Review Article
Neural invasion: a scenic trail for the nervous tumor and hidden therapeutic opportunity

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Abstract: Neural invasion (NI) is the invasion of cancer cells into nerves, influencing the pathological characteristics of malignant tumors. NI promotes metastasis and is associated with reduced survival of affected patients. Although known for decades, its prognostic and therapeutic implications have not been not much appreciated due to the scattered information available on its clinical complications. The use of multiple nomenclatures to describe NI also generated confusions among researchers to understand this pathological process. Here, we discuss the multiple classifications of NI and review its clinical complications. Recent findings of the regulatory roles of nerves on tumor growth have fuelled research in this field, and there has been several attempts to molecularly define the NI interface and the cancer cells involved. Therefore, in this review, we discuss the large datasets available to characterize the cancer cells in NI and also discuss the roles of Schwann cells and macrophages participating in NI.

Keywords: Neural invasion, perineural invasion, intraneural invasion, endoneurium, tumor, nerves

Introduction

Neural invasion (NI) is the invasion of cancer cells into and around nerves [1]. Although first reported in the early 19th century, NI's prognostic value was unrecognized over many decades due to a lack of established guidelines to assess it quantitatively. It was earlier thought that the nerves serve only as physical hosts to cancer cells in NI. However, recent studies revealed that nerves indeed promote tumor dissemination, which paves the way for revisiting neural milieu as much more than mere physical cues to cancer cells [2, 3].

The physical orientation of NI is better understood by understanding the structural organization of peripheral nerves. The nerve architecture involves multiple layers; the inner *endoneurium*, mid *perineurium*, and outer *epineurium*. *Endoneurium* accommodates an axon, supporting Schwann cells (SCs) and myelin sheath. A collection of *endoneurial* units is called a fascicle, which is enclosed in the *perineurium*. The outermost connective tissue lay-er enclosing multiple *perineuria* is called the *epineurium*. Classically, invasion of cancer cells into the *perineurial* space is known as perineural invasion (PNI). However, since the prefix 'peri' means 'around', many researchers considered invasion of cancer cells around nerves also as PNI. Accounting this fact, Liebig et al. defined PNI as the presence of tumor cells in any layer of the nerve sheath and/or in close proximity to the nerve involving more than one-third of its circumference [1]. This broader definition often limits delineating the independent contribution of cancer cells that either invade the layers or invade around the nerves on tumor prognosis. An emerging consensus defines the process of cancer cells invading the outer surfaces of nerves as perineural tumor spread (PNTS) while their invasion into the interior as PNI [4]. Some researchers again subclassify PNI into cancer cells strictly occupying the *perineurial* space as PNI, whereas their occupancy at the *endoneurium* or fascicles as intraneural invasion (INinv). Since INinv alone is a rare event and mostly accompanied by *perineurial* invasion, in this review, we refer to the nerve
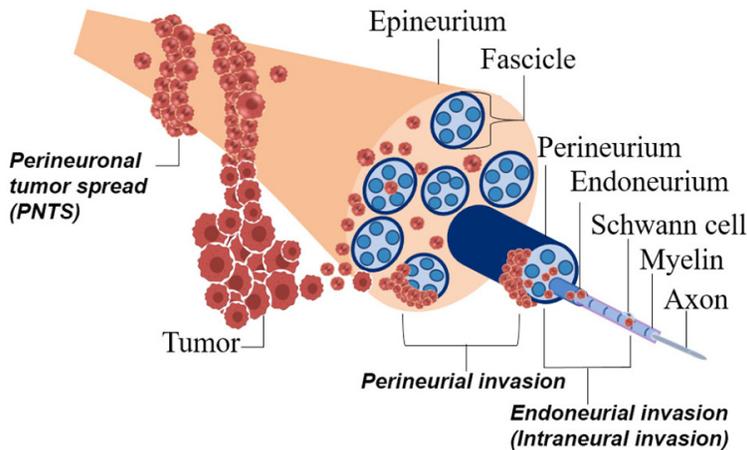
Figure 1. Schematic representation of neural invasion and the multiple terminologies involved.

interior and exterior invasion of cancer cells as PNI and PNTS, respectively. Whenever both are referred together, the term 'NI' is used. The physical orientation of NI and the multiple nomenclatures used to describe it are summarized in **Figure 1**.

The PNTS and PNI follow a distinct course of progression due to their characteristic physical orientation. Their effect on tumor prognosis may also be different. Regardless, they both occur at the intra and extra-tumoral sites. PNTS can be diagnosed using MRI and PET/CT imaging, whereas PNI is a histological finding [4, 5]. The histological description of PNI involves cancer cells orienting as full circular structures (onion bulb-like or circular) covering the entire perineurium, half-circular structures (crescent-like) partially covering the perineurium, or distributing into the endoneurium (rarely), while PNTS appears as tumor cells sandwiching the entire nerve from both sides or spreading between nerves [6]. Thus, in PNTS, tumor cells exploit nerve skin as an adherent migratory matrix, which could be visible during surgical resection whereas PNI progresses through coordinated cellular and molecular signaling between nerves and cancer cells. PNI may not be visible to surgeons during surgical resection raising the risk of missing surgical margins. Here we review the clinical complications of NI with attention to the individual class, wherever such information is available. We also discuss unique characteristics of neurotropic cancer cells based on data available from high-content transcriptomics and proteomics studies. Fi-

nally, we discuss the critical roles of SCs and macrophages in facilitating NI.

Clinical complications of NI

NI in sensory and motor dysfunctions

Although NI has been recognized in cancers over several decades, its effect on neural functions has not been studied in great detail. Nonetheless, it has been noted that patients with NI develop neurological symptoms, such as formication, pain, paraesthesia, and motor deficits, indicating that NI-mediated neuronal dysfunctions have varying presentations and magnitude [7]. Rodent experiments also demonstrated that PNI induces motor deficits. Gil et al. showed that nude mice developed hind limb paralysis when sciatic nerve is invaded by pancreatic cancer cells. However, in this study, the authors implanted the cancer cells directly into the nerve, allowing direct entry of a high concentration of cells into both *perineurium* and *endoneurium*, representing severe cases of NI [8]. Nevertheless, the study demonstrated that PNI could induce severe neuro-deficits.

Cancer pain involves nociceptive and neuropathic components [9]. NI contributes to neuropathic pain in cancers [10]. A direct association between NI and otalgia was observed by Hechler et al. in tongue cancer patients. Among 50 patients that developed otalgia in their study, 56% had NI, whereas NI was absent in 63% of patients that did not complain of otalgia [11]. A clear association between pancreatic cancer pain and NI has also been demonstrated. It was found that pancreatic cancer patients complained of severe pain had higher NI scores than those with mild or no pain [10]. The impact of NI in pancreatic cancer pain is reviewed in great detail by Bapat et al. [12]. There may be multiple mechanisms accountable for NI-induced pain. Degeneration of nerves from tumor mediated crush injuries may sensitize axons to mechanical thresholds [9]. Pain may also originate from nerves that regenerate to counteract fiber losses from crush injuries. Intact nerves also regrow at the nerve-tumor

interface to feed tumors [13]. The axonal sprouts thus generated may share molecular signals with invading cancer cells. For example, cancer cells and SCs secrete nerve growth factor (NGF). The NGF then acts on TRKA and p75NTR receptors on growing neurons to generate pain [12]. NGF also acts on the transient receptor potential cation channel, subfamily V, member 1 (TRPV1), which also contributes to cancer pain [14, 15].

NI occurs at early stages of several cancers, but whether nerve dysfunctions develop at the early stages is unknown. Theoretically, neuronal dysfunctions may result from *endoneurial* invasion due to direct encounter of cancer cells with conducting fibers (axons) occupied within the *endoneurium*. Alternatively, a growing tumor that participates in *perineurial* invasion or PNTS can crush myelin and underlying fibers to induce neuronal abnormalities. In any case, if neuro-deficit symptoms develop at early stages of cancer, it can be exploited for diagnostic purposes. However, it may require a special attention of clinicians to capture the overlapping symptoms, which are otherwise involved with well-defined neurodegenerative conditions, to suspect a cancer diagnosis early-on based on neuronal dysfunction. In most cases, milder presentations of these symptoms may be missed, but revisiting clinical guidelines to consider PNI in early diagnostic measurements may be worth considering.

NI in disease recurrence

Several studies showed that NI is an independent risk factor for the recurrence of head and neck [16, 17], pancreatic [18], prostate [19], vulvar [20], and colorectal cancers [21]. Indeed, NI induced recurrence was initially well-documented in head and neck cancers. Ballantyne et al. reported that NI occurs at the early stages of head and neck cancers, and hence early intervention may prevent recurrence [22]. The authors presented multiple cases of PNTS and PNI, but detailed descriptions of the cases indicated that PNI was more frequently associated with disease recurrence. For example, a 57-year-old cylindroma patient, that recurred later, showed islands of cancer cells in the inferior alveolar nerve. Similarly, a patient with skin carcinoma at the infra-orbital region recurred within months after surgical treatment, which involved PNI of the infra-orbital nerve and sub-

sequent cancer cell containment in the gasserian ganglion [22]. Similarly, in a small number of head and neck cancer patients, Teymoortash et al. found that six out of nine PNI positive cases had recurrence compared to no recurrence in PNI negative cases [6]. On the other hand, Chinn et al. observed that 79% of the recurrent head and neck cases in their study had PNTS involving small-diameter nerves, indicating that PNTS is also critical as PNI in promoting recurrence [17].

NI occurs at the early stages of prostate cancer too [19]. Because of its early occurrence, some researchers considered additional indices, such as PNI diameter and overall diameter of the involved nerves for prognostic measurements. In line with this, Maru et al. noted that PNI diameter of >0.25 mm adversely affects the progression-free survival [19]. Fagan et al. supports the idea that the involvement of small-diameter nerves is critical for recurrence. For example, 23% of their NI positive head and neck cases that recurred involved small-diameter nerves (<1 mm) [16]. Substantiating the involvement of small-diameter nerves, Chinn et al. showed that NI, especially PNTS of small-diameter nerves, is a risk factor for head and neck cancer recurrence [17].

PNI location, whether it is *perineurial* or *endoneurial* (INinv), also plays a critical role in determining cancer recurrence. It was noted that PNI positive pancreatic cancer cases have less median disease-free survival (DFS) and overall survival (OS) compared to PNI negative cases. Strikingly though, INinv was found to be more detrimental than *perineurial* invasion. In a study by Chatterjee et al. in pancreatic cancer, 94.3% of INinv cases showed recurrence with a mean DFS and OS of 13.4 and 28.1 months compared to only 71.6% cases of recurrence noted after *perineurial* invasion, which show a higher median DFS and OS of 32.9 and 41.2 months [23].

NI in metastasis

Peripheral nerves communicate with multiple tissues in the body, as do blood and lymphatic capillaries. Hence, it is conceivable that nerves could serve as a third channel for the dissemination of cancers, along with blood capillaries and lymphatics, by offering a smooth trail for cancer cells [24]. Pooled evidence that demon-

strate NI as a risk factor for nodal/distant metastasis in head and neck [16, 25], pancreatic [23], prostate [19], esophageal [26], cervical [27], and penile cancers [28] indicates that this is true.

NI has demonstrated to have a profound effect on nodal metastasis in head and neck cancers. Fagan et al. demonstrated that 73% of their NI positive head and neck cases accompanied nodal metastasis compared to only 43% of such incidence noted in NI negative cases [16]. Similarly, Shen et al. showed that NI promotes cancer cell invasiveness and nodal metastasis in tongue cancers. The authors found that NGF is involved with the occurrence of both PNTS and PNI, indicating that both these events use similar molecular machinery for facilitating local and regional spread of tongue cancer [29].

In a comparative analysis, Barbetta et al. showed that NI is more critical than tumor size and location for nodal metastasis of esophageal adenocarcinoma. However, the authors did not advocate NI as an independent risk factor [26]. Similarly, a definite association between PNI and nodal metastasis was shown in pancreatic cancers by demonstrating a higher incidence of nodal metastases (>80%) in PNI$^+$ cases [23]. PNI also facilitates distant metastasis. For example, PNI in inferior alveolar nerve in a head and neck cancer patient had shown to develop pulmonary metastasis [22].

Nerve diameter is also a critical determinant to promoting metastasis. It is demonstrated that increased PNI diameter (>0.5 mm) is a poor prognostic indicator for nodal metastasis in prostate cancer [19]. Fagan et al. noted that PNI in nerves of <1 mm diameter in head and neck cancers promoted cervical metastasis [16]. NI density, defined as the number of NI foci per tissue, also determines metastasis. It was shown that NI with >1 foci density has a 19 fold increased risk of spreading tongue carcinoma. However, the authors of the study noted that small-caliber nerves (<0.5 mm) are critical for facilitating metastasis, and both PNTS and PNI are involved [25].

Due to the frequent association of NI with nodal metastasis, it was believed that lymphatic vessels drain cancer cells into the *perineurium*. However, this concept was ruled out later due to the fact that the *perineurium* lacks lymphat-

ics. Then there were curiosities about the order of events, i.e., whether NI facilitates nodal metastasis. An interesting study by Kayahara et al., in a small number of pancreatic tumor samples, demonstrated that this is true. The authors showed that cancer cells in the *perineurium* follow nerve branches and eventually access the hilum of the lymph node to facilitate nodal metastasis [30].

The clinical complications of NI are summarized in **Table 1**. Overall, it is beyond doubt that NI is a risk factor for cancer progression and serves as a poor prognostic marker. Therefore, understanding the mechanisms underlying NI is of paramount interest to researchers at present to eliminate this event and successfully manage solid tumors.

Unique features of nerve-invaded cancer cells

It is noteworthy that the cancer cells participating in NI acquire survival advantage. It was shown that the PNI-competent cancer cells have a relatively lower apoptotic index compared to their counterparts [31]. Therefore, understanding the unique characteristics of NI-competent cancer cells deserve merit. Below, we discuss the unique characteristics of cancer cells and the nerve segment that participate in NI.

The general molecular mediators of NI are shown in **Figure 2**. In addition, high-content transcriptomics and proteomics studies from several research groups gather profound insight into the molecular properties of NI-competent cancer cells. For instance, Abiatari et al. used a microarray platform. The authors allowed pancreatic cancer cells to migrate through isolated cervical nerves ex vivo, and then the cells were collected from the other end of the nerves for the microarray analysis [32]. They found a differential expression of 680 mRNAs in PNI-competent cells and identified KIF14 (a kinesin family member) and ARHGDI β (Rho-GDP dissociation inhibitor) as an inhibitor and promoter of PNI, respectively. The authors also found downregulation of KIF14 and upregulation of ARHGDI β in PNI positive pancreatic tumors, confirming the regulatory roles of these proteins in PNI [32]. Similarly, Koide et al. selected PNI-competent cells based on their ability to invade subcutaneously implanted nerves in mouse. Microarray showed

Table 1. Clinical complications of neural invasion

| Cancer	Overall/Disease free/Disease specific survival	Metastasis	Recurrence
Pancreatic	Independent prognostic factor [18, 23]	higher rate of lymph node metastasis [23]	Independent prognostic factor [18]
Head and neck	Independent prognostic factor [63]	higher lymph node metastasis and distant recurrence [22, 25]	Independent prognostic factor [16, 17, 63]
Gastric	Independent prognostic factor [64]	-	high rate of recurrence [64]
Colorectal	Independent prognostic factor [21]	-	Independent prognostic factor [21]
Prostate	Independent prognostic factor [65]	Increased PNI diameter is a risk factor for lymph node metastasis [19]	Independent prognostic factor [19]
Cervical	Independent prognostic factor [66]	higher risk of nodal metastasis [27, 66]	high-risk factor [66]
Vulvar	High-risk factor [67]	High-risk factor [67]	Independent prognostic factor [20]
Penile	-	higher risk of nodal metastasis [28]	-
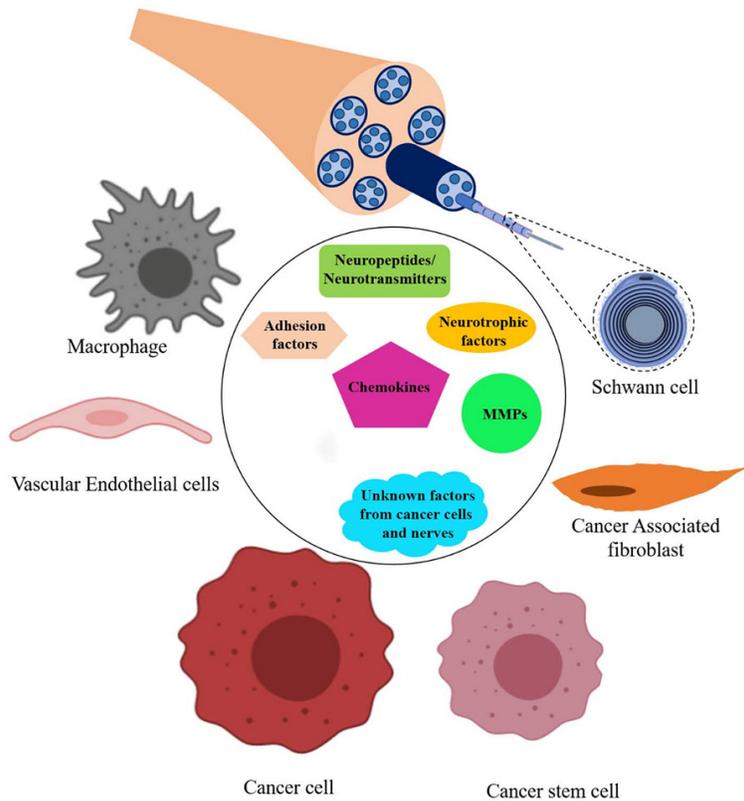
Figure 2. The cellular and molecular mediators of neural invasion at the tumor-nerve interface.

that these cells differentially expressed 49 mRNAs compared to PNI-incompetent cancer cells. CD74 was then identified to be a critical regulator of PNI. CD74 was known to express in antigen-presenting cells previously, however, in this study, the authors found that it is expressed in PNI competent cancer cells in pancreatic tumors, and associated with PNI incidence [33].

To examine the protein signature of NI-competent cells, Alrawashdeh et al. performed LC-MS/MS using cancer cells isolated from the PNI and non-PNI regions of human pancreatic tumors [34]. The authors found that only 19 proteins were differentially expressed in PNI-cancer cells, indicating a less robust change at the protein level. However, they found that 61 proteins were differentially expressed in PNI positive nerves compared to non-participating nerves. Ingenuity Pathway Analysis (IPA) showed that these proteins are involved with neurological dysfunctions, immunological disorders, and inflammatory responses. The authors then identified secretogranin II (SCG II)

and neurosecretory protein VGF as critical molecules involved with PNI [34].

Another study showed that microRNAs are also altered in NI-competent cells. It was found that 19 microRNAs and 34 mRNAs were differentially expressed in NI positive prostate tumors. These molecules were associated with metabolism and transport of carboxylic acids, fatty acids, amino acids, and polyamines, along with neurogenesis and anti-apoptosis. The authors of the study also found upregulation of the microRNAs, miR-224, miR-10, miR-125b, miR-30c, miR-100 and downregulation of metallothioneins and coxsackie adenovirus receptor (CXADR) in NI positive samples, indicating a regulatory involvement of these molecules in NI [35].

Chen et al. used a combination of laser capture microdissection (LCM) and high-throughput cDNA microarray to profile gene expression associated with PNI in salivary adenoid cystic carcinoma (ACC). They eventually identified 53 genes that were differentially expressed in the PNI group. The upregulated genes are related to transmembrane protein (NETO1), metabolic process (APOC1), metalloprotease (ADAM30), oncogene (MCAM), extracellular matrix (COL4A3, COL4A6), apoptosis (CARD12) and cell cycle (CCNB1, CCNB3). Others were from the SOX (SOX2, SOX5, and SOX11), Notch (NOTCH4), and WNT (WNT2B) families. The downregulated genes were from the oncogene (MAGEA2, MAGEA3, MAGEA4, MAGEA6, and MAGEA9) and metalloprotease (MMP7) class along with some related to the cytoskeleton (KRT4, KRT6B) [36]. Overall, their study suggests that targeting Notch and Wnt/β-catenin may be promising in preventing PNI. Mays et al. also used DNA microarray to characterise PNI competent skin cancer cells. Using BRB-Array Tools, they found that 24 genes are involved with PNI. However, only 4 of them (GAL3ST4, TMEM140, TXNIP and SCPEP1) were more striking [37].

Several researchers used The Cancer Genome Atlas (TCGA) data to predict the molecular signature of PNI-competent cancer cells. For example, Zhang et al. performed Weighted Gene Co-expression Network Analysis (WGCNA) and showed that 357 genes were differentially expressed in NI positive head and neck cancers. The authors identified 12 hub genes such as TIMP2, MIR198, LAMA4, FAM198B, MIR-4649, COL5A1, COL1A2, OLFML2B, MMP2, FBN1, ADAM12, and PDGFRB as potential facilitators of NI. These genes are known to be involved with epithelial-mesenchymal transition (EMT), metastasis, and invasion. The authors also claimed that fibroblast is the major stromal cell type involved with NI, followed by endothelial cells and macrophages [38]. Similarly, another study that used head and neck cancer data from the TCGA identified that the transcription factors MYF6, MYOG, MYF5, and MYOD1 are critical for NI. These transcription factors are otherwise known to modulate muscle cell activities [39].

Several other researchers also mined large data repositories to characterize NI-competent cancer cells. For instance, Jia et al. constructed a PNI gene signature (104 genes) of gastric cancer by performing a meta-analysis of the data retrieved from Gene Expression Omnibus (GEO). The authors used pathway studio database for the analysis and concluded that CXCL8 could serve as a prognostic biomarker for gastric cancer. They also predicted that therapies targeting CXCL8/CXCR2 and MMP9 might be promising for preventing PNI [40]. Similarly, Huang et al. analysed the genomic datasets of colon, pancreatic, cutaneous, and prostate cancers by performing weighted gene co-expression network analysis (WGCNA). The authors reported that 30 hub genes, most of which are associated with cell cycle, are critical for PNI [41].

The newly identified molecular mediators of NI are summarized in **Table 2**. Although these are overwhelming data to represent a molecular signature of NI-competent cancer cells, whether this is unique to individual cancers is unknown and may need additional investigation.

Additional cellular players of NI

Schwann cells (SCs)

The general cellular mediators of NI are shown in **Figure 2**. Among those, the SCs and macro-

phages play critical roles in facilitating NI. SCs are glial cells of the peripheral nervous system (PNS) and regulate nerve homeostasis, survival, and repair [42]. They are highly plastic cells and reprogram themselves molecularly and phenotypically during nerve repair and regeneration [43]. These properties of SCs are exploited by cancer cells in PNI. In general, the SCs remain in an active, undifferentiated state in tumor-infiltrated nerves [44]. In co-culture experiments, Deborde et al. found that SCs recruit cancer cells to neurites that emerge from DRG explants for the efficient migration of cancer cells towards the DRGs [44]. Further, in their 3D co-culture model, the authors found that SCs interact with individual pancreatic cancer cells, which helps restructuring of the clustered cancer cells into more linear (chain like) form. In other words, SCs interrupt cell to cell contact within the cancer cell cluster to disperse and redirect individual cancer cells away from clusters for facilitating migration, indicating a direct physical involvement of SCs in modulating cancer cell migration [44].

SCs also modify growth response of cancer cells. For example, SCs promote cancer cell proliferation in co-cultures, indicating that growth-stimulatory molecular cross-talks occur between cancer cells and SCs [41]. Neural cell adhesion molecule (N-CAM) has been shown as a connecting link between SCs and cancer cells, and a promoter of NI [45, 46]. It was shown that knockdown of N-CAM limits SC activity, tumor progression and NI [44]. Neurotrophins also play critical roles in promoting NI. SC is chief source of neurotrophins. It was found that SCs secrete BDNF when co-cultured with cancer cells. The BDNF then act via TrkB receptor on cancer cells to induce EMT and invasive properties desirable for NI [47].

On the other hand, Fujii-Nishimura et al. demonstrated that SCs also induce mesenchymal-epithelial transition (MET) in cancer cells at NI locations. For instance, co-culture of pancreatic cancer cells with SCs has shown to induce MET-like structural changes accompanied by upregulation of E-cadherin and downregulation of Smad3 and vimentin. Similar changes in expressions of E-cadherin and Smad3 was also observed at PNI locations in pancreatic tumors. The authors suggested that SC-dependent MET might be required for cancer cells to colonise in

Table 2. High-content molecular analysis of NI-competent cancer cells and NI interface

Cancer type	Technique used	Cell/Tissue analyzed	Differentially expressed molecules	Potential molecules identified as critical for NI	References
Pancreatic cancer	Microarray (mRNAs)	Cancer cells invaded and migrated out of nerves	680	KLF14 and ARHGDI β	[32]
Pancreatic cancer	Oligonucleotide microarray	Cancer cell lines	49	CD 74	[33]
Pancreatic cancer	Proteomics analysis	Cancer cells from the PNI site	31 (based on 1.5-fold change)		[34]
		Nerve involved with PNI	Total 167 (61 molecules based on ≥2-fold change)	SCG II, VGF	
Prostate cancer	MicroRNA array Microarray	Tumor tissues	19 MicroRNAs 34 mRNAs	miR-224, miR-10, miR-125b, miR-30c, miR-100 Metallothionein 1M, 1F, CXADR	[35]
Adenoid cystic carcinoma	Combination of laser capture microdissection and high-throughput cDNA microarray	Tumor tissues	53	NETO1, APOC1, ADAM30, MCAM COL4A3, COL4A6, CARD12, cycle CCNB1, CCNB3, SOX2, SOX5, SOX11, NOTCH4 and WNT2B, MAGEA2, MAGEA3, MAGEA4, MAGEA6, MAGEA9, MMP7, KRT4, KRT6B	[36]
Head and Neck Squamous Cell Carcinoma (HNSCC)	DNA microarray technique and BRB-Array Tool for the analysis.	Cutaneous HNSCC tissues	24 genes	GAL3ST4, TMEM140, TXNIP and SCPEP1	[37]
HNSCC	Network analysis using TCGA (The Cancer Genome Atlas) data	Gene expression data from 351 HNSCC patients	357 genes with 12 hub genes	Metalloproteins, laminins, integrins. Enriched signaling pathway: PI3K-Akt	[38]
HNSCC	Network analysis of TCGA data	Data from 528 HNSCC patients	263 genes (26 with >2-fold change in expression)	MYOD1, MYF5, MYF6 and MYOG.	[39]
Gastric cancer	Data retrieved from the Gene Expression Omnibus (GEO) and analysis using pathway studio database.	Gene expression data from 13 studies	104 genes	CXCL8/CXCR2 axis and MMP9	[40]
Colon, pancreatic duct adenocarcinoma, cutaneous SCC and prostate cancer	Data retrieved from the GEO database.	Gene expression data from four PNI related dataset (one for each mentioned cancer type)	30 hub genes	Directly regulating cell cycle: AURKA, CCNB1, CCNB2, CDK1, CDKN3, and RRM2 Contributing in the DNA replication and cell mitosis: TOP2A, MAD2L1, MCM2, Polycomb-repressive complex, TTK, BUB1B, KIF11, ASPM, NUSAP1, and PCLAF	[41]

the perineurium [48]. SCs are also a chief source of TGF-β, which is another promoter of NI [49].

Since SCs play critical roles in promoting NI, disruption of SCs-cancer cells interaction can be therapeutically relevant. The repulsive protein SLIT2 has shown to regulate SC-cancer cell interaction in NI. Expression of SLIT2 in either SCs or cancer cells inhibits their mutual interaction [50]. However, it was shown that SLIT2 expressed in cancer associated fibroblasts (CAF) induces SCs migration towards cancer cells by regulating β-catenin or cadherin-2 signalling, which in turn promote the mutual interaction of SCs and cancer cells in NI [51]. Similarly, Swanson et al. demonstrated that an interaction of mucin 1 on cancer cells with myelin-associated glycoprotein on SCs promote SCs-cancer cells partnership in NI [52]. All these evidences clearly indicate that SCs play a major role in promoting NI. However, how the distinct physical locations of SCs and perineurium aligns with SC-dependent NI remains obscure and need additional investigations.

Macrophages

Endoneurial macrophages are resident immune cells of the peripheral nervous system (PNS) and are essential for nerve homeostasis and regeneration. The endoneurial macrophages are abundantly present in tumor-infiltrated nerves and also recruited by tumor cells to the NI sites. For example, it was shown that pancreatic cancer cell-secreted colony stimulating factor-1 (CSF-1) recruit endoneurial macrophages, which in turn secrete GDNF to promote NI [53]. In addition, tumor cells recruit hematogenous macrophages by secreting several chemotactic mediators such as CCL2, CCL5, CCL7, CCL8, CXCL12, vascular endothelial growth factor (VEGF) and CSF-1 [54]. The tumor milieu also have tumor associated macrophages (TAMs) with self-renewal capabilities. Interestingly, PNS resident macrophages also self-renew [55]. Thus, it is likely that TAM and PNS resident macrophages share some molecular and phenotypic characteristics. TAMs promote angiogenesis and immune suppression to facilitate tumor survival [54]. In addition, the TAMs secrete several cytokines such as TNF-α, IL-6, IL-8 and angiopoetins (ANG1 and ANG2), MMPs, FGF, VEGF, all associated with tumor invasion and proliferation [54]. All these factors promote

invasive properties of cancer cells and indirectly pave way for NI.

Bakst et al. showed that macrophages recruited to the nerve-tumor interface disrupts perineurium by secreting Cathepsin B and thus promote NI, indicating that hematogenous macrophages of the monocytes origin are more critical than resident macrophages of embryonic progenitor cell origin for NI [56]. The authors' observation that recruited macrophages induce disruption of perineuria suggests that both perineurial invasion and INinv are likely when macrophages are involved. Nevertheless, although macrophages are well-known to exist in two phenotypes, the pro-inflammatory MI and the anti-inflammatory M2, the phenotypic identify of macrophages that participate in NI is not known. Overall, with the current data available, it is suggested that SC-macrophage-cancer cell crosstalk plays a critical role in facilitating NI. However, it needs additional investigations to understand the exact modulatory functions of macrophages in NI.

Future directions

Despite having ample literature on the incidence and complications of NI, it is still a challenge to delineate the individual contribution of PNI and PNTS in cancer prognosis. PNI diameter could be a measurable tool for determining prognosis, however, an increase in PNI diameter may overlap with histological diagnosis of PNTS. Therefore, clear guidelines on defining PNI and PNTS in tissue specimens is a mandate to use this knowledge in cancer management effectively. NI-induced cancer pain is another under-explored area. Since nerves are critical mediators of cancer pain, the specific role of NI in facilitating cancer pain would be something of interest to pursue in the future.

Here, we reviewed the molecules at the NI interface that are mostly specific to NI-competent cancer cells. It is however understood that stromal cells in the tumor microenvironment are also critical regulators of NI [57]. Therefore, understanding the overall molecular signature of the stromal cells that participate in NI might also be worthwhile to define the process overall. The glial cells and macrophages localised in nerves are also rich sources of growth factors [42, 58]. These cells respond to nerve injuries by self-renewal and may thus

amplify proliferative response of cancer cells within neural milieu [55]. This is particularly important considering their specific location at the *endoneurium,* generating much interest on their involvement in INinv. Nerve injuries also promote production of growth factors in abundance [59]. In addition, we have shown that the peripheral nerves are equipped with tumor suppressor and DNA repair molecules [43, 60-62]. Since tumor invasion could partly mimic injury response in nerves, it would be interesting to study how a balanced milieu of nerve-derived growth factors, tumor suppressors and repair molecules regulate survival and transit of tumor cells in NI. Overall, a much deeper understanding of the cellular and molecular drivers of NI would facilitate developing strategies to eliminate this pathological process and promote survival of affected individuals.

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Disclosure of conflict of interest

None.

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