MINI REVIEW

Tumor Neurobiology and the War of Nerves in Cancer

Sam Faulkner1,2, Phillip Jobling1,2, Brayden March2,3, Chen Chen Jiang2,3, and Hubert Hondermarck1,2

ABSTRACT

Nerves are emerging regulators of cancer progression. Cancer cells induce the outgrowth of nerves in the tumor microenvironment through the release of neurotrophic factors, and in return nerves liberate neurotransmitters that activate cancer growth and dissemination. Although sympathetic nerves drive tumor angiogenesis via the liberation of noradrenaline, sensory and parasympathetic nerves stimulate cancer stem cells. Interestingly, recent evidence indicates that parasympathetic nerves can eventually inhibit tumor progression, suggesting a yin–yang type of regulation of cancer by nerves. From a broader perspective, the question of a higher level of control of cancer development by the central nervous system should be raised.

Significance: Nerves are emerging regulators of cancer initiation, progression, and metastasis. Here, we review the evidence to date and explore the basic and clinical ramifications of these findings.

INTRODUCTION

Peripheral nerves constitute an essential component of cellular microenvironments. With the exception of cartilage and lens, all human tissues are infiltrated by nerves of sensory, autonomic (sympathetic and parasympathetic), and/or motor origin. Nerves connect all body parts to the central nervous system (CNS) and are essential not only to locomotion, sensation, and cognition, but also to physiologic regulation of internal organs. However, nerves also have a trophic effect during tissue development, repair, and regeneration, a role that has been underestimated. Nerve dependence in tissue growth was initially established over 200 years ago in the context of limb regeneration in the salamander, where denervation of the limb prevents regeneration (1). This was later confirmed in embryogenesis and various processes of tissue repair where it was shown that the outgrowth of nerve endings (axonogenesis) in the cellular microenvironment is required for tissue growth (2). Although nerve endings are known to release a variety of neurotransmitters, hormones, and growth factors, the growth-stimulatory mechanisms of nerves during development and regeneration have remained unclear (3). Illustrative of the compartmentalization in science and medicine, the role of nerves in cancer growth has been understudied, and until recently nerves were not regarded as major contributors in tumorigenesis. Although nerves were known to be eventually surrounded and invaded by cancer cells, a process called perineural invasion (4), the perception was nevertheless that nerves were essentially passive bystanders in cancer. However, in the last 5 years, there has been a series of pioneering studies that have demonstrated the driving role of nerves in cancer initiation and progression. In this review, we describe the evidence for the role of nerves in cancer and discuss how it could affect both research and clinical practice.

DENERVATION AND THE DISCOVERY OF NERVE INVOLVEMENT IN TUMORIGENESIS

An overview of the current evidence demonstrating the impact of nerves in tumorigenesis is presented in Table 1. The initial demonstration that denervation can inhibit cancer progression was performed in mouse models of prostate cancer (5). The prostate gland is essentially innervated by autonomic nerves of sympathetic (adrenergic) and parasympathetic (cholinergic) origin, and surgical or chemical denervation of the prostate was found to result in a complete inhibition of prostate cancer growth and dissemination (5). On the one hand, denervation of adrenergic nerves or knockout of adrenergic receptors beta 2 (ARβ2) and beta 3 (ARβ3) inhibited the proliferation of stromal and cancer cells at early stages of prostate tumor development. On the other hand, denervation of cholinergic nerves or knockout of type 1 muscarinic acetylcholine receptors (CHRM1) inhibited tumor cell dissemination at later stages of the disease. The crucial role played by stromal cells, which express both ARβ and CHRM1 receptors, as a relay that promotes cancer cell...
growth was also noted. The authors also reported that the density of nerve infiltration in the tumor microenvironment of prostate cancer, indicative of axonogenesis, was increased in high-grade cancers compared with low-grade cancers or benign prostatic hyperplasia (5). The conclusion of this early study was that the activation of adrenergic signaling by the release of catecholamines from sympathetic nerves stimulated tumor growth, whereas cholinergic signaling that was activated by parasympathetic nerves stimulated tumor dissemination (5). Incidentally, this landmark demonstration of nerve dependence in cancer also provided a rationale for the long-reported lower incidence of prostate cancer in patients with spinal cord injuries where a functional denervation of the prostate occurs (6, 7).

Soon after the initial discovery of the protumorigenic role of nerves in prostate cancer, similar findings were reported in other malignancies. In mouse models spontaneously developing gastric cancer, denervation of the vagus nerve decreased tumor initiation and progression (8). The investigators analyzed the pharmacologic inhibition and genetic knockout of the type 3 muscarinic acetylcholine receptor (CHRM3) and found that it produced an inhibitory effect on both tumor initiation and progression that was similar to vagal denervation (8). In pancreatic cancer, it was demonstrated that the ablation of sensory neurons in mouse models of pancreatic adenocarcinoma can slow both the initiation and progression of cancer and prolong survival (9). It was also shown that the denervation of adrenergic nerves resulted in the inhibition of pancreatic tumor progression, and that adrenergic nerves release catecholamines that activate ADRB2-mediated adrenergic signaling in cancer cells (10). Interestingly, cholinergic nerves and cholinergic signaling have recently been shown to inhibit pancreatic tumor progression (11), raising the possibility of a more complex neural regulation in pancreatic cancer. More than 80% of axons running in the vagus nerve are sensory (12). Although sensory nerves have been shown to promote pancreatic cancer (9), vagal denervation accelerates pancreatic cancer (11). This observation argues for a distinct suppressive effect by the vagal cholinergic axons. It is important to note that whereas autonomic nerves are implicated in prostate (5) and gastric (8) cancers, sensory nerves are also involved in the stimulation of pancreatic cancer progression (9, 13, 14). Interestingly, a dense substance P–positive sensory innervation has been described in precancerous pancreatic lesions, and neuroendocrine cells were found to express the substance P receptor neurokinin 1-R (NK1R), suggesting neuroendocrine cells as the mediators of sensory nerve stimulation in early pancreatic tumorigenesis (14). The stimulatory role of sensory nerves has also been found in basal cell carcinomas, a nonmelanoma form of skin cancer emerging from

### Table 1. Evidence for neural regulation in cancer and cancer cell–induced axonogenesis

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Finding</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>Prostate</td>
<td>Adrenergic and cholinergic nerves stimulate tumor progression</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>Adrenergic nerves activate an angiogenic switch</td>
<td>(38)</td>
</tr>
<tr>
<td></td>
<td>Botulinum toxin–based denervation induces cancer cell apoptosis</td>
<td>(74)</td>
</tr>
<tr>
<td></td>
<td>Neurogenic expression in stem cells</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>Neurotrophic factors drive tumor axonogenesis</td>
<td>(26, 27)</td>
</tr>
<tr>
<td></td>
<td>Cancer incidence is lower in spinal cord injuries</td>
<td>(6)</td>
</tr>
<tr>
<td>Gastric</td>
<td>Vagus nerve stimulates cancer initiation and progression</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>Cholinergic signaling stimulates cancer stem cell growth</td>
<td>(8, 28)</td>
</tr>
<tr>
<td></td>
<td>Cholinergic signaling induces NGF secretion that in turn drives tumor axonogenesis</td>
<td>(28)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Sensory nerves stimulate tumor progression</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerve/NGF feed-forward loop promotes cancer progression</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>Parasympathetic nerves suppress tumorigenesis and cancer stemness</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>Neuronal cross-talk promotes tumorigenesis</td>
<td>(13, 14, 29)</td>
</tr>
<tr>
<td>Skin</td>
<td>Sensory innervation is necessary to tumor initiation and cancer stem cell growth</td>
<td>(15)</td>
</tr>
<tr>
<td>Breast</td>
<td>Axonogenesis is associated with tumor aggressiveness and driven by NGF</td>
<td>(30, 31)</td>
</tr>
<tr>
<td>Colon</td>
<td>Nerve infiltration is associated with tumor aggressiveness</td>
<td>(33, 34)</td>
</tr>
<tr>
<td></td>
<td>Neuroimmune regulation of cancer progression</td>
<td>(62)</td>
</tr>
<tr>
<td>Ovary</td>
<td>Tumor axonogenesis is driven by BDNF</td>
<td>(71)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>Axonogenesis is stimulated by cancer cell–released exosomes containing Ephrin B1</td>
<td>(73)</td>
</tr>
<tr>
<td>Glioma</td>
<td>Neurons stimulate cancer cell growth through the release of neuroligin-3 and pleiotropin</td>
<td>(16, 17)</td>
</tr>
</tbody>
</table>

NOTE: Nerves can stimulate cancer cells directly or indirectly through the tumor microenvironment.
epithelial cells, where surgical ablation of sensory cutaneous nerves in hair follicles blunts tumor formation via a mechanism involving the activation of nerve-derived hedgehog signaling in epithelial cells (15).

Brain cancer also provides an illustration of neural regulation in cancer progression. Neuronal cells have been shown to promote glioma growth through neuroligin-3 (NLGN3; ref. 16). NLGN3 is a synaptic protein, and soluble NLGN3 can be released from neuronal endings to stimulate glioma cell proliferation through a PI3K–mTOR signaling pathway (16). Interestingly, NLGN3 release is activated by neuronal activity (16) and can be targeted in vivo to inhibit glioma development (17). The release of other proteins, such as pleiotropin, from neural cells can promote glioma cell invasion (18), and in return glioma cells can also affect neuron activity (19). These studies in the CNS emphasize the tumor-promoting effect of neuronal cells.

Together, the pioneering studies described above have revealed the active role of nerves in cancer (Fig. 1). It is also clear that nerves can stimulate cancer growth and dissemination either directly or indirectly through the tumor microenvironment. Given the presence of nerves in most tissue microenvironments, it is anticipated that nerves may play a role in other if not all solid tumors, but this remains to be experimentally demonstrated.

**TUMOR AXONOGENESIS: AN EMERGING HALLMARK OF CANCER**

As nerves were not considered to be important for tumor progression, the outgrowth of nerves in the tumor microenvironment, or axonogenesis, has been overlooked. The other reason why nerves have been understudied in cancer is that they are difficult to observe in regular histology. Big nerve trunks can be seen in regular histology and constitute the basis for assessing perineural invasion in pathologic examination (4). However, most nerves in the tumor microenvironment are small trunks or even individual axons that require specific neuronal biomarkers to be detected in IHC. The pan-neuronal marker PGP9.5 (protein gene product 9.5/UCH-L1/PARK5) can be used as an IHC marker to detect all nerve types. Other neuronal biomarkers include peripherin, a type III intermediate filament protein expressed mainly in neurons of the peripheral nervous system, as well as tubulin beta-3. Autonomic nerves can be differentiated by using tyrosine hydroxylase for adrenergic nerves and vesicular acetylcholine

![Figure 1. Molecular basis and functional impact of tumor innervation. The outgrowth of nerves in the tumor microenvironment (axonogenesis) is driven by the secretion of neurotrophic factors (NTF) by cancer cells and takes place from peripheral nerves in the surrounding tissues that emerge from the CNS and associated neural ganglia. In return, nerve endings in the tumor microenvironment, which can be of adrenergic, cholinergic, or sensory origin, release neurotransmitters (NT) that stimulate corresponding receptors in stromal cells, immune cells, and cancer stem cells, resulting in the regulation of cancer growth and metastasis. Therefore, the stimulation of cancer cell growth can be direct and indirect through the stimulation of other cell types in the tumor microenvironment. Of note, the stimulation of endothelial cells by noradrenalin (NA) released from adrenergic nerves induces an angiogenic switch that fuels tumor growth and metastasis. The presence of sensory nerves in the tumor microenvironment can also participate in cancer pain.](cancerdiscovery.aacrjournals.org)
transporter (VACHt) for cholinergic nerves. Additionally, peripheral glial cells (Schwann cells) can also be identified in the tumor microenvironment by immunostaining for glial fibrillary acidic protein or S100. Assessment and quantification of nerves by IHC in the tumor can be done by direct microscopic observation, but quantification of nerve density may also necessitate digital computer-based analysis as illustrated in prostate cancer (20). The discovery of the regulatory role of nerves in cancer progression has led to more recent insightful explorations of the distribution of nerve subtypes in human tumors, using the above neuronal biomarkers and methodologies.

In prostate cancer, adrenergic and cholinergic nerves are found around and inside the tumors, and the density of nerves is increased with tumor aggressiveness (5, 21). Prostate cancer areas of higher histologic grade present with more nerves than cancer areas of lower grade or benign prostatic hyperplasia. During embryonic development or tissue regeneration, axonogenesis is stimulated by the release of neurotrophic growth factors from tissues in order to attract nerve terminals. The mechanisms underlying axonogenesis involve neurotrophic growth factors such as those of the neurotrophin family, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4/5 (NT4/5), which have been shown to drive axonogenesis through the stimulation of tyrosine kinase receptors expressed in nerve terminals (22). Further, the expression of axon guidance molecules, such as Robo-Slit or semaphorins (23), has been described in human tumors (24, 25) and may also contribute to tumor axonogenesis. Interestingly, in the tumor microenvironment, a similar contribution of both neurotrophic growth factors and axon guidance molecules produced by cancer cells could drive axonogenesis. An increased production of the precursor for NGF (proNGF) in prostate cancer cells was shown to be associated with nerve density in the tumor microenvironment (26). In addition, cancer cells were able to induce axonogenesis in vitro, suggesting that proNGF/NGF are molecular mediators of tumor axonogenesis in prostate cancer (26). The off-target effect of granulocyte colony-stimulating factor (G-CSF) on nerve outgrowth also appears to promote prostate cancer development (27). In gastric cancer, a similar increase in nerve density in the tumor microenvironment induced by the release of NGF from cancer cells was observed (28). Gastric cancer cell-derived NGF promotes parasympathetic tumor innervation that stimulates cancer cell growth through cholinergic-regulated WNT signaling (28). NGF also drives a feed-forward loop in pancreatic cancer, whereby the upregulation of NGF and BDNF increases sympathetic innervation and local accumulation of noradrenaline to stimulate the growth of pancreatic cancer cells (10). In pancreatic cancer, the production of the leukemia inhibitory factor (LIF), a member of the interleukin 6 family, by cancer cells has also been shown to contribute to tumor innervation (29). Importantly, in other malignancies such as breast and colorectal cancers, where nerve dependence has not yet been formally reported, increased axonogenesis in the tumor microenvironment has also been described. In breast cancer, nerves are detected in approximately a third of invasive ductal carcinomas, and nerve density is associated with tumor aggressiveness as well as NGF release by cancer cells (30, 31). Early research suggests the involvement of sympathetic signaling in breast cancer progression (32); however, molecular mechanisms underlying these findings are yet to be elucidated. In colorectal cancer, axonogenesis is also identified as a characteristic of aggressive tumors (33, 34). It has been shown that NGF-induced cholinergic innervation may potentially stimulate colorectal cancer (28) and that CHRM3 activation enhanced intestine tumorigenesis in vivo (25), but the molecular mechanisms driving axonogenesis in colon cancer are still to be clarified.

Overall, axonogenesis is emerging as a new hallmark of cancer, and neurotrophic growth factors released by cancer cells appear to be the drivers of nerve infiltration in the tumor microenvironment. Although an increased number of neurons (neurogenesis) in neural ganglia has been shown to be associated with axonogenesis in prostate cancer (35), the occurrence of neurogenesis in other cancer types has not been reported, and therefore the extent of neurogenesis in human tumors is unclear. Taken together, tumor axonogenesis can be compared with axonogenesis during embryonic development, where the release of neurotrophic growth factors by organs drives axonogenesis. To date, neurotrophic growth factors of the NGF family have been clearly identified, but, as in development, it is likely that a combination of neurotrophic growth factors and axon guidance molecules drive the growth of different types of neurons in tumor axonogenesis.

**SYMPATHETIC NERVES DRIVE TUMOR ANGIOGENESIS**

Sympathetic/adrenergic nerves are known to be closely associated with blood vessels, and the release of noradrenaline by sympathetic nerves mediates blood vessel constriction through the contraction of the surrounding smooth muscle cells. During development, sympathetic nerves and blood vessels grow simultaneously, and sympathetic nerves are necessary to structure the vascularization of tissues and organs (36, 37). In cancer, a recent study by Zahalka and colleagues has demonstrated that adrenergic nerves activate tumor angiogenesis by promoting an angiometabolic switch in endothelial cells (38) and has attracted considerable interest (39–41). This study has shown that the infiltration of adrenergic nerves in the microenvironment of prostate cancer results in the liberation of noradrenaline by nerve endings that can subsequently stimulate Adrb2 expression in endothelial cells. Upon stimulation by noradrenaline, Adrb2 receptors induce a change in endothelial cell metabolism toward the inhibition of oxidative phosphorylation. As endothelial cells rely on aerobic glycolysis for angiogenesis, the noradrenaline-induced Adrb2 activation promotes a burst of tumor angiogenesis that fuels cancer progression. It had previously been shown that the activation of the sympathetic nervous system can stimulate cancer progression (32), but the mechanisms remained unclear. Circulating adrenaline released from the adrenal medulla (eventually in response to stress) was thought to contribute, but evidence against this hypothesis has been provided (42). The study by Zahalka and colleagues (38) elegantly provides a clear mechanism whereby sympathetic innervation directly activates cancer progression through the liberation of noradrenaline in the microenvironment, resulting in the activation of endothelial cells and angiogenesis.
Importantly, the stimulatory influence of sympathetic nerves on tumor angiogenesis provides a rationale for the reported potential impact of beta blockers on the survival of patients with cancer. Beta blockers are currently prescribed for cardiovascular diseases and anxiety disorders, but some retrospective studies have suggested a positive impact on the survival of patients with prostate (43), breast (44, 45), and ovarian cancers (46), as well as multiple myeloma (47). Up until now, it was unclear how beta blockers could improve the survival of patients with cancer, but the results of Zahalka and colleagues (38) provide a possible explanation: The inhibition of ADRβ2 signaling induced by sympathetic nerves in endothelial cells by beta blockers results in the inhibition of angiogenesis. Evidence supporting this mechanism has been demonstrated in pancreatic cancer, where the use of beta blockers was associated with significantly improved survival of patients with pancreatic cancer undergoing surgery, compared with no beta blocker use (10). Therefore, prospective clinical trials are investigating the therapeutic value of beta blockers in prostate (NCT02944201 and NCT03152786), gastrointestinal (NCT03245554), and breast (NCT01847001) cancers, as well as melanoma (NCT02962947) and multiple myeloma (NCT03245554). The outcome of these clinical trials is highly awaited, as it may lead to the repurposing of this commonly used class of drugs as anticancer therapeutics.

The discovery that sympathetic nerves drive tumor angiogenesis reveals that the regulation of angiogenesis is more complicated than previously thought. To date, the concept was that tumor angiogenesis was essentially driven by the secretion of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), by cancer cells and secondarily immune cells in the tumor microenvironment. The novel concept introduced by Zahalka and colleagues (38) is that the neural compartment is regulated by neural inputs, and further investigations are expected to clarify the molecular contribution of nerves to cancer stem cell expansion.

The case of pancreatic cancer is particularly interesting, as a recent study has highlighted that cancer stemness is suppressed by cholinergic nerves (11). In contrast to previous denervation experiments in prostate (5) and gastric (8) cancers, the authors have observed that denervation of cholinergic nerves stimulates pancreatic cancer progression (11). The stimulatory impact of cholinergic denervation was due to a mechanism involving the release of acetylcholine by cholinergic nerves that activates the muscarinic receptor CHRM1 in pancreatic cancer stem cells. CHRM1 signaling inhibits downstream MAPK/EGFR and PI3K/AKT pathways in pancreatic cancer cells (11). Together, enhanced cholinergic signaling led to a suppression of the cancer stem cell (CSC) compartment, CD11b+ myeloid cells, TNFα levels, and liver metastases. These data suggest that cholinergic signaling can suppress the growth of pancreatic cancer cells. Therefore, pancreatic cancer cells appear to be under a balanced yin-yang type of neural influence (Fig. 2). On the one hand, sensory (9, 14) and sympathetic (10) nerves stimulate the growth of pancreatic cancer cells. Therefore, pancreatic cancer cells to cancer stem cell expansion through muscarinic receptors and downstream cholinergic signaling involving YAP and WNT pathways in stem cells (8). In prostate cancer, a neurogenic gene-expression profile is associated with stem cells, suggesting that cancer stem cells can develop a molecular profile oriented toward the stimulation of tumor angiogenesis (54). Therefore, there is increasing evidence showing that the cancer stem cell compartment is regulated by neural inputs, and further investigations are expected to clarify the molecular contribution of nerves to cancer stem cell expansion.

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A HIGHER REGULATION LEVEL OF CANCER DEVELOPMENT

All nerves are connected to the CNS and ultimately the brain through direct and indirect neuronal networks. So far, and in contrast to other systemic diseases such as cardiovascular or endocrine disorders, the notion of a brain-mediated regulation was largely missing in cancer. However, now that tumor innervation and the impact of nerves have been uncovered, thereby highlighting a physical and functional connection between the tumor and the CNS, the question of a potential higher level of regulation of cancer development should be raised.

The brain is not only the central hub for neural communications in the body, but it also integrates signals from the outside world. On the one hand, cancer-associated pain is the illustration that neuronal information from tumors can travel to the brain (56). On the other hand, external stimuli and psychosocial interactions are functionally integrated at
The War of Nerves in Cancer

In innervation, the development of pancreatic cancer cells through the liberation of substance P (SP) and noradrenaline (NA), and the subsequent activation of the neurokinin-1 receptor (NK1R) as well as the adrenergic receptor beta 2 (ADRB2), respectively. Sympathetic nerves also activate the release of NGF by cancer cells that activates the corresponding receptor tyrosine kinase (RTK) in neurons, leading to more axonogenesis in the tumor microenvironment [10]. In contrast to sensory and sympathetic nerves, parasympathetic nerves inhibit pancreatic cancer cell growth via the liberation of acetylcholine (ACh) and the activation of cholinergic muscarinic receptor 1 (CHRM1), leading to the inhibition of PI3K/AKT and EGFR/ERK [11]. Cholinergic signaling also leads to the suppression of the cancer stem cell compartment, CD11b+ myeloid cells, TNFα levels, and metastatic growth in the liver [11]. This opposite impact of sensory and sympathetic vs. parasympathetic nerves suggests that the development of pancreatic cancer is regulated through a balance of neural innervation.

Figure 2. Yin–yang type of neural regulation in pancreatic cancer. In pancreatic cancer cells, sensory [9, 14] and sympathetic [10] nerves activate the growth of pancreatic cancer cells through the liberation of substance P (SP) and noradrenaline (NA), and the subsequent activation of the neurokinin-1 receptor (NK1R) as well as the adrenergic receptor beta 2 (ADRB2), respectively. Sympathetic nerves also activate the release of NGF by cancer cells that activates the corresponding receptor tyrosine kinase (RTK) in neurons, leading to more axonogenesis in the tumor microenvironment [10]. In contrast to sensory and sympathetic nerves, parasympathetic nerves inhibit pancreatic cancer cell growth via the liberation of acetylcholine (ACh) and the activation of cholinergic muscarinic receptor 1 (CHRM1), leading to the inhibition of PI3K/AKT and EGFR/ERK [11]. Cholinergic signaling also leads to the suppression of the cancer stem cell compartment, CD11b+ myeloid cells, TNFα levels, and metastatic growth in the liver [11]. This opposite impact of sensory and sympathetic vs. parasympathetic nerves suggests that the development of pancreatic cancer is regulated through a balance of neural innervation.

The potential clinical applications of tumor axonogenesis are in relation to cancer diagnosis, prognosis, and treatment. At this stage, the extent of nerve infiltration in human tumors and the relationship between cancer diagnosis and prognosis remain to be ascertained in large clinical cohorts. Similarly, strategies...
to target nerves are already in the making, but translation to the clinical arena will require the precise description of the neural landscape across the many different human cancers. **Diagnostic and Prognostic Value of Nerves and Neurotrophic Factors**

Various studies have shown that nerve density is increased in primary human tumors and that there is an association with metastatic or aggressive disease (66). Therefore, the value of quantifying nerve infiltration by pathologic examination of clinical specimens to predict patient outcome or treatment response should now be investigated in large retrospective and prospective cohorts of patients with cancer. In cancers where diagnosis and prognosis are intimately linked, quantifying nerve density may also help differentiate benign tumors and indolent cancer from aggressive disease. For instance, given that autonomic nerve density is increased in prostate cancer versus benign prostatic hyperplasia, as well as in aggressive versus indolent disease (5, 21), the quantification of autonomic nerve density in prostate cancer could ultimately improve histologic grading and prognosis of the disease. Differentiating the subtypes of nerves may be required and would necessitate the use of IHC detection of specific neuronal biomarkers. Furthermore, detection and quantification of neurotrophic growth factors driving tumor axonogenesis may also have a diagnostic or prognostic value. In prostate cancer, the expression of proNGF by prostate cancer cells contributes to axonogenesis in the tumor microenvironment and correlates with histologic grade (26), and in thyroid cancers proNGF is increased in cancer as compared with normal thyroid tissue (70). In pancreatic (10) and gastric (28) cancers, where NGF production by cancer cells drives axonogenesis, the value of NGF as a prognostic biomarker should be investigated. In breast cancer, the expression of NGF is associated with lymph node invasion (30), and in ovarian cancer the production of BDNF drives adrenergic nerve infiltration (71). Therefore, given their role in tumor innervation, neurotrophic factors may be used as diagnostic and prognostic cancer biomarkers. In addition, because neurotrophic factors can be released into the blood, what remains to be determined is if the increased expression of neurotrophic factors in various cancers could also be detected and quantified in the blood of patients with cancer. Neuropeptides in general are increasingly regarded as new potential biomarkers in cancer (72), and the recent demonstration that exosomes produced by cancer cells can stimulate tumor innervation (73) raises the possibility that exosomes containing neurotrophic growth factors or neuronal guidance molecules may represent novel cancer biomarkers of clinical importance and utility. **Tumor Denervation–Based Cancer Therapies**

In terms of therapeutic strategies, although successfully applied in animal models, surgical denervation would probably be challenging to implement in humans. Not only does organ innervation originate from multiple entry points from the spinal cord and neural plexuses outside of the CNS, but also innervation is important to the metabolic and physiologic regulation of most organs, and therefore surgical denervation would have unwanted side effects. However, the injection of neurotoxic drugs into the tumor may be more feasible, as it can be targeted to specific subtypes of neurons with limited side effects. For instance, a neurotoxic drug such as the botulinum toxin (Botox), an inhibitor of cholinergic signaling used in a range of clinical situations from muscle spasms to cosmetics to resorb wrinkles, could be used to mimic autonomic denervation without affecting sensory or motor afferences. Botulinum toxin has already been tested in animal models to denervate gastric tumors (8), and clinical trials are ongoing to test this approach in gastric cancer. In prostate cancer, the results of the first phase I clinical trial of botulinum toxin–based denervation of the prostate are already available and have found that nerve density in the tumor was decreased by botulinum toxin injection, resulting in an increased apoptosis of prostate cancer cells (74). These results confirm the involvement of cholinergic signaling in the growth of human prostate cancer, hence confirming data obtained in mouse models and suggesting that botulinum toxin could be used in prostate cancer treatment. The results of ongoing as well as future clinical trials in the areas of surgical and chemical denervation of human tumors are highly awaited. **Targeting Neurotrophic Factors**

As compared with surgical or chemical denervation, preventing tumor innervation by targeting neurotrophic factors involved in tumor axonogenesis may be a more suitable therapeutic strategy, as preexisting innervation of the organ would remain intact. As demonstrated during the development of the peripheral nervous system, neurotrophic growth factors such as NGF are necessary to neuronal growth, but once the innervation of a tissue is established, they are not involved in neuronal maintenance. This is well illustrated with NGF, which has no significant neurotrophic activity in the adult and essentially acts as a mediator of pain through the stimulation of its tyrosine kinase receptor TrkA in sensory neurons (75). Animal experiments and clinical trials have already shown that systemic injections of anti-NGF antibodies have no significant impact on neuronal and cognitive activities (75). NGF has a neurotrophic effect that drives tumor axonogenesis (10, 28, 30); thus, targeting NGF could prevent the outgrowth of nerves in the tumor microenvironment (76). The use of anti-NGF blocking antibodies and inhibitors for TrkA (77) or NGF-targeting siRNA encapsulated into nanoparticles (78) has already demonstrated the ability to decrease cancer growth and metastasis in animal models, with further translation into clinical trials warranted. As anti-NGF blocking antibodies are already in clinical trial for the treatment of chronic and arthritic pain (75), they could be repurposed to inhibit tumor axonogenesis and therefore tumor growth. In addition to targeting cancer development, anti-NGF antibodies may also decrease cancer pain, and there are already animal experiments showing the value of targeting NGF for the purpose of alleviating cancer-induced pain (79). Together, these findings show that, albeit at an early stage in humans, targeting neurotrophic factors in cancer therapy offers the unique potential to simultaneously address cancer progression and cancer-induced pain. **CONCLUSION**

The newly discovered role of nerves in regulating cancer initiation and progression opens the way for more basic and
clinical research in the emerging area of cancer neurobiology. Defining the extent and the functional impact of neuronal outgrowth in human tumors now needs to be carefully explored. After decades of cancer cell-centered genetic investigations of tumorigenesis, the essential role of the tumor microenvironment has progressively emerged, and in particular the long-underestimated role of nerves now appears to form a cornerstone. Of particular interest is the fact that neurosignaling can regulate cancer cell growth directly, or indirectly through the tumor microenvironment, including the control of angiogenesis. From a broader perspective, a promising ramification of the role of nerves in tumorigenesis is that it provides an opportunity to develop a more holistic understanding of cancer biology within the larger context of neurophysiological regulation of the human body. Thus, cancer neurobiology appears as a new angle for developing innovative clinical strategies for the diagnosis, prognosis, and treatment of cancer, including cancer pain, and translating the concept of neural regulation of cancer into clinical practice is the next challenge.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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