Received: 4 June 2021 | Revised: 18 August 2021 | Accepted: 25 August 2021

DOI: 10.1096/fba.2021-00066

FASEB BioAdvances WILEY

REVIEW

Neural regulations of the tumor microenvironment

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This article is part of the Neuroscience of Cancer, Regeneration, and Immunity special collection.

Abstract

The identification of nerves in the tumor microenvironment has ushered in a new area of research in cancer biology. Numerous studies demonstrate the presence of various types of peripheral nerves (sympathetic, parasympathetic, sensory) within the tumor microenvironment; moreover, an increased density of nerves in the tumor microenvironment correlates with worse prognosis. In this review, we address the current understanding of nerve-mediated alterations of the tumor microenvironment and how they impact disease through a variety of processes, including direct nerve-cancer cell communication, alteration of the infiltrative immune population, and alteration of stromal components.

KEYWORDS

cancer, immune escape, neuro-immune communication, tumor innervation, tumor microenvironment

1 | INTRODUCTION

The tumor microenvironment (TME) is a complex mixture of cancer cells, immune cells, vasculature, and cancer-associated fibroblasts (CAFs). Within this unique environment, these varied cell types communicate and interact with each other generating conditions optimal for tumor growth. Importantly, metabolic changes associated with cancer result in a low pH within this microenvironment. This alteration inhibits activated immune cells allowing cancer cells to escape immune detection.[1–3] In addition, cancer cells express key check point signals that further aide in disarming the immune response and promoting immune escape.[4–6] These mechanisms of immune protection are major contributing factors to disease progression. Cancer-associated metabolic changes also create a hypoxic environment generating signaling molecules that promote angiogenesis, further fueling cancer growth and progression.[7] While much work has led to our mechanistic understanding of how these changes promote disease progression, more recent findings indicate that the TME is even more complex than initially imagined. Pioneering studies have heralded in the importance of tumor-infiltrating nerves to the TME.[8,9] While the role of nerves in tissue regeneration and embryogenesis has been, and continues to be, well studied, only recently have nerves been identified as key components of the TME. The emerging field of tumor innervation is focusing attention on the neural regulation of cancers and the complexity of the TME.

The nervous system is a large and complex component of biological organisms and plays a variety of functions, including the maintenance of homeostasis, immune regulation, tissue organization, and development. Intra-tumoral nerves are newly formed or recruited fibers that infiltrate the TME. Similar to other nerves, intra-tumoral fibers likely retain diverse functions which are utilized to enhance tumor progression. To date, three types of neurons

(sympathetic, parasympathetic, and sensory) have been identified within tumor tissues.[10,11] Their overwhelming presence in most solid tumors has put the nervous system center stage in the field of cancer biology. However, how intra-tumoral nerves mechanistically contribute to disease remains largely undefined. Here, we will review key published works that provide compelling evidence for the presence of peripheral nerves within the TME and discuss how they may impact disease.

2 | IDENTIFICATION OF VARIOUS NERVES IN CANCER

A summary of studies focused on intra-tumoral nerves in cancer and the TME are catalogued in Table 1.

While the well-studied presence of nerves within developing and regenerating tissues indicates they are necessary for proper organogenesis and patterning, only recently have nerve ablation studies suggested that nerves may critically contribute to cancer initiation and progression.[8,9,12–18] The first well-established evidence for a pro-tumorigenic function of nerves was reported in prostate cancer.[8] Facilitating the study of nerves in this particular cancer is the discrete origin of both sympathetic and parasympathetic nerves that innervate the gland. This anatomical design allows easy manipulation and ablation of nerves, thereby enabling the study of their downstream effects on prostate cancer initiation and progression. Both chemical and surgical ablation of autonomic nerves innervating the prostate gland inhibit tumor proliferation and metastasis.[8] Additionally, a distinctive role for adrenergic nerves, promoting early cancer proliferation and growth, and cholinergic nerves, promoting tumor dissemination, was demonstrated. These findings establish a potential for neuropeptide signaling in tumor biology. Histological staining of patient samples indicates a correlative relationship between nerve density and pre-cancerous and cancerous staging in prostate cancer suggesting that densely innervated tumors grow faster than sparsely innervated disease.[8,9] This seminal study provides the foundation for additional studies confirming the contribution of intra-tumoral nerves to cancer. For instance, in the context of pancreatic ductal adenocarcinoma (PDAC), recurrent or chronic pancreatitis is driven in part by inflammation promoted by afferent nerves in the pancreas. Ablation of these sensory nerves decreases inflammation, delays the formation of PDAC in a Kras-driven mouse model and increases survival.[18] Furthermore, sensory innervation of the pancreas and its direct communication with cancer cells promotes pancreatic intraepithelial neoplasms (PanIN).[19] Additionally, patient survival has been negatively correlated with nerve density and nerve size in patients with pancreatic cancer, associating increased innervation with pancreatic cancer staging.[20]

A similar denervation study in squamous cell carcinoma of the tongue shows that removal of the superior cervical ganglia inhibits tumor growth and invasion.[21] Additionally, innervation of breast cancer correlates with tumor severity, while ablation of the nerves supplying the TME decreases tumor volume and growth.[22,23] Furthermore, *in vitro* experiments with immortalized breast cancer cells demonstrate their production of NGF and the promotion of neurite outgrowth when in co-culture with PC12 cells, suggesting a mechanism whereby breast cancer cells mediate tumor innervation.[24] In a transgenic melanoma mouse model, chemical ablation of adrenergic nerve terminals with 6-hydroxydopamine not only slows tumor growth but also alters the genetic profile of the tumor suggesting that intra-tumoral nerves

TABLE 1 Presence of nerves in the tumor microenvironment

Cancer type	Parasympathetic	Sympathetic	Sensory	Citation
Breast cancer	✓	✓		22–24
Cervical cancer			✓	39
Colorectal cancer		✓		28,29
Esophageal cancer		✓		31
Gastric cancer	✓	✓	✓	26,27,74
Head and Neck Squamous Cell Carcinoma (HNSCC)		✓	✓	38,40
Melanoma			✓	25,61
Pancreatic cancer	✓	✓	✓	18,20,33,57
Prostate cancer	✓	✓		8,9
Basal cell carcinoma			✓	43
Thyroid cancer		✓		30,67,83

influence epigenomic cancer regulation.[25] Gastric adenocarcinomas are highly innervated by cholinergic nerves and the tissue density of nerves increases with disease progression from a pre-neoplastic stage to fully formed adenocarcinoma.[26] Surgical denervation of the vagal nerve impedes this progression while also inhibiting tumor growth at later stages.[26] Similarly, in carcinogen-induced gastric cancer ablation of the myenteric plexus hinders tumor growth.[27] In colorectal cancer, nerve density is associated with aggressive tumor behavior and poor patient prognosis. *In vitro* studies using dorsal root ganglia demonstrate that the addition of colorectal cancer cell supernatant increases axonogenesis suggesting the presence of cancer-derived signals promoting this morphological change.[28,29] More recently, innervation has been described in papillary thyroid cancer and esophageal cancer. In papillary thyroid cancer nerve density was increased in cancerous tissue compared to adjacent benign tissue and was also positively associated with perineural invasion and tumor aggressiveness.[30] Similarly, innervation of esophageal cancer is a negative prognostic factor for patients.[31] Additionally, production of NGF by esophageal cancer cells *in vitro* increase neurite outgrowth of PC12 cells, suggesting that esophageal cancer cells themselves promote innervation of the tumor environment.[31] Taken together, these studies indicate a relationship between the presence of functional nerves in the tumor environment and disease prognosis.

While we have discussed how the presence of nerves in the TME promotes tumor growth and development, it is also important to understand the deviations from this observation. For example, while the presence of sympathetic nerves in the TME of breast cancer patients promotes disease progression, parasympathetic nerves, also present in this TME, exhibit an anti-tumor effect.[32] Similarly, while adrenergic signaling promotes pancreatic cancer development and progression, cholinergic signaling inhibits PDAC initiation and progression.[33] This difference in the function of distinct nerve types suggests the presence of an environmental or cell-specific determinant(s) mediating the effect(s) nerves impose on cancer progression. Further studies will shed more mechanistic light on this possibility.

Another pertinent deviation in the contribution of nerves to cancer initiation and progression occurs in hematological cancers and myelodysplasias. The bone microenvironment, where hematological stem cells (HSCs) are housed, consists of a complex mixture of mesenchymal, nervous, and hematological cells and tissues. Sympathetic nerve signaling in the bone microenvironment contributes to the maintenance of HSC differentiation, replication, and egress.[34,35] Ablation of sympathetic nerves innervating the bone marrow *induces* development of myeloproliferative diseases.[36,37] Additionally, similar to how various tumor types promote their own innervation, loss of sympathetic innervation and neuropathy in the bone marrow appears to be instigated by the developing cancer itself.[36–42] This is further compounded by evidence that some solid tumors arise preferentially from highly innervated microenvironments.[43] This dichotomy in the role of nerves in the development and progression of cancer is poorly understood and potentially results from differences in the biological needs of specific cancers or differences in the microenvironment in which the cancer propagates. As this area of research continues to mature, a clear mechanistic picture of contributions of nerves to hematological malignancies will emerge.

3 | FUNCTION OF NERVES IN CANCER CELL BEHAVIOR AND THE TUMOR MICROENVIRONMENT

While nerves have been identified in numerous solid tumors, and the increase of fiber density correlates with increased tumor size and stage, how nerves influence and change the cancer progression remains largely undefined. Identified functions of nerves in the tumor microenvironment have been summarized in Table 2. There are two main aspects of this relationship we will review: the interaction between nerves and cancer cells and the interaction between nerves and the non-cancerous cellular components of the TME.

3.1 | Nerve-cancer cell communication: adrenergic innervation

Denervation studies indicate that loss of functional nerves from associated tumor tissue inhibits tumor growth suggesting that direct nerve-cancer cell communication may control cancer progression. Indeed, numerous studies identified neurotropic receptors expressed across various cancer cell lines as well as in patient tumor samples.[10,44–46] However, recent studies suggest that neural signaling in cancer may be more complex. For instance, the *in vitro* treatment of cancer cells with adrenergic agonists promotes their proliferation, while adrenergic antagonists impede it.[47] Epinephrine and norepinephrine are the main signaling molecules that bind adrenergic receptors. These catecholamines are associated with states of stress and can be modulated at the local and systemic levels. Much of the research involving the immune system, cancer and adrenergic signaling focuses on systemic catecholamine changes and how states

TABLE 2 Summary of described functions of nerves on cancer cells and in the tumor microenvironment

Mechanism	Parasympathetic	Sympathetic	Sensory	Citation
Cell proliferation	✓	✓	✓	47,50–53,63,64,66,73,75
Regulation of immune cells	✓	✓	✓	32,61,80–85
Cancer pain			✓	72
Angiogenesis		✓	✓	89–92
Metastasis		✓	✓	57,63,66,69
Innervation	✓	✓	✓	55,56,58,65,74
Lymphangiogenesis		✓		93,94
Regulation of extracellular matrix		✓	✓	95
Transdifferentiation		✓		45,54

of sustained stress influence these systems. More recent studies focused on catecholamine signaling in cancer indicate that *local* increases in catecholamine levels within the tumor environment, rather than systemic changes, significantly influence cancer progression.[48,49] In determining the necessity of circulating catecholamines in breast cancer progression, mice underwent splanchnic denervation to block catecholamine synthesis and release from the adrenal glands. Analysis of circulating catecholamines confirmed successful denervation as circulating epinephrine levels significantly reduced and stress did not significantly change them. Despite this loss of circulating epinephrine, breast cancer growth and metastasis did not change following splanchnic denervation. Moreover, intra-tumoral catecholamine levels did not diminish significantly. This study indicates the importance of *local* catecholamine release during stress events and its contributions to breast cancer progression.[48]

Epinephrine and norepinephrine signaling through β-adrenergic receptors expressed on cancer cells increases cAMP (Figure 1).[50,51] β2 adrenergic signaling also promotes cell proliferation through downstream activation of sonic hedgehog (Shh) and its targets.[52] Consistent with this, inhibition of Shh signaling or knockdown of the Gli1 transcription factor, decreases proliferation following activation of β2 adrenergic signaling.[52] The effects and influence of adrenergic signaling are not limited to proliferation but extend to more complex processes such as cellular differentiation and dormancy. A major challenge for cancer patients is the reactivation of dormant cancer cells which develop into new tumors following treatment. Dormant prostate cancer cells are stimulated to re-enter the cell cycle by adrenergic signaling, secondary to cAMP-mediated downregulation of GAS6.[53] Furthermore, adrenergic signaling mediates a neuroendocrine trans-differentiation process that is characteristic of prostate cancer. Trans-differentiation of prostate adenocarcinoma cancer cells to a neuroendocrine state occurs in castration-resistant prostate cancer following treatment and designates a shift towards treatment resistance and poor patient prognosis.[45,54] The level of mRNA expression of ADRB2, which encodes the β2-adrenergic receptor, correlates with the metastatic potential of prostate cancer. Under androgen-deprivation conditions, high levels of ADRB2 were necessary for the LNCaP prostate cancer cells to undergo neuroendocrine transdifferentiation. Moreover, high levels of ADRB2 correlate with a higher incidence of neuroendocrine transdifferentiation in xenograft models.[54] Additionally, downregulation of ADRB2 in prostate cancer cells increases canonical Wnt signaling and decreases neuroendocrine genes, suggesting ADRB2 signaling contributes to the induction of neuroendocrine transdifferentiation of prostate cancer cells via inhibition of canonical Wnt signaling.[54]

While in prostate cancer intracellular β-adrenergic signaling contributes to disease progression, its mechanistic contribution to tumor growth is different in pancreatic cancer. NGF and its precursor forms, namely ProNGF, have also been implicated as a driving mechanism of tumor innervation and are associated with high Gleason scores in prostate cancer.[55] Additionally, neurite outgrowth of PC12 cells increase when cocultured with prostate cancer cells.[55] Similarly, β2 adrenergic signaling promotes NGF production and secretion by pancreatic epithelial cells. NGF secretion drives increased tumor innervation; this enhanced nerve density contributes to PDAC formation.[56] Together, this indicates that various cancers can produce NGF and its precursor forms, promoting tumor innervation and increased tumor aggressiveness. Mechanistically, treatment of pancreatic cancer cells with norepinephrine results in phosphorylation of STAT3, stimulating increased production and secretion of NGF. Consistent with this mechanism, inhibition of β2 adrenergic signaling decreases nerve density in pancreatic tissue and reduces PDAC initiation and tumor growth in a Kras/Trp53/PDx1 mutant mouse model.[57] Interestingly, norepinephrine signaling in pancreatic cancer cells promotes

their migratory behavior by increasing expression of matrix metalloproteinases (MMPs) which ultimately foster perineural invasion and, subsequently, metastasis[57] Importantly, the pro-tumorigenic functions of neurons are not limited to the release of neuropeptides and growth factors. Intra-tumoral nerves also influence the metabolic environment of the TME. Under serine deprivation, PDAC cells increase translation and production of NGF, stimulating the innervation of the TME.[58] Having penetrated the TME, intra-tumoral nerves release serine, creating a nutrient-rich environment that promotes a shift from the high production of NGF to a state of increased cell proliferation.[58] Thus, intra-tumoral nerves utilize various molecular mechanisms that culminate in disease progression.

3.2 | Nerve-cancer cell communication: sensory innervation

Two of the main neurotropic factors released from sensory neurons are substance P and calcitonin-gene related peptide (CGRP). Substance P is a tachykinin neuropeptide that functions primarily in mediating inflammation in response to noxious stimuli. Its main receptor, neurokinin receptor 1 (NK-R1), is expressed across various human tissues as well as cancer cell lines.[59,60] Innervation of the TME by sensory nerves occurs in various cancer models and these intra-tumoral nerves significantly contribute to cancer progression through various mechanisms (Figure 2).[18,38–40,61] In oral squamous cell carcinoma (OSCC), a subtype of head and neck squamous

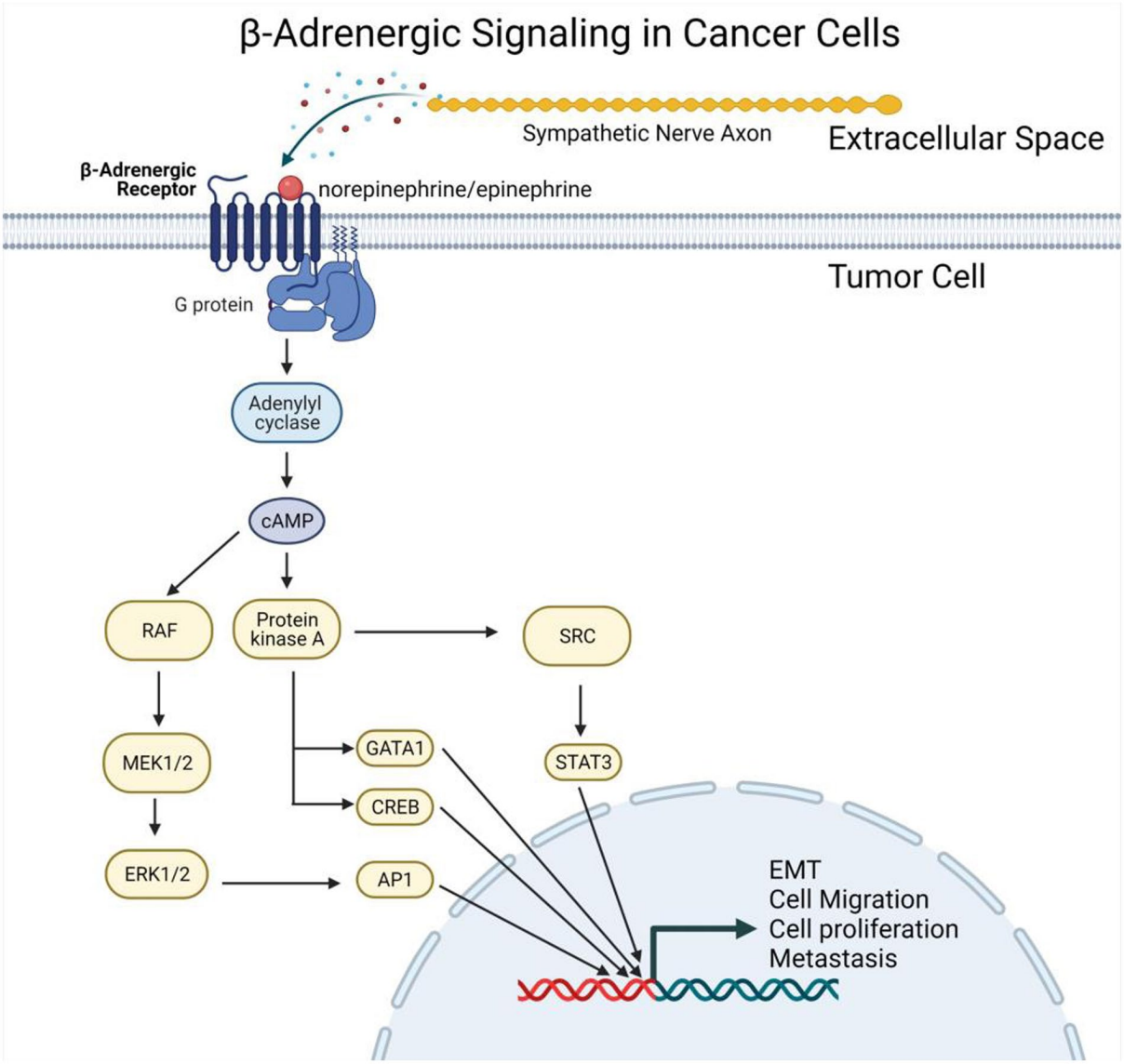

FIGURE 1 β-adrenergic signaling in tumor cells. Various studies have identified β-adrenergic signaling as a mediator of tumor cell proliferation. Here we outline downstream components of the β-adrenergic signaling pathway involved in these aspects of tumor biology. Created with BioRender.com

cell carcinoma, immunohistochemical staining of patient samples indicates that increased substance P correlates with poorly differentiated tumors suggesting a correlative relationship between substance P and patient prognosis.[62] In gastric cancer, a similar correlation between substance P-expressing nerves and cancer differentiation state was observed. Together, these studies suggest that substance P may serve as a potential prognostic marker in some cancers.[63] Moreover, substance P binding to its NK-R1 receptor promotes cell proliferation and migration across various cancers. For instance, treatment of OSCC cell lines with increasing concentrations of substance P increases their proliferation rate.[64] In gastric cancer models, the presence of substance P-expressing neurons is associated with poorly differentiated tumors: treatment of MKN45 cancer cells, an immortalized human gastric adenocarcinoma cell line, with substance P increases cell proliferation, migration, and invasive; these effects were all mediated through increased intracellular calcium.[63] In a pancreatic cancer model, similar responses to substance P treatment were evident with two different cell lines.[65] Importantly, these effects were attenuated with the addition NK-R1 antagonists. Furthermore, co-culture of pancreatic cancer cell lines with dorsal root ganglia (DRG, sensory) increases migration of cancer cells towards DRG axons following treatment with substance P, indicating a potential role for promoting perineural invasion of pancreatic cancer cells.[65] Similar substance P mediated

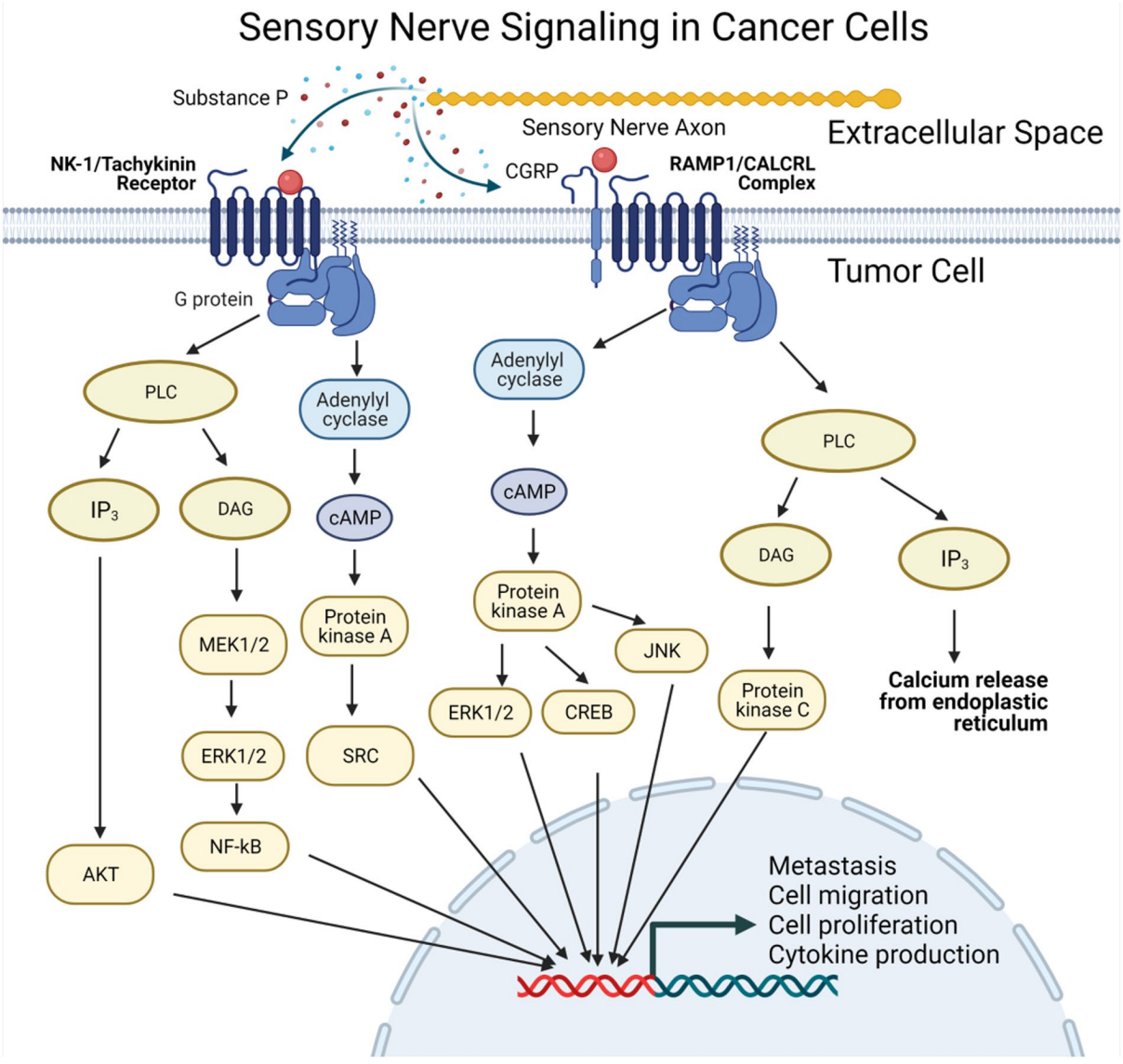

FIGURE 2 Sensory nerve signaling in tumor cells. Various studies have identified sensory neuropeptide signaling as a mediator of tumor cell proliferation and cancer progression. Here we outline downstream components of the sensory nerve signaling pathway involved in these aspects of tumor biology through substance P and CGRP signaling. Created with BioRender.com

increases in cancer cell proliferation and migration occur in other cancer cell lines, including gallbladder cancer.[66]

CGRP, another neurotrophic factor released by sensory neuron, is also interesting in the context of cancer. While most of the research focused on CGRP revolves around its function in the treatment of migraines, some studies address its potential contributions in cancers both as a diagnostic marker and as a direct regulator of cancer behavior. In metastatic non-small cell lung cancer and thyroid carcinoma, increased levels of circulating CGRP strongly associate with poor patient prognosis.[67,68] Additionally, the CGRP associated receptors, RAMP1 and CALCRL, are expressed in many tissues as well as cancer cell lines; tissues of the head and neck region harbor especially high CGRP levels.[59] Unlike substance P, however, studies focused on the direct effects of CGRP on cancer progression are limited. What is known is that CGRP treatment of PC-3 prostate cancer cells potentiate their invasiveness, providing a functional role for CGRP in metastasis.[69] Additionally, CGRP signaling promotes the proliferation of Ewing sarcoma, while its inhibition blocks cellular growth. While various tissues express the CGRP receptors, future studies will elucidate the relationship between these receptors, the CGRP neuropeptide and tumor behavior.

An additional aspect of sensory nerve functions in the TME is their contribution to cancer pain. Cancer pain is a prognostic factor for HNSCC patient survival and is traditionally viewed as resulting from inflammatory processes or from physical compression of tissues surrounding a tumor.[70,71] However, recent evidence in a murine model of HPV+HNSCC indicates that cancer pain arises prior to the emergence of tissue compression and may not be solely dependent on cytokine signaling.[72] These results suggest a potential role for intra-tumoral nerves in cancer-associated pain. Additional studies of other sensory innervated cancers will further define the contribution of these intra-tumoral nerves to cancer pain.

3.3 | Nerve-cancer cell communication: cholinergic innervation

Cholinergic signaling, mediated through acetylcholine binding muscarinic or nicotinic receptors, is another potential neural regulator of cancer progression, though its contributions have not been analyzed to the same scrutiny as adrenergic or sensory nerve peptides. In cervical cancer cell lines, where several nicotinic receptor subunits are expressed at the cell membrane, treatment with a nicotinic receptor agonist increases cell proliferation suggesting a contribution of cholinergic signaling in cervical cancer cell proliferation.[73] In gastric cancer, a symbiotic relationship between cholinergic nerves and tumorigenesis has also been detailed. Here, stimulation of the gastric epithelium with acetylcholine increases their production of NGF which promotes increased innervation of the tissue. This increased cholinergic innervation of the epithelial layer promotes carcinogenesis of the gastric epithelium by phosphorylation of YAP.[74] Non-small cell lung cancer (NSCLC) patient tumors also harbor cholinergic nerves within the microenvironment; these cancers express both nicotinic and muscarinic receptors that participate in cholinergic signaling.[75] Treatment with either muscarinic or nicotinic antagonists inhibits tumor growth *in vitro*, further implicating cholinergic nerves in disease progression.[75] Additional studies are needed to establish the mechanisms utilized by these cholinergic signals to induce these observed changes. Cancer models of particular interest that could benefit from further cholinergic analysis include lung cancers and HNSCC, which are mediated in part by cigarette smoking which contains nicotine, nicotinic receptor agonist.

3.4 | Neuro-immune communication

Regulation of the immune system and immune cells is well documented and explored in separate reviews.[76–79] However, the potential neural regulation of immune cell populations within the TME is less well defined. A major mechanism promoting tumor development and progression is the establishment of an immunosuppressive environment. Interestingly, a large population of immune cells express neuropeptide receptors and respond to neuropeptides. Moreover, several biological processes involved in immune responses and inflammation (e.g., blood vessel dilation, swelling, and pain) are mediated, in part, by neural signaling. Thus, it is logical to investigate the contributions of tumor-infiltrating nerves in immune regulation in cancer (Figure 3).

A major cell type associated with immunosuppressive and immune escape events characteristically associated with cancers are myeloid-derived suppressor cells (MDSCs). These immature cells of the myeloid blood lineage share physical and functional similarities to other myeloid lineage cells but also exhibit unique characteristics. One similarity is ADRB2 expression. Adrenergic signaling in MDSCs increases their sequestration within the TME.[80] Additionally, MDSCs are functionality altered by adrenergic signaling. For example, knock-out of the ADRB2 in MDSCs slows breast cancer growth *in vivo* suggesting that MDSC adrenergic signaling is necessary to mount an effective immunosuppressive response. *In vitro* treatment of MDSCs with isoproterenol (an ADRB2 agonist) or norepinephrine (an ADRB2 ligand) activates STAT3, an effect that is abrogated when ADRB2 is

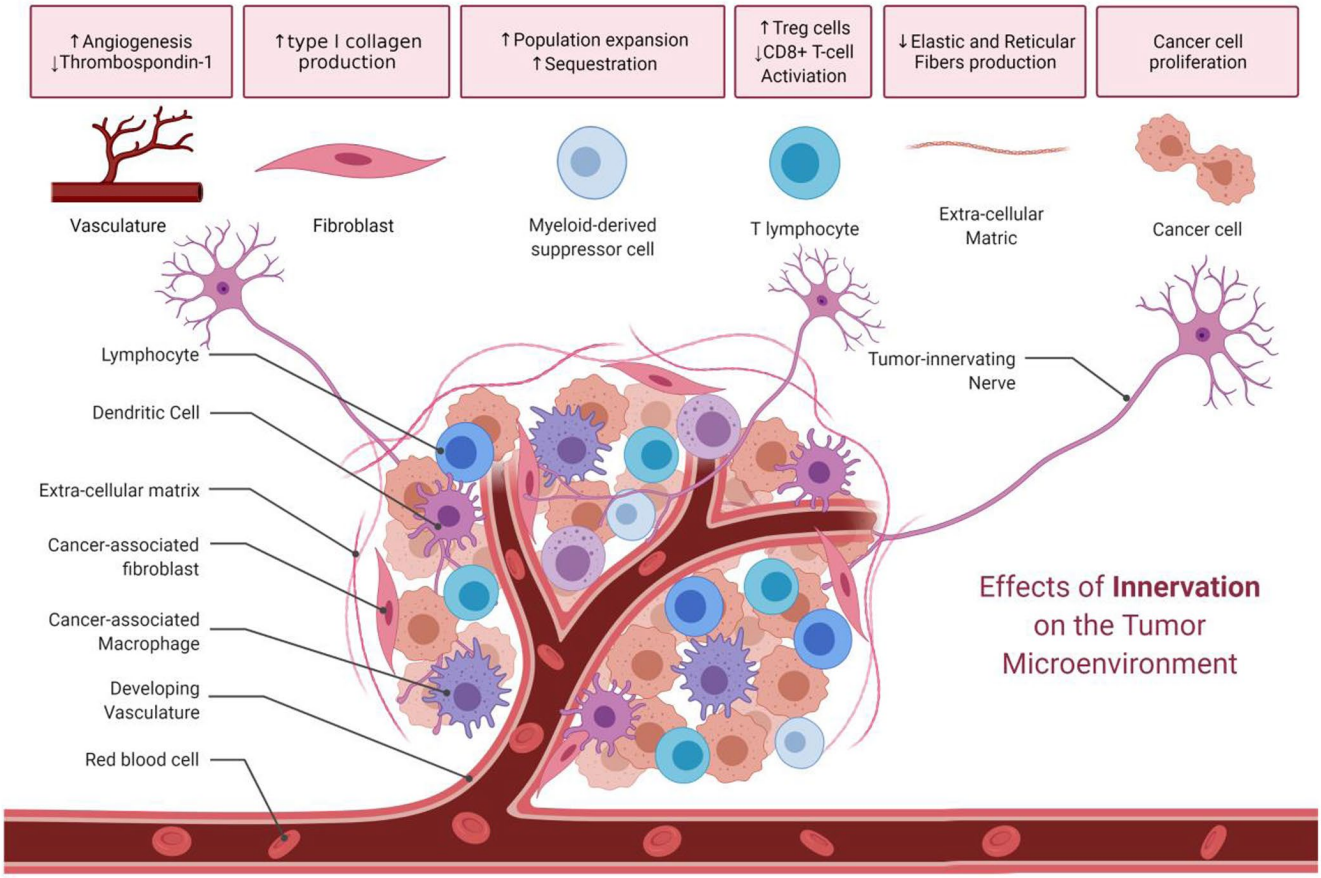

FIGURE 3 Neural regulation of the cellular components of the tumor microenvironment. During development solid tumors become innervated with a variety of nerves. These nerves, in turn, interact with and regulate various components of the tumor microenvironment, including promoting angiogenesis, production of an immunosuppressive microenvironment, and direct promotion of cancer cell proliferation and metastasis. Created with BioRender.com

knocked-out. Moreover, *in vivo* studies using a STAT3 inhibitor decreases tumor growth and MDSCs at the tumor bed. This same study showed that treatment of wildtype MDSCs with isoproterenol increased their expression of PD-L1, an immune checkpoint molecule, thereby enhancing anti-CD8 activity *in vitro*. Together, these data suggest that β2-adrenergic signaling in MDSCs regulates their immunosuppressive functions.[80]

Surprisingly, CD8+ cytotoxic T cells can also contribute to an immunosuppressive TME mediated, at least in part, by adrenergic innervation of secondary lymphoid organs which regulate their metabolic function. Treatment of CD8+ T cells with adrenergic agonists promotes metabolic reprogramming, suppressing their activation.[81] In two transgenic tumor mouse models, inhibition of adrenergic signaling promotes a shift in intratumoral T-cell populations from immunosuppressive to anti-tumorigenic.[82] Additionally, loss of adrenergic signaling improved tumor response to immune checkpoint inhibitor treatment.[82] Together, these studies suggest that adrenergic signaling inhibits CD8-mediated anti-tumor functions. While much research looks at systemic catecholamine regulating immune and tumor functions, these findings suggest that *local* release of catecholamines into the TME may critically regulate the functions of infiltrating immune cell populations. Furthermore, the role of sympathetic nerves in the TME on anti-tumor T-cell function in breast cancer was illustrated more recently, with denervation of sympathetic fibers reducing the expression of immune checkpoint molecules, including PD-1 and PD-L1.[32] Additionally, decreased expression of immune checkpoint molecules was more pronounced following complete sympathetic denervation as opposed to treatment with β-blockers, suggesting that non-adrenergic signals released from nerves may also influence the TME.[32]

Adrenergic signaling is not alone in the regulation of immune functions at the TME. Wang et al. demonstrate the juxtaposition of cholinergic neurons and CD133+ thyroid cancer cells in patient tumors, suggesting a potential direct communication between the two cell types. They show that acetylcholine treatment promotes activation of the PI3K-AKT pathway resulting in the increased

expression of PD-L1 on CD133+ thyroid cancer cells. PD-L1 is a ligand that functions to inhibit apoptosis by cytotoxic T-cells. The increased expression of PD-L1 enhances immune escape of CD133+ thyroid cancer cells. Additionally, following inhibition of cholinergic signaling with receptor antagonists, loss of PD-L1 was observed concurrent with the decreased immune escape of CD133+ cancer cells.[83] Together, these studies suggest that intratumoral nerves can directly signal to cancer cells, promoting their expression of check-point inhibitory molecules and driving immune escape. Similarly, nociceptive neurons also regulate the infiltrating immune population in the TME by promoting an increase in the infiltrating immunosuppressive cell population or by inhibiting the function of cytotoxic cells.[84,85]

As already mentioned, neuropeptides released from afferent sensory nerves stimulate proliferation and migration of cancer cells directly, resulting in increased tumor burden. However, this is not the only mechanism utilized by sensory nerves to promote tumor growth. For example, *in vitro* co-culture of melanoma cells with DRG (sensory) neurons does not alter cellular proliferation. However, *in vivo* increased tumor burden is evident in intact mice as compared to nerve-ablated animals. These studies suggest that intra-tumoral sensory nerves contribute to tumor growth indirectly (i.e., tumor growth is not mediated via nerve-driven increased tumor proliferation). Instead, alterations in chemokine production in non-ablated mice increase tumor infiltration of MDSCs and, in this way, promote a pro-tumorigenic immune environment.[61] Of note, different immune cells express varying levels of sensory neuropeptide receptors, potentially indicating multifaceted roles of nerves in the immune regulation of the TME.[85–88]

3.5 | Nerve-supportive functions of stromal components

As alluded to previously, nerves are essential for proper tissue organization, development, and healing.[10–15] Additionally, during these processes, nerves and neuronal signaling is necessary for the proper development of both the stromal organization as well as the proper formation of a blood supply (Figure 3).[89] Sensory nerves that travel alongside blood vessels promote angiogenesis via substance P mediated signaling during inflammation.[89,90] A similar neuronal-mediated process promoting angiogenesis appears to function in the TME. Adrenergic nerves in the TME release norepinephrine triggering an angiogenic switch by signaling through the ADRB2 on endothelial cells, thereby promoting increased vascularization of the TME.[91] Consistent with this, deletion of the endothelial adrenergic receptor inhibits the nerve-promoting induction of angiogenesis.[91] ADRB2 signaling also provides a similar pro-angiogenic function in prostate cancer. Stimulation of prostate cancer cells with adrenergic agonists induces HDAC2 activation. This histone deacetylase regulates the expression of thrombospondin. When adrenergically activated, HDAC2 suppresses expression of thrombospondin-1, an angiogenesis inhibitor, the ultimate result is the promotion of angiogenesis.[92]

Much like blood vessels, the lymphatic system is an important potential metastatic route for several cancers, and, like blood vessels, their function can by influenced by neurotrophic factors and nerve stimulation.[93] Treatment of a transgenic mouse model of breast cancer with an adrenergic agonist does not alter tumor growth but rather promotes metastasis through loco-regional lymph nodes and enhanced lympangiogenesis.[94] While the regulation of the stromal compartment of the TME by invading nerves is relatively unexplored, there is some evidence that innervation is involved in regulating the extracellular matrix (ECM) and stroma of other structures. Sensory innervation of lymph nodes plays a key role in mediating immune responses, in part by regulating gene expression of the endothelium, stromal cells, and leukocytes in peripheral lymph nodes.[94] Evidence from this study indicates that the presence of a specific sensory nerve-stroma communication axis could critically contribute to peripheral immune responses. Given that some tumors are innervated by sensory nerves, it is possible that newly recruited nerves to the TME modulate the local stroma or existing vasculature.[18,38–40,61] Myenteric nerve ablation in gastric lesions alters ECM fibrillar structure, increasing the presence of both reticular and elastic fibers.[95] Similarly, fibroblast function alters in response to adrenergic signaling.[96] In the case of liver fibrosis, fibroblasts increase the production of type I collage in response to increased norepinephrine levels.[97] Although these studies have not been conducted directly with cancer-associated fibroblasts, the function of both adrenergic and sensory nerves in the regulation of fibroblasts during wound healing, tissue patterning, and tissue regeneration point to additional directions of research in this developing field.[10–15,98]

4 | THERAPEUTIC POTENTIAL: TARGETING NEURAL INVOLVEMENT IN TUMORS

While our understanding of the specific functions and roles of nerves in the TME continues to develop, the potential therapeutic benefit of targeting nerves and neuronal signaling the TME has already been tested. As mentioned briefly, various aspects of tumor innervation have been

correlated to cancer progression and prognosis. Nerve density in prostate cancer has been shown to increase with progression and tumor development, from low innervation in precancerous lesions to high nerve density in metastatic disease.[8] Similar correlations between nerve density and cancer progression has been identified in various other cancers.[22,23,26,30,31] Therapeutically, measurement of nerve density is difficult in patients as biopsies only provide a narrow view of the TME. Measurement of neuropeptides and neurotransmitters, however, can be utilized as prognostic markers. As mentioned earlier, CGRP has been correlated to patient prognosis and cancer progression in non-small cell lung cancer and thyroid carcinoma.[67,68] Studies investigating the relationship between chronic stress and cancer have also reinforced the value of catecholamines as potential diagnostic markers, with studies repeatedly demonstrating negative correlations between catecholamine concentrations and cancer occurrence and progression. Not only do these neuropeptides and neurotransmitters act as potential tumor biomarkers, but due to their described role in cancer progression they provide for easily testable therapeutic targets.[99–102] The existence of drugs approved for human use that target neuropeptide receptors allows for a faster transition from bench to bedside. A major drug category of focus in this endeavor has been beta-blockers, which are adrenergic antagonists that block adrenergic receptor functions. These therapeutics have been used in the treatment of various medical conditions including angina and hypertension. While levels of significance vary, several studies show that even incidental use of beta-blockers increases survival and decreases rates of secondary cancers in patients undergoing cancer treatment.[103–105] However, off target effects for these drugs are well documented and underlying patient medical history could make use of these drugs in oncology more limited in scope. While the use of adrenergic antagonists has been widely addressed in retrospective and cohort studies, the use of antagonists to other neuropeptide receptors remains more limited. Few clinical trials have tested the efficacy of tachykinin antagonists in the treatment of cancer, though case studies have shown positive results.[106] Similarly, cholinergic receptor antagonists have few clinical trials indicated for the treatment of cancer. Additionally, non-pharmaceutical approaches have been investigated as potential means of specific targeting of infiltrating nerves by genetically engineered viruses.[107] Alternatively, additional approaches to targeting the nervous system in the treatment of cancer can come from therapies that target the development and protection of the nerves. One example includes therapies targeting the proper growth and function of nerves and synapses. For example, recent studies in high-grade gliomas provide evidence for the necessity of neuroligin-3, a synaptic protein, in glioma growth. Neuroligin-3 secretion can be inhibited with ADAM10 inhibitors, resulting in decreased glioma growth.[108,109] Secretion of neuroligin-3 has also been shown to necessary for the proper function and formation of synapses in the peripheral nervous system, providing a potential mechanism for targeting the developing nervous system in the periphery in non-central nervous system tumors.[110] A majority of the interest in the use of neurological compounds for the treatment of cancer has been almost exclusively limited to commercially available β-blockers but developing technologies in the field of neuroscience will only further expand the potential therapeutic options in targeting tumor-infiltrating nerves.

5 | CONCLUSION AND FUTURE DIRECTIONS

In this review, we have addressed the current understanding of how nerves invading the TME influence tumorigenesis and modulate the local environment. While current studies provide insights into the roles that various nerves play in regulating tumor biology and the surrounding microenvironment, many important questions remain. Moreover, though many studies demonstrate the presence of nerves in the TME and their promotion of tumorigenesis and disease progression, this is not always the case. Even amongst the same nerve type and signaling molecule, the function of nerves in the TME appears to be dependent on inherent factors to the cancer or to the environment or both. These differences are not well understood and need to be addressed further to better examine the potential intrinsic factors mediating the functions nerves play in the TME. Additionally, a major concern in numerous cancers is the risk of perineural invasion (PNI) for metastatic spread. While studies show a relationship between cancer cells promoting tumor innervation and a progression towards PNI, to date no direct studies mechanistically define this relationship.[18,57] Furthermore, while various studies show that inhibiting nerve signaling pathways presents a positive effect on patient survival and prognosis, these studies have been limited in both the scope of the target signaling pathways and the drugs used. Studies that further define key molecular pathways will provide much needed insights into potential targets and treatment options. Finally, while not addressed here, numerous studies and reviews have also attempted to determine the origin of the infiltrating nerve fibers.[38–41] The determination of nerve origin is of particular importance as this knowledge may influence treatment options. In summary, infiltrating nerves are an emerging hallmark of the TME and future mechanistic studies will better define the roles that nerves play in tumorigenesis and the TME while providing further insights into these complex diseases.

ACKNOWLEDGMENTS

We gratefully acknowledge David C. Restaino for assisting with editing of the manuscript. We are also grateful to Jeffrey Barr, Austin Walz, Allison Kruse, Caitlin Williamson, Craig Welbon, Payal Ghosh, William C. Spanos, and Steven Powell for critical discussion of topics included in the article.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

ACR and PDV designed the focus of the manuscript together. ACR wrote and revised the manuscript. PDV assisted in manuscript revisions.

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How to cite this article: Restaino AC, Vermeer PD. Neural regulations of the tumor microenvironment. *FASEB BioAdvances*. 2022;4:29–42. https://doi.org/10.1096/fba.2021-00066