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# Cancer-associated neurogenesis and nerve-cancer crosstalk

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## Abstract

In this review, we highlight recent discoveries regarding mechanisms contributing to nerve-cancer crosstalk and the effects of nerve-cancer crosstalk on tumor progression and dissemination. High intratumoral nerve density correlates with poor prognosis and high recurrence across multiple solid tumor types. Recent research has shown that cancer cells express neurotrophic markers such as nerve growth factor, brain-derived neurotrophic factor, and glial cell-derived neurotrophic factor and release axon guidance molecules such as Ephrin B1 to promote axonogenesis. Tumor cells recruit new neural progenitors to the tumor milieu and facilitate their maturation into adrenergic infiltrating nerves. Tumors also rewire established nerves to adrenergic phenotypes via exosome-induced neural reprogramming by p53-deficient tumors. In turn, infiltrating sympathetic nerves facilitate cancer progression. Intratumoral adrenergic nerves release noradrenaline to stimulate angiogenesis via vascular endothelial growth factor signaling and enhance the rate of tumor growth. Intratumoral parasympathetic nerves may have a dichotomous role in cancer progression and may induce Wnt-β-catenin signals that expand cancer stem cells. Importantly, infiltrating nerves not only influence the tumor cells themselves but also impact other cells of the tumor stroma. This leads to enhanced sympathetic signaling and glucocorticoid production, which influences neutrophil and macrophage differentiation, lymphocyte phenotype, and potentially lymphocyte function. Although much remains unexplored within this field, fundamental discoveries underscore the importance of nerve-cancer crosstalk to tumor progression and may provide the foundation for developing effective targets for the inhibition of tumor-induced neurogenesis and tumor progression.

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## Keywords

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## Introduction

Mounting evidence suggests that cancer prognosis is associated with intratumoral neural infiltration. This phenomenon is most commonly observed in cancers that arise in highly innervated organs, including nearly all pancreatic cancers, 80% of head and neck cancers, 75% of prostate cancers, and 33% of colorectal cancers (1). Studies on patient tumor samples have revealed that intratumoral nerve density is associated with increased metastasis, morbidity, and mortality. Consistently, the presence of nerve fibers is an independent prognostic factor for overall survival in pancreatic ductal adenocarcinoma (PDAC) (1–4), gastric carcinoma (5,6), biliary tract tumors (7), head and neck cancer (8–10), and cervical cancer (11,12) and an indicator for recurrence risk in pancreatic cancer (13), prostate cancer (11,14), gastric cancer (15), and colorectal cancer (16–21). Tumor innervation may play an important direct role in facilitating metastasis, as tumor-associated nerves may extend into the central nervous system and cultivate pre-metastatic niches (19,22). Tumor innervation may also affect patients' quality of life by causing pain, paresthesia, numbness, and paralysis. The purpose of this review is to help clinicians and researchers gain a deeper mechanistic understanding of nerve-cancer crosstalk. Of note, this nerve-cancer crosstalk is distinct from perineural invasion, the process by which cancer cells disseminate through lymphatic vessels within the perineural space (23,24).

## Fundamental Discoveries in Nerve-Cancer Crosstalk

Despite the known impact of denervation in reducing cancer growth, investigation of nerve-cancer interaction has been slow (25–27). However, knowledge regarding nerve-cancer crosstalk has been greatly advanced by a few landmark discoveries that have elucidated three key mechanisms by which tumors regulate nerves: axonogenesis, neurogenesis, and neural reprogramming (Figure 1A).

### Cancer Progression Drives Axonogenesis

Following their observation that murine dorsal root ganglia form neurite outgrowths towards prostate cancer cells (28), Ayala et al. (29) investigated the symbiotic relationship between nerves and cancer. Through 2- and 3-dimensional reconstructions of entire prostates, Ayala and colleagues uncovered and confirmed cancer-related axonogenesis (the enlargement of nerves or increase in nerve density) and demonstrated an association between prostate cancer and neurogenesis (an increased number of neurons). These findings were supported by the observation that the axon-guidance molecule semaphorin 4F (S4F) is highly expressed—and may be secreted-- by DU-145 cells when co-cultured with neurons, and S4F induced neurite sprouting and increased neurite length in neurons compared to controls. Furthermore, a reduction of S4F via small interfering RNA transfection decreases neurite

outgrowth. Together, these findings demonstrated, for the first time, that cancer cells produce known neurotropic molecules capable of driving axonogenesis.

Around the same time as this discovery, the revelation that chronic stress promoted angiogenesis and malignant cell growth through sympathetic β-adrenergic activation (30) caused researchers to further understand the mechanistic effects of stress on tumor growth. A closer investigation of the role of sympathetic β-adrenergic activation in tumor progression led to the landmark demonstration that tumor progression is promoted by nerve stimulation (31). In this study, surgical or pharmacological denervation of both parasympathetic and sympathetic nerves led to prostate tumor suppression in mice. Genetic deletion of sympathetic $\beta_2$- and $\beta_3$-adrenergic receptors in stromal cells also prevented early tumor progression. In contrast, parasympathetic stimulation contributed to later tumor progression, invasion, and metastasis through pharmacological or genetic disruption of the muscarinic 1 receptor. Additionally, clinical sympathetic and parasympathetic nerve densities were greatest in patient tumors and surrounding tissues, respectively, and were associated with poor clinical outcomes (32). This study suggests that both sympathetic and parasympathetic signaling may serve as potential therapeutic targets and that cancer-related neurogenesis may drive tumor progression.

## Cancer Progression Drives Neurogenesis

In addition to studying cancer's role in axonogenesis, Ayala et al. and Magnon et al. proposed that cancer may drive neurogenesis, the outgrowth of neural progenitors to the tumor. Mauffrey et al. (33) expounded on this hypothesis, demonstrating that neural progenitor cells expressing the neural stem cell marker doublecortin (DCX+) migrate from neurogenic regions of the brain's subventricular zone to tumorous and metastatic niches via the bloodstream, differentiating into noradrenergic, mature neuronal phenotypes. Furthermore, DCX+ cells were observed in greater numbers in high-risk versus low-risk human prostate cancer specimens. DCX+ cell depletion also decreased incidence of neoplastic lesions and increased tumor growth in tumors with the addition of DCX+ neural progenitor cells. Additional studies to identify the mechanisms by which DCX+ progenitor cells migrate will be important next steps to confirm the conclusion of this study, cancer-related neurogenesis.

Cancer is also capable of forming de novo neurons from cancer stem cells. Lu et al. (34) showed that cancer stem cells derived from patients' gastric and colorectal carcinomas were able to differentiate into both tyrosine hydroxylase (TH)-producing sympathetic and vesicular acetylcholine transporter-producing parasympathetic neurons. These neurons, in turn, were able reciprocally communicate with cancer cells within xenografts to facilitate tumor growth. Knockdown of the neuron-generating capacity of these stem cells by MAP2 inhibited tumor xenograft growth, thereby underscoring the importance of these de novo nerves to cancer progression.

## Cancer Stimulates Neural Reprogramming

Concurrently with Mauffrey et al.'s study, Amit et al. (35) made a surprising discovery regarding cancer: reciprocal neural crosstalk. Specifically, the authors observed exosome-

induced neural reprogramming--- a phenomenon typically only observed during development---by tumors and showed that cancer-derived extracellular vesicles (EVs) play a role in cancer-related axonogenesis. They discovered that genetically aberrant, p53-knockout or -mutant ($p53^{C176F}$ and $p53^{A161S}$) oral cavity squamous cell carcinoma (OCSCC) cells release EVs that promote neuritogenesis in dorsal root ganglia. This release was dependent on *Rab27A* and *Rab27B*, GTPases both necessary for EV release (35,36–39). Analysis of micro RNA (miRNA) array revealed a decrease in the p53-deficient, cancer-derived EV-packaging of miR-34a and miR-141. The knockdown or antagonism of miR-34a in $p53^{WT}$ cancer cells produced similar EVs to those seen in p53-deficient cancer cells. Furthermore, Amit and colleagues were the first to report that daily intratumoral injections of $p53^{WT}$ OCSCC EVs can suppress noradrenergic neurogenesis. Decreased levels of miR-34a not only promoted the neuritogenesis of sensory nerves, but also induced transdifferentiation of these nerves into noradrenaline-producing adrenergic nerves, which are commonly enriched in head and neck tumors and have been shown to promote tumor growth (38). To demonstrate that neural reprogramming, rather than outgrowth of existing adrenergic nerves, drove this process, the authors performed lingual denervation prior to OCSCC implantation in mice, and found lower intratumoral tyrosine hydroxylase-positive nerve density than sham controls. In contrast, global chemical sympathectomy before OCSCC implantation in mice did not affect tumor growth. Similarly, TH+ nerve density in patients with OCSCC was associated with lower overall survival rates and thus it may potentially serve as an independent prognostic marker. Overall, these results demonstrate a novel mechanism by which cancer cells induce nerve density and initiate adrenergic neurogenesis.

## Reciprocal Communication Between the Tumor, Microenvironment, and Nerves

The mechanisms that drive axonogenesis through nerve-cancer crosstalk have not yet been fully elucidated. However, evidence described in this section indicates that axonogenesis is stimulated by cancer cells' release of neurotrophic growth factors and EVs containing altered miRNA levels. Reciprocally, nerves release neurotransmitters that stimulate cancer growth, possibly through either bona-fide or pseudo-tripartite synapses (resembling a tripartite synapse, in which a synapse consisting of two neurons is closely associated with a third, non-neural cell). Together, tumor-associated, stromal, endothelial, and immune cells are nurtured and primed for angiogenesis and inflammation (Figure 1B).

### Tumor-derived EVs Induce Axonogenesis through miRNA

EVs play an important role in establishing a tumor microenvironment that can positively regulate tumor initiation and metastasis. EVs, including exosomes, which are assembled and released through a transcriptionally driven process, are released from nearly every cell type and carry complex cargos, consisting of DNA, RNA, miRNA, transfer RNA, long non-coding RNA, lipids, and proteins, across long distances (38–40). As described below, cancer-derived exosomes carry different cargo than do non-cancer-derived exosomes and signal inflammation, angiogenesis, and axonogenesis.

Exosomes extracted from human head and neck cancer samples and from murine oropharyngeal squamous cell carcinomas (OPSCC) induce increased neurite outgrowth in

PC12 cells (a rat pheochromocytoma cell line) compared to exosomes derived from control plasma and tissue. In contrast, the pharmacological exosome inhibitor GW4869 and genomic knockout of *Rab27A* and *Rab27B*, genes that control the exosome secretion pathway, decrease neurite outgrowth (40). High concentrations of at least one exosome cargo, the axon-guidance molecule Ephrin B1, drive axonogenesis, also seen in cervical cancer cell lines (41). In addition, neuritogenic effects have been seen when PC12 cells treated with cancer-derived exosomes were used to recruit sensory nerves to the tumor microenvironment. These results demonstrate that cancer-derived EVs promote tumor innervation.

It is likely that additional signals packaged in exosomes drive axonogenesis. For example, cancer commonly presents with aberrant regulation of gene expression by miRNAs, which bind to mRNA to reduce its translation or increase its degradation. This binding to mRNA regulates many cellular pathways and transcription factors, such as those involved in proliferation, differentiation, and apoptosis, all of which may be corrupted in cancer. Thus, the genomic aberrations, epigenetic changes, or mutations of miRNA that affect its processing or activity may nurture a microenvironment conducive to tumorigenesis or metastasis (42–44). As such, miRNAs can be classified as oncoMIRs or tumor-suppressor miRNAs (45,46). In support of this concept, Amit et al. revealed that a combination of stimulatory miR-21 and miR-324 with scramble miRNA increased axonogenesis more than did a cocktail including inhibitory miR-34a (35). This suggests the presence of cancer-orchestrated signaling to other cells in the tumor microenvironment through specific exosome packing and release of miRNAs.

Additionally, the promotion of tumor progression through tumor-derived EVs and dysregulated miRNA has been demonstrated by a number of groups studying various cancer types. In lung cancer, low levels of miR-100-5p have been shown to increase levels of mTOR (47). In breast cancer, modified EVs and miR-23a, miR-222, miR-452, and miR-24 alter their respective targets—Sprouty2, PTEN, APC4, and p27—to confer drug resistance (48,49). Tumor-derived EVs can also provide nutrients to the tumor and mediate tumor-stem cell and tumor-progenitor cell communication (50–52). Thus, there are abundant opportunities to investigate the roles of other molecules within tumor-derived EVs in regard to nerve-cancer crosstalk.

## Neurogenic Factors Promote Axonogenesis in Cancer Progression

Cancer cells increase secretion of neurogenic factors promoting axonogenesis, while nerves increase expression of the complementary receptors. Deregulation of NGF, which is responsible for the survival, differentiation, and neurite outgrowth of neurons, has been implicated in a number of cancer types that express the tyrosine-receptor kinases (Trks) TrkA, TrkB, and TrkC and the p75 neurotrophin receptor (53).

These receptors are also expressed on nerves, and p75 neurotrophin receptor has recently been reported to act as a chemoattractant for cancer cells (54). Indeed, cancer-derived NGF drives neurite growth and cancer proliferation and migration and is correlated with nerve-cancer crosstalk (1, 55–64). One notable study demonstrated an association between expanded enteric nerves and increased NGF expression in gastric cancer and the NGF/Trk

signaling regulation of Dclk1+ tuft-cell coordinated crosstalk between nerves and gastric cancer (64, 65). Migration of pancreatic cancer cells toward dorsal root ganglia was reduced when NGF signaling was blocked through NGF knock down or NGF-neutralizing antibodies (66,67). Similarly, in breast cancer cells, there is a correlation between nerve fibers and NGF expression, and breast cancer cells are capable of driving axonogenesis in PC12 cells, a process partly reversed by blocking NGF (57).

The expression not only of neurogenic factors, but also their cognate receptors, influences nerve-cancer crosstalk (58). NGF's receptor, nerve growth factor receptor (NGFR), is expressed across multiple cancer types; for example, it is expressed in luminal breast cancer in rare, basal-like cells resistant to antiestrogens (59). NGFR inhibits p53 activity within tumor cells in a negative feedback loop across multiple tumor types. This process is central to maintaining melanoma stem cells *in vitro* and melanoma growth *in vivo* (60). Through it, NGF signaling from nerves via NGFR expression on cancer stem cells may drive cancer stem cell renewal and proliferation.

Like NGFR, L1 cell adhesion molecule (L1CAM), a surface receptor central to proper cell adhesion and migration during neural development, is highly upregulated in several different tumor types. It promotes cancer cells' motility and invasiveness, leading to cancer metastasis and chemo- and radioresistance (61,62). Recently, it has been found that L1CAM expression, in conjunction with CD133 expression, defines a cancer stem cell population in glioma and ovarian cancer. In *in vivo* ovarian cancer models, L1CAM expression elicited cancer stemness in several ways, e.g., through the enhancement of spherogenicity, the tumor take rate, cancer cells' self-renewal capacity, and tumor growth (62).

Like NGF, brain-derived neurotrophic factor (BDNF) is induced by noradrenergic signaling to stimulate axonogenesis through Trk receptors (70,71) and is associated with the promotion of angiogenesis and increased tumor cell proliferation (72). Another NGF, NT-3 , is overexpressed in PDAC and its nerves, and NT-3 inhibition led to decreased growth of PDAC in a murine xenograft model (64,65,73).

Glial cell-derived neurotrophic factor (GDNF) is robustly expressed in human PDAC and is significantly correlated with neural invasion, and associated with an increase in pain levels (74–76). The cancer-promoting effects of GDNF, including neural invasion, are likely mediated through GDNF receptors including GDNF family receptors α1-3 and through *RET*, initiating downstream activation of the RAS/ERK, MAPK, JNK, and PI3-K/Akt signaling pathways (75,76). GDNF is also known to promote integrin expression, activate matrix metalloproteinase (MMP)-9, and increase nuclear factor κ B, which plays a significant role in nerve adhesion and invasion (77–80).

Neurturin and artemin also signal through *RET* and promote innervation (81). They are also highly expressed in PDAC and are associated with tumor invasiveness and nerve alteration, respectively (3,58,82,83). Artemin has been found primarily in the hypertrophic nerves of PDAC tissue samples, as determined through Western blotting and immunohistochemistry (84).

Lastly, when upregulated, the axon-guidance molecule netrin-1 acts as an oncogene in a number of cancer types (85,86) and promotes gastric cancer cell navigation along sensory dorsal root ganglia cells and sciatic nerve invasion in vitro (87). In contrast, in PDAC, restoration of pathologically decreased expression of the axon-guidance molecule, Slit2, to normal levels reduces metastasis and neural invasion (88). While these studies reveal promising therapeutic targets, further investigation will be important to translate their feasibility in clinical application.

## Synapses Facilitate Nerve-cancer Crosstalk

Communication between nerves and brain cancer cells has recently been characterized as taking place via either bona-fide chemical synapses, as in glioblastomas, or via pseudo-tripartite synapses as in breast-to-brain metastases (B2BM). Gene expression of synaptic markers has been found in primary glioma cells (89). Neuron-glioma interaction via bona-fide chemical synapses have electrophysiological properties similar to those seen in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) signaling (89,90). Spontaneous excitatory postsynaptic currents from AMPA-receptor-expressing glioma cells demonstrates a functional neuroglioma synapse (90). It was also determined that these synapses do not result from a direct connection to neurons. Rather, tumor microtubules are found in glioma tissue and facilitate gap-junction signal transmission (89,91). In addition, the optogenetic stimulation of glioma cells promote glioma growth and proliferation. Furthermore, it has been demonstrated that activated glioma increases neuronal hyperexcitability in the tumor microenvironment in a reciprocal interaction (89).

Similarly, activation of glutamate ligand receptors, N-methyl-D-aspartate receptors (NMDARs), facilitates breast cancer metastasis to the brain (22). Previous reports have demonstrated tumor growth via NMDARs in neuroendocrine and ductal pancreatic cancers, and this growth is thought to result from autocrine secretion. Breast cancer transcriptional signatures have also implicated NMDARs in metastasis and NMDAR subunits have high expression levels in B2BM cell lines. It was also discovered that the extracellular glutamate in the B2BM tumor microenvironment was not sufficient to substantiate autocrine signaling. B2BM cells express adhesion molecules, such as postsynaptic density protein 95, that form pseudo-synapses between non-neuronal cells and axons and contribute to astrocytic synaptogenesis. Non-disruptive B2BM processes establish pseudo-tripartite synapses to access glutamate. The knockdown of NMDAR subunits resulted in fewer brain tumors and increased subject brain metastasis-free survival but did not affect their primary tumor burdens or lung metastases, which were rescued with the re-expression of NMDAR subunits. Together, these finding describe how tumors can co-opt neural signaling in the brain to promote tumor progression.

## Sympathetic and Parasympathetic Nerves Cultivate a Microenvironment to Support Cancer

Neurotransmitters are signaling molecules that allow neurons to communicate with other neurons or cells. Because various cancers and components of their stroma express corresponding neurotransmitter receptors, the induction of a pro-tumor microenvironment can be supported by the peripheral nervous system. Autonomic nervous system responses promote tumor progression. Sympathetic stimulation is involved in the early stages of

carcinogenesis and angiogenesis, while parasympathetic stimulation promotes invasion and metastasis. The sympathetic nervous system is also involved in immunomodulation and cancer-associated neuropathic pain.

**Sympathetic Signals Promote Tumor Progression—**Sympathetic nerve innervation has been demonstrated in human tumors and murine tumor models. Increased levels of noradrenaline have been observed in solid tumors and implicated in stress-mediated tumor progression. Furthermore, noradrenergic receptors are widely distributed in various cancer types, and modulating them has known effects on tumorigenesis and progression, described below.

**Stress-induced Noradrenaline and the Angiogenic Switch:** The growth of a new vascular network, angiogenesis, is marked by pro-angiogenic molecules (such as interleukin(IL)-8, tumor necrosis factor(TNF)-α, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-α, TGF-β, angiogenin, platelet-derived growth factor, and fibroblast growth factor), the levels of which indicate tumor aggression and are important factors in prognostic outcomes (91–94). Peripheral neurons participate in vascular organization during development and wound repair and contribute to tumor angiogenesis (95–97).

The catecholamines noradrenaline and dopamine have opposing roles in angiogenesis; respectively, they stimulate and inhibit vascular networks. Noradrenaline stimulates angiogenesis by signaling increased VEGF expression in tumor-associated macrophages in primary mammary tissues and amplifying the expression of cytokines known to stimulate angiogenesis (34,98–100). Noradrenergic signaling in a $\beta_2$-adrenergic and $\beta_3$-adrenergic knockout in a xenogeneic orthotopic prostate cancer model in immunodeficient mice resulted in reduced tumor-associated vascular density (101). Furthermore, $\beta_2$-adrenergic knockout in a spontaneous transgenic prostate cancer mouse model also exhibited decreased vascular density, migration, and branching compared to that seen in controls. Dopamine, on the other hand, downregulates the VEGF receptor-2-mediated signaling pathway, diminishing proliferation and migration in colon cancer cell lines and impairing tumor growth in mouse models of gastric cancer and ovarian cancer (98,102–106). These studies indicate that tumor innervation promotes angiogenesis and neovascularization of the tumor microenvironment.

As previously mentioned, surgical and pharmacological sympathectomy decreases prostate tumor growth and lung metastasis (33, 107). Pro-tumorigenic properties have been observed in Rv1 and LNCaP prostate cancer cell lines that express $\alpha_{1A}$-adrenergic receptors and in PC3 and DU-145 cells lines that express $\alpha_{1B}$- and $\alpha_{1D}$-adrenergic receptors. Blocking $\beta_2$- and $\beta_3$-adrenergic signaling was found to arrest tumor growth and angiogenesis, and it was later shown that $\beta_2$-adrenergic signaling activates the angiogenic switch by inhibiting the expression of mitochondrial cytochrome c oxidase assembly factor 6, which decreases oxidative phosphorylation (aerobic glycolysis) (101). It has also been shown that noradrenergic signaling indirectly stimulates angiogenesis by stimulating M2 macrophages to secrete VEGF, which was inhibited in mice with propranolol (107). Together, these findings suggest that stress-induced sympathetic signaling drives angiogenesis and tumor progression.

**Adrenergic Signaling Promotes Chemoresistance:** Like dense, intratumoral sympathetic nerves, enhanced adrenergic signaling may drive primary or secondary resistance to cytotoxic chemotherapies. Eng et al. (108) noted that pancreatic cancer biology and treatment responses in their mouse colonies were heavily dependent on temperature, and that the sympathetic cold stress response enhanced noradrenaline and activated β-adrenergic receptors, driving resistance to cisplatin and paclitaxel (109). In head and neck cancer cells treated with cisplatin, the half-maximal inhibitory concentration of cisplatin was strikingly increased in oral cancer cells cocultured with neurons compared to oral cancer cells cultured alone. In addition, oral cells treated with a β2-adrenergic receptor blocker (ICI 118,551) demonstrated significantly diminished cell viability 48 hours after treatment with cisplatin (unpublished data). Similarly, blockading of β2-adrenergic receptor (ADRB2) and NGF pathways improved gemcitabine efficacy in KPC pancreatic cancer models (73). In a recent study by Chen et al. (109), signaling through upregulated β2 receptors on cervical cancer cells led to upregulation of Sirt1; inactivation of its target, p53; and downregulation of p53 target genes. As a result, these tumor cells were resistant to doxorubicin-induced p53 acetylation. Additional work needs to be done to fully determine how nerves influence cancer response and resistance to chemotherapy and other treatment modalities.

**Dichotomous Role of Parasympathetic Fibers in Tumors—**In addition to stimulating tumors, parasympathetic signals can also suppress tumor progression. In gastric, prostate, and breast cancers, parasympathetic signals serve as specific markers for tumors, and the corresponding receptors are expressed in gastric, pancreatic, lung, cervical, and colon cancer cells (71,110–112).

**Parasympathetic Tumor Suppressors:** Vagotomies performed in patients with gastric cancer and mice with pancreatic cancer have increased tumor progression (92,113). However, the activation of muscarinic cholinergic receptor 1 (Chrm1) reduces tumor incidence, perturbs cancer cell signaling pathways through the suppression of EGFR/MAPK and PI3K/AKT, and suppresses cancer stem cells (114–117). Cholinergic stimulation also prevents colorectal cancer progression by inducing trefoil factor 2 secretion by memory T cells and suppresses breast cancer by reducing PD-1 expression levels in CD4+ and CD8+ lymphocytes (118,119). Furthermore, cholinergic deprivation increases macrophage influx and TNF-α production, promoting cancer progression (107). These studies suggest a contribution of parasympathetic signaling in tumor suppression.

**Parasympathetic Tumor Promoters:** Conversely, cholinergic promotion of tumorigenesis and metastasis has been demonstrated in prostate and gastric cancers, respectively (32,92). Chrm1 expression in prostate cancer stromal cells is essential for metastasis (34,71). Other studies have indicated that Chrm3 mechanisms drive the pro-tumor effects of the parasympathetic nervous system. Chrm3 activation in gastric cancer induces Wnt-β-catenin signaling downstream of YAP (70,92,117). The Wnt-β-catenin signals expand cancer stem cells and induce NGF's promotion of nerve innervation in gastric cancer (92,117). In small cell lung carcinoma, Chrm3 activates MAPK/Akt signaling (115). In colon cancer, Chrm3 activates ErbB receptors downstream of the MMP-7 adhesion molecules that promote tumor

cell invasion (116). Converse to the previous section, these findings suggest parasympathetic signaling can promote tumor progression, depending on the type and stage of tumor.

**Implications of Neural Signaling on Intratumoral Immune Cells—**Stress, as manifested by enhanced sympathetic signaling and glucocorticoid production, contributes to many disorders, both benign and malignant. Although acute bouts of stress may enhance a CD4+ T-cell and B-cell mediated immune response, chronic repeated stress diminishes the immune response and renders important immune effectors anergic. Since many innate and adaptive immune cell types express or upregulate neural receptors including adrenergic receptors, it follows that immune cells are sensitive to neural signaling during oncogenesis.

We are now beginning to understand that the neuroregulation of inflammation plays a critical role in cancer, as inflammatory changes in nerves are observed in the early stages of some cancers (107,120). In breast cancer, CD11b+F4/80+ macrophages have been shown to infiltrate the tumor parenchyma upon pharmacologic sympathetic activation of adrenergic receptors and to contribute to a 30-fold increase in metastasis to the lymph nodes and lung. The β-adrenergic receptor blocker propranolol reversed this effect (120). CD11b+F4/80+ macrophages also secrete higher levels of GDNF in pancreatic adenocarcinoma cells compared to resting endoneurial macrophages, and a *CCR2*-deficient model of perineural invasion of cancer cells with reduced recruitment and activation of tumor-associated macrophages showed reduced CD11b+ F4/80+ macrophages and nerve invasion (75). Similarly, neuropathy of the sympathetic nervous system induced leukemic bone marrow infiltration in an acute myelogenous leukemia mouse model through the $\beta_2$-adrenergic receptor expressed in stromal cells (121). In another study, splenic vagal denervation suppressed cytotoxic T cells and promoted carcinogenesis (119). It has also been shown that noradrenaline stimulates IL-6 production and activates macrophages and other stromal cells in the tumor microenvironment (95). Macrophages are recruited by cancer cells through cancer cell-secreted colony stimulating factor and release GDNF to promote cancer migration and nerve invasion (75). Consistently, $\beta_3$-adrenergic signaling induces ovarian cancer cells to secrete brain-derived neurotrophic factor, which ultimately signals axonogenesis and plays a role in switching macrophages and neutrophils into immunocompetent M1- and N1-types and in recruiting and maintaining hemopoietic and mesenchymal stem cells (111,112).

Nerves can also interact with immune cells in an immune tolerizing fashion, allowing for tumor evasion and escape. Sensory neurons have been shown to attract myeloid-derived suppressor cells in melanoma, promoting an immune-tolerant, pro-tumor milieu (122). Within prostate cancer, regions rich in autonomic tumor-infiltrating nerves express high levels of the immune checkpoint protein programmed cell death ligand-1 (PD-L1). This inhibitory immune checkpoint ligand which binds and promotes anergy of cytotoxic CD8+ T-cells expressing the cognate receptor, PD-1. Regions high in PD-L1 expressing nerves were inversely correlated with those expressing CD8+ T-cells, and high density of PD-L1 nerves was associated with recurrence (123). Also supporting crosstalk between the autonomic nervous system and T-cell function, genetic parasympathetic stimulation of tumor decreased PD-L1 expression in tumors and PD-1 expression in T-cells, and enhanced CD8+/Treg ratios (110). In an analysis of breast cancer specimens, sympathetic nerve density was

associated both with high expression of immune checkpoints and poor prognosis. Ultimately, a connection between nerve crosstalk and immune effectors may have important ramifications for the future of immune checkpoint blockade and other immunotherapies, but warrants further investigation.

### Conclusions

Although advances in cancer treatment have improved patient outcomes, we still have much to learn about how tumors interact with their microenvironment. We are now beginning to realize that, in some solid tumors, infiltrating nerves and catecholaminergic signaling may play an important role in tumor initiation and progression. A deeper understanding of the mechanistic basis of cancer progression, specifically in regard to nerve-cancer crosstalk, will reveal new therapeutic targets and allow the repurposing of existing treatments, including neuromodulatory therapies to slow or stop cancer progression, or to be used in conjunction with chemo- or immunotherapies. As a greater understanding of nerve-cancer crosstalk and the neuro-immune axis emerges, new anti-neurogenic targets hold tremendous potential as novel opportunities for treating cancer (Table 1).

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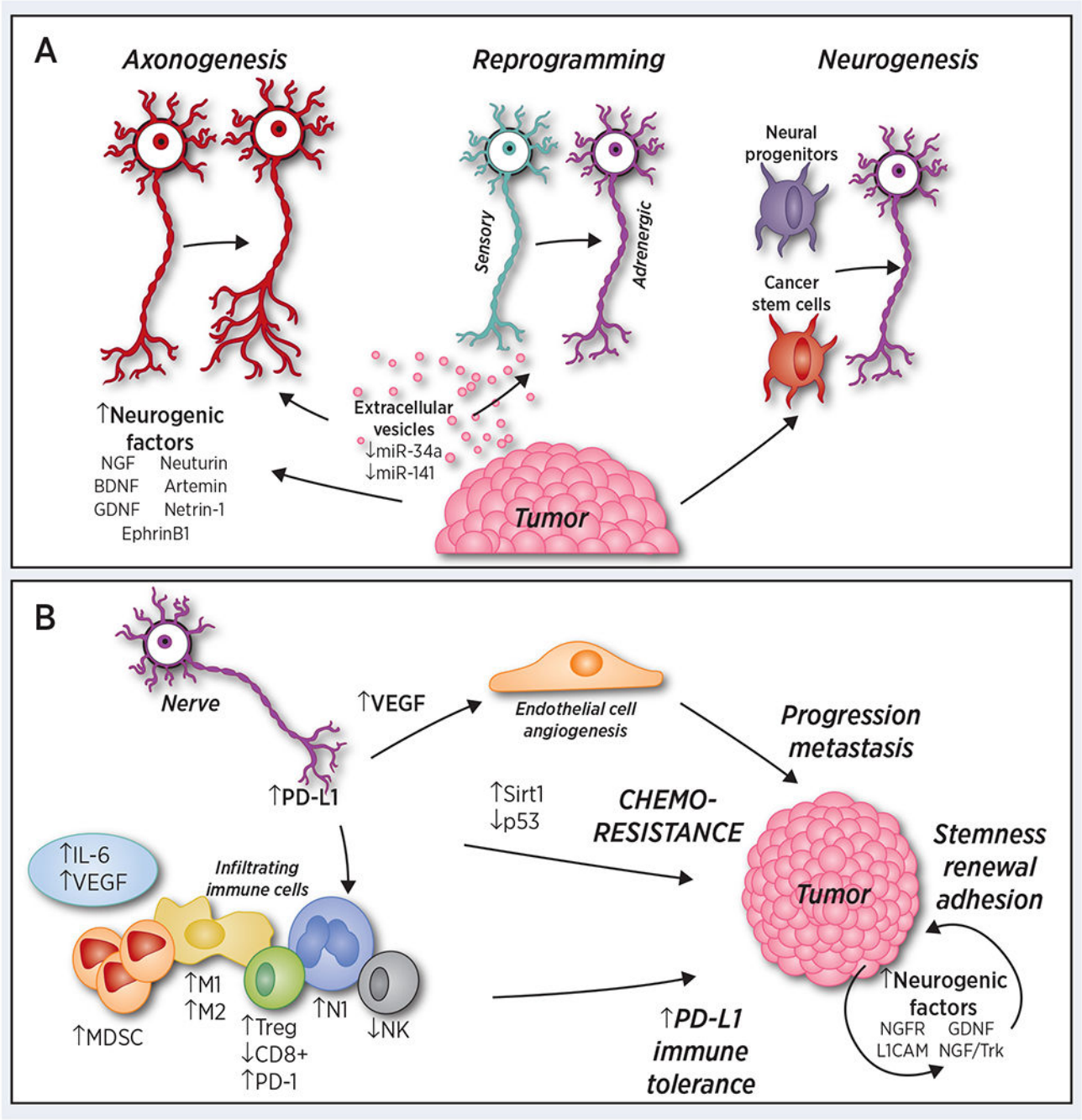


**Figure 1.**

**A) Cancer cells drive nerve alteration.** Cancer signals to induce nerve growth and innervation through multiple mechanisms. Cancer-induced axonogenesis includes the secretion of numerous neurogenic factors, axon-guidance molecules, and extracellular vesicles containing increased levels of axonal guidance molecules. Neural reprogramming occurs through extracellular vesicles containing orchestrated levels of miR-34a and miR-121, transforming a sensory nerve into an adrenergic nerve. Finally, cancer communicate with distant organs to recruit neural progenitor cells to initiate neurogenesis,

while cancer stem cells drive *de novo* neurogenesis. **B) Sympathetic innervation promotes the tumor microenvironment and tumor growth.** Sympathetic signaling induces an angiogenic switch through increased vascular endothelial growth factor (VEGF) levels and the induction of aerobic glycolysis. It also promotes the infiltration of CD11b+F4/80+, FoxP3+ Tregs, and myeloid-derived suppressor cells (MDSCs). It stimulates the secretion of interleukin (IL)-6 and decreases the numbers of CD8+ cells and natural killers (NK) cells. It participates in a tumor-type dependent M2-type/M1-type macrophage shift and N2-type/N1-type neutrophil shift. Sympathetic nerves also express protein programmed cell death-1 (PD-1) in some cancer types, potentially contributing to immune suppression. In addition, sympathetic signaling drive chemoresistance via p53-dependent Sirt1 signaling among other mechanisms, and expression of neurogenic ligands and receptors on tumor cells promote stemness and self-renewal within the tumor.

**Table 1.**

Emerging anti-neurogenic treatments

| TREATMENT | MECHANISM | OUTCOME | CLINICAL TRIAL | REFERENCE |
|---|---|---|---|---|
| Tanezumab; fulranumab | Neutralizing NGF antibody | Reduces secretion of NGF and migration of cancer cells, induction of neurites, and sympathetic nerve sprouting.<br>Has a limited impact on neural or cognitive function. | NCT02609828 (ongoing) | (57,124,125) |
| GW441756; carozantinib | Tyrosine kinase inhibitor | Decreases cancer cell migration and induction of neurites.<br>Has limited cognitive effects.<br>Previously failed to demonstrate survival benefit in men with metastatic castration-resistant prostate cancer. | NCT01522443 (completed)<br>NCT02219711 (ongoing) | (57) |
| Propranolol; carvedilol | β-blocker | Reduces nerve-cancer interaction and neurotrophin secretion.<br>Increases prostate cancer survival and reduced cancer-specific mortality. | NCT02944201 (ongoing)<br>NCT03152786 (ongoing) | (66,126,127) |
| Surgical denervation | | Causes gland atrophy and functional and structural deterioration of prostate epithelial cells. | | (128) |
| Botulinum toxin (BOTOX) | Neurotoxin | Increases apoptosis in cancer cells. | NCT01520441 (withdrawn) | (129) |
| siRNA encapsulated nanoparticles | Gene silencer | Reduces neural invasion. | | (4) |

Abbreviations: NGF, neuron growth factor; siRNA, small interfering RNA.