lines, such that the only passage for the pancreatic cancer cells to the tissue culture plate below was thorough the nerves. Any invasive cancer cells appearing on the tissue culture plate were grown as nerve-invasive clones of the parent pancreatic cancer cell lines. Genome-wide transcriptome analyses identified several differentially expressed genes between the highly nerve-invasive and non-invasive cancer cell clones[83]. The kinesin family member 14 (KIF14) was one of the most significantly downregulated genes in the highly neuro-invasive cells. KIF14 is a mitotic kinesin that has been linked to poor prognosis in various malignances[84]. Knockdown of KIF14 disrupted cytokinesis and the cell cycle and caused the cancer cells to undergo apoptosis[83–85]. Although KIF14 was upregulated at the mRNA level in bulk tissue samples of pancreatic cancer and pancreatitis compared to normal pancreatic tissues, further gene expression analyses of microdissected pancreatic cancer tissue showed low expression of KIF14 in neuro-invasive cells as opposed to non-invasive pancreatic cancer cells. Downregulation of KIF14 using small interfering RNA (siRNA) increased the invasiveness of previously non-invasive pancreatic cancer cells[83]. These results prompted the authors to propose an anti-invasive role for KIF14 in pancreatic

cancer[83]. It is possible that increased expression of KIF14 in the bulk of the cancer cells results from a negative feedback loop that negates the invasiveness of the tumour cells[83].

RHO–GDP dissociation inhibitor-β (ARHGDIβ) was also upregulated in the highly nerve-invasive pancreatic cancer cells compared to the less-invasive cells[83]. ARHGDIβ prevents the dissociation of GDP from the GTPases, thereby keeping them in their inactive states[86]. Knockdown of ARHGDIβ expression in pancreatic cancer cells did not affect the survival or invasiveness of these cells, but did diminish their perineural invasive ability[83]. Increased expression of ARHGDIβ in breast cancers has been linked to chemotherapeutic resistance, and downregulation of its expression resulted in decreased growth and invasiveness of the breast cancer cells[87,88]. ARHGDIβ was also independently identified by another group using a different model of PNI in pancreatic cancer, further confirming its importance[80] (FIG. 2).

Synuclein-γ, which is expressed in the peripheral nervous system, has been found to be overexpressed in metastatic and infiltrating breast cancer cells[89]. Increased expression of synuclein-γ has also been seen in pancreatic cancer cell lines and correlates with PNI and lymph node invasion in patients with pancreatic cancer[89,90]. Hibi *et al.*[90] showed that synuclein-γ expression was a strong indicator of reduced disease-free survival and a poorer overall prognosis in patients, in addition to being significantly correlated with PNI in an *in vivo* mouse model of PNI. Furthermore, downregulation of synuclein-γ using short hairpin RNA (shRNA) sequences reduced the extent of PNI and liver metastasis. Synuclein-γ can associate with centrosomes and spindles during mitosis and, through its chaperone function, can increase the expression of MAPK. This leads to increased MAPK signalling, which is also downstream of the NGF–TRKA pro-survival signalling pathway that is implicated in PNI[90,91]. Synuclein-γ may additionally contribute to the process of PNI and tumour progression by upregulating the expression of MMPs through MAPK[90,91]. Synuclein-γ can be detected in serum and urine samples from patients with pancreatic cancer and thus has the potential to become a biomarker for this disease[89,90]. The involvement of synuclein-γ in multiple facets that contribute to PNI and the metastatic spread of pancreatic cancer makes it a promising target for the development of new therapeutics[90,91] (FIG. 2).

Other cell-surface molecules in PNI. Nerve-invading pancreatic cancer cells are known to associate with Schwann cells through cell-surface proteins[2,5,8,9]. For example, mucin 1 (MUC1), a type I transmembrane protein, is overexpressed in pancreatic cancer cells and has an anomalously glycosylated extracellular domain. This domain acts as a specific ligand for myelin-associated glycoprotein (MAG), a membrane glycoprotein that is predominantly expressed by Schwann cells in the peripheral nerves[92]. In a recent study, Swanson *et al.*[45] showed that an increased interaction between MUC1 on pancreatic cancer cells and MAG on the Schwann cells correlated with PNI. In addition to acting as a preferred ligand for MAG, differential phosphorylation of the cytoplasmic tail of MUC1 in pancreatic cancer cells leads to the activation of signalling pathways that are involved in proliferation and metastasis[44]. Therefore, the MUC1–MAG signalling pathway not only contributes to increased invasiveness and proliferation of pancreatic cancer cells but may also have a role in PNI by aiding the association of pancreatic cancer cells with the Schwann cells[44,45] (FIG. 2).

Two cell adhesion molecules, neural cell adhesion molecule (NCAM) and L1 cell adhesion molecule (L1CAM), have also been investigated in pancreatic cancer in the context of PNI. NCAM is post-translationally modified by polysialic acid, which inhibits the ability of NCAM to mediate cell–cell adhesion through cadherins. Increased expression of polysialic acid-linked NCAM has been associated with PNI in pancreatic cancer[93,94]. However, the expression level of NCAM itself in pancreatic tumours seems to correlate with better patient survival[95]. Therefore, it is likely that the polysialylation of NCAM plays a part in the PNI and metastasis[93,94]. L1CAM is required for normal neuronal functions, such as migration and adhesion[96]. Ben *et al.*[96] reported that L1CAM and GDNF were overexpressed in PDA: these molecules are positively correlated with PNI and poor prognosis[6,38,69,96] (FIG. 2). Currently, functional studies on both molecules in PNI are still lacking.

PNI and pain generation in PDA

A prominent consequence of PNI in PDA is the generation of pain[29,97]. Many patients with pancreatic cancer complain of abdominal or back pain at the site of the cancer. The use of traditional pain medications, such as analgesics, opiates and non-steroidal anti-inflammatory drugs (NSAIDs), is somewhat effective in the treatment of pancreatic cancer pain. Unfortunately, they do not always work, and the severe and detrimental side effects can affect patients' quality of life[98,99]. The use of neurolytic blocks as a means to relieve cancer-related pain have been occasionally effective; however, these procedures can have many undesirable side effects[100]. For example, coeliac plexus blocks, which are used to alleviate the pain that is associated with pancreatic cancer[100], have several side effects and complications in addition to being technically challenging and relatively short lived[100–103]. Approaches that specifically target PNI and pain can potentially overcome these drawbacks and provide improved treatment outcome.

The reasons for pain generation in patients with pancreatic cancer are thought to be many-fold. For example, invading cancer cells damage the neuronal sheath, leaving the nerve processes vulnerable to noxious stimuli from the ECM, and signalling between pancreatic cancer cells and nerves leads to the accelerated growth of pancreatic cancer cells and the growth and enlargement of nerves[10,104]. Furthermore, invasion of the perineurial space may promote the growth of cancer cells, thereby contributing to their metastatic spread and the resultant pain[104]. Vascularization of the tumour, coupled with the growth of new nerve fibres as the tumour progresses, also contributes to pain generation[104,105]. One of the reasons that has been postulated for this correlation is

Neurolytic blocks
A process in which a neurolytic or analgesic agent is injected into or near nerves that are involved in pain signalling. Neurolytic blocks are used to combat chronic pain states or pain that is caused by cancer.

Coeliac plexus blocks
A process in which the nerves of the coeliac plexus are subjected to neurolysis using neurolytic agents, such as a 50–100% solution of alcohol or a 10% solution of phenol, that are injected into the coeliac plexus guided by ultrasound and computed tomography (CT) imaging.

that molecular factors — such as vascular endothelial growth factor (VEGF), ARTN, interleukin-1, ephrin B2 and prostaglandins — influence the growth of new blood capillaries and nerve fibres.

Although various neurotrophins and their receptors have been shown to promote pancreatic cancer growth and neural invasion, functional and molecular analyses of their role in pain generation in pancreatic cancer are sparse. One of the widely studied pathways that is related to pain generation is the NGF signalling pathway[40]. It has been proposed that NGF, which is secreted by tumour-associated immune cells and fibroblasts, can directly activate and sensitize sensory nerves that are in close proximity to the pancreatic cancer by interacting with TRKA and/or $p75^{NTR}$, both of which are expressed on the perineurium. This is thought to contribute to pain generation through a process called neurogenic inflammation[10,104] (FIG. 3). Recently, using a mouse model of pancreatitis, Zhu *et al.*[106] showed that NGF can modulate the expression and function of transient receptor potential cation channel, subfamily V, member 1 (TRPV1), a non-selective cation channel that stimulates sensory neurons on its activation. TRPV1 is expressed in the central nervous system and in sensory ganglia, and is involved in mediating pain responses[107,108]. TRPV1 is overexpressed in pancreatic cancers, colocalizes with TRKA and correlates with severe pain in pancreatic cancer patients[10,109]. TRPV1 is activated by chemicals such as capsaicin, a component of chilli peppers, or by thermal and mechanical stimuli, resulting in pain signalling[107,108,110]. In response to capsaicin, TRPV1 functions as an ion channel for Na^+ and Ca^{2+}, leading to neuronal depolarization and the release of pain-related neurotransmitters such as calcitonin gene-related peptide (CGRP) and substance P, which in turn signal pain through the central nervous system[109,111,112]. Similarly, GDNF signalling also increases the expression

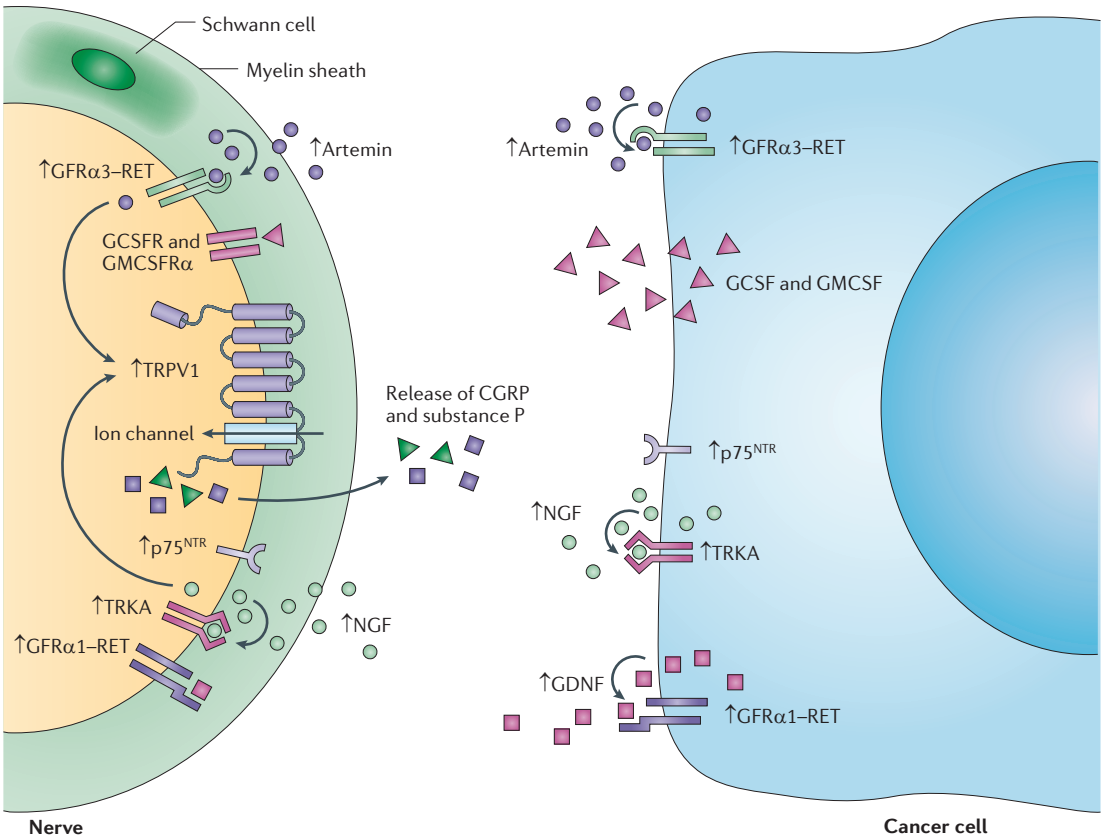

Figure 3 | **Signalling molecules involved in pain generation in pancreatic cancer.** The presence of pain is a mainstay in pancreatic cancer and influences patient prognosis and quality of life. The incidence of pain is higher in those cancers that also present with perineural invasion (PNI), and some of the molecular mechanisms that are responsible for PNI have also been implicated in pain generation in pancreatic cancer. This figure shows the major molecules and interactions that are known to be involved in the process of pain generation in pancreatic cancer. These include the secreted factors nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), artemin, granulocyte colony stimulating factor (GCSF) and granulocyte–macrophage colony stimulating factor (GMCSF), and their respective receptors tropomyosin-receptor kinase A (TRKA) and p75 neurotrophin receptor ($p75^{NTR}$), the GDNF receptor-α1 (GFRα1)–rearranged during transfection (RET) complex (GFRα1–RET), GFRα3–RET, GCSF receptor (GCSFR) and GMCSF receptor-α (GMCSFRα). Also shown is transient receptor potential cation channel, subfamily V, member 1 (TRPV1), which functions as an ion channel for Na^+ and Ca^{2+}, leading to neuronal depolarization and the release of the pain-related neurotransmitters (calcitonin gene-related peptide (CGRP) and substance P) that signal pain through the central nervous system. Upwards-directed arrows indicate an increase in the expression of the stated protein in pancreatic cancer based on literature review. Semi-circular arrows represent an autocrine effect. .

of TRPV1 and induces capsaicin-related responses through TRPV1 in isolated neurons to generate pain[113,114]. Increased expression of ARTN in pancreatic cancer cells and Schwann cells also increases the expression of TRPV1 and its capsaicin-associated response[7,10,114]. Furthermore, colocalization of the ARTN receptor, GFRα3, with TRPV1 in neurons perhaps also increases TRPV1 function and pain signalling[7,10,114]. Thus, a multifactorial role for neurotrophins in PNI and pain generation makes them favourable targets for reducing PNI and the associated pain[10,104,105] (FIG. 3).

In a report by Schweizerhof *et al.*[115], increased expression of granulocyte colony stimulating factor (GCSF) and granulocyte–macrophage colony stimulating factor (GMCSF) was seen in human PDA samples and increased expression of their receptors, GCSFR and GMCSFRα, was found in the surrounding pancreatic nerves. Interestingly, overexpression of GCSF and GMCSF and their receptors coincided with increased expression of CGRP in the nerves, which is known to contribute to neurogenic inflammation and pain[115]. Furthermore, signalling through the GCSF–GCSFR and GMCSF–GMCSFRα pathways between nerves and PDA cells led to an increased release of CGRP in nerves in response to capsaicin and was shown to contribute to pain generation in a mouse model of bone cancer pain[115]. The authors also determined that GCSF and GMCSF signalled through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, as inhibiting this pathway reduced pain[115]. Further evidence of the involvement of the GCSF and GMCSF signalling pathways in pain came when the sprouting of peripheral nerves, and the severity of thermal hyperalgesia and mechanical hyperalgesia, was reduced following treatment with either neutralizing antibodies or shRNAs targeting these pathways[115]. Thus, the involvement of PDA-derived haematopoietic signalling molecules can be exploited to develop novel therapeutics to target the pain that is directly related to cancer, as well as pain that is a substantial side effect in various other diseases[115,116] (FIG. 3).

Therapeutic strategies to combat PNI and pain

Considering the clinical consequences (that is, metastatic spread and pain) that are associated with PNI in pancreatic cancer, targeting PNI presents a potentially attractive therapeutic approach for patients with pancreatic cancer[28,31–36]. Therapeutic strategies that target PNI could conceivably not only arrest the spread of cancer cells but also alleviate pain and improve the quality of life of patients[28,31–36]. Although not all of the strategies or agents discussed below are currently being evaluated in pancreatic cancer models or patients with pancreatic cancer, it is our hope that they soon will be, owing to the involvement of their intended targets in pancreatic cancer.

To date, the NGF–TRKA signalling pathway has been the most studied and is the most promising for therapeutic intervention. One common strategy in targeting this pathway is the inhibition of NGF using neutralizing antibodies to prevent its interaction with TRKA[117–121]. A mouse monoclonal NGF-targeted antibody, muMab911, greatly reduces bone cancer-related pain in mouse models[118,120]. Tanezumab (also known as RN624) is a humanized version of muMab911 and is currently in Phase II clinical trials. These include trials for relieving pain in patients with osteoarthritis and to treat the severe pain that is associated with bone metastases in patients with prostate cancer, breast cancer, multiple myeloma or other cancers (for example, ClinicalTrials.gov identifier NCT00545129)[119,121]. In addition to tanezumab, other humanized neutralizing antibodies against NGF are being developed for the treatment of pain[120]. Similarly, MNAC13, a selective and high-affinity antibody against the TRKA receptor, prevents the binding of NGF to TRKA in both *in vitro* and *in vivo* systems[122,123]. MNAC13 has been shown to function synergistically with opiates in alleviating pain in a mouse model of neuropathic pain and inflammation[120,122–124]. Furthermore, as the activation of survival signalling by TRKA is facilitated by NGF binding exclusively to the d5 extracellular domain of TRKA, recombinant TRKAd5 protein can be used to sequester NGF and thus prevent subsequent downstream pain signalling[125]. In fact, a mature recombinant TRKAd5 can attenuate pain signalling in a pancreatitis and interstitial cystitis model and in several *in vivo* inflammation models[120,126,127]. Additionally, sequestration of NGF by TRKAd5 was shown to reduce neurite extension in PC12 cells[126]. A fusion protein of the extracellular domain of TRKA and the Fc tail of IgG immunoglobulin was constructed as another way to capture and segregate NGF, and this molecule can capture NGF with picomolar affinity. Although this fusion protein successfully reduced pain and thermal and mechanical hyperalgesia, the large size of the fusion protein and the possibility of an immune response as a result of the modified TRKA–IgG domains may limit its clinical use[118,120]. NGF peptibodies, which sequester NGF, were also shown to reduce highly sensitized pain states such as tactile allodynia and thermal hyperalgesia in rats[128]. However, a short half-life and the possibility of triggering an immune response could limit its clinical use[118,120,128].

Given the role of TRKA in pro-survival signalling, various small-molecule inhibitors of the kinase activity of TRKA have been developed as anticancer agents, several of which have progressed into clinical trials (reviewed in REF. 129). These agents could also be potentially effective in inhibiting PNI and reducing pain in pancreatic cancer[130]. For example, a pan-TRK inhibitor, ARRY-470, has been shown to profoundly reduce bone cancer pain and to reduce the cancer pain-associated remodelling of nerve fibres in a mouse model of sarcoma[131]. Additionally, PHA-848125, a dual inhibitor of cyclin-dependent kinases (CDKs) and TRKA, has been shown to be effective at inhibiting tumour growth in pancreatic xenograft models and is currently in multiple Phase I and Phase II clinical trials[132–135]. PHA-848125 is potentially able to target PNI and reduce pain generation in the treatment of pancreatic cancer patients[136]. These findings suggest that NGF–TRKA-targeting agents may not only have a direct effect on tumour growth but could also inhibit PNI and thus alleviate the associated pain in pancreatic cancer.

The development of TRPV1 antagonists has also shown promise in the treatment of pain. Several TRPV1

Thermal hyperalgesia
Increased pain responses following an increase in temperature.

Tactile allodynia
Pain sensations caused by mechanical stimuli such as touch that usually do not invoke pain responses.

antagonists are in clinical development for the treatment of various pain states, including pancreatic cancer-associated pain (reviewed in REFS 137,138). One such compound, resiniferatoxin (RTX), which is an analogue of capsaicin, has been shown to induce apoptosis in pancreatic cancer cells and may have potential in controlling the pain that is generated as a result of pancreatic cancer[109]. Furthermore, Ghilardi *et al.*[139] demonstrated that an antagonist of TRPV1 reduces pain in a bone cancer model of pain. Thus, targeting TRPV1 could provide another avenue of new treatments for PNI and associated pain in pancreatic cancer.

Recently Dorgham *et al.*[140] showed that an antagonist of CX3CR1 reduces the pain that is associated with various inflammatory processes. Furthermore, administration of a neutralizing antibody to CX3CR1 attenuated the pain that is associated with cancer in a rat model of bone cancer pain[141]. Thus, considering its important role in the PNI of pancreatic cancer, the CX3CL1–CX3CR1 signalling axis presents another promising target for developing new therapeutics for pancreatic cancer.

Concluding remarks

Despite some advances in the diagnosis and treatment modalities, pancreatic cancer is still a deadly disease[142–144]. Many lines of evidence indicate that PNI is associated with the high incidence of disease relapse and the poor survival of patients with pancreatic cancer[11,12,28,31–35,145,146]. PNI is also closely associated with pain generation in patients with pancreatic cancer. Thus, targeting PNI presents a potentially attractive therapeutic approach for pancreatic cancer. However, it is not clear at this point whether or not such therapeutics will have a positive effect on patient survival. Furthermore, although targeting a single signalling pathway, such as the NGF pathway, may reduce PNI and pain generation, targeting multiple signalling pathways — those that are involved in PNI, in addition to those that underlie other tumour growth and metastasis processes — may be needed to improve the treatment outcome of patients with pancreatic cancer, including their quality of life and survival. Therefore, a better understanding of the mechanisms that are involved in PNI, and the role of PNI in pancreatic cancer progression, is essential.

Much of the current research on PNI has focused on the interaction between cancer cells and nerves. Very few studies have examined the roles of tumour stromal elements — including pancreatic stellate cells, fibroblasts, infiltrating immune cells and ECM proteins — in the PNI process. Two recent reports described the effects of pancreatic stellate cells and the associated fibrotic reaction on neuroplasticity and pancreatic neuropathy in pancreatic tumours[97,147]. However, it is not clear how this relates to PNI. Clearly, further studies are needed to explore this aspect of PNI in pancreatic cancer. Furthermore, improved animal models that closely recapitulate the development of PNI and the pain that is associated with PNI are needed. There have been few studies on the functional analysis of pain generation and maintenance in pancreatic cancer, presumably owing to the lack of suitable animal models. Recently, genetically engineered mouse models (GEMMs) that recapitulate the full range of human PDA subtypes have been developed, and PNI has been noted in some of these models[148,149]. Further characterization of PNI in these GEMMs and the development of other new models will be of great value. Such models will help to advance our understanding of the biology of PNI and to facilitate the evaluation of new targeted agents against PNI and pain in pancreatic cancer and other cancers, thereby expediting the translation of new findings into the clinic.

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DATABASES
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