Neuronal activity in ontogeny and oncology

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Abstract

The nervous system plays a central role in regulating the stem cell niche in many organs and thereby critically modulates development, homeostasis and plasticity. A similarly powerful role for neural regulation of the cancer microenvironment is emerging. Neurons promote the growth of cancers of the brain, skin, prostate, pancreas and stomach. Parallel mechanisms shared in development and cancer suggest that neural modulation of the tumor microenvironment may prove a universal theme, although the mechanistic details of such modulation remain to be discovered for many malignancies. Here, we review what is known about the influences of active neurons on stem cell and cancer microenvironments across a broad range of tissues and discuss emerging principles of neural regulation of development and cancer.

The neural regulation of development and cancer

Direct growth-promoting effects of active neurons in the tumor microenvironment have now been described for an increasing list of cancers, and tumor innervation is emerging as an important therapeutic target. For a broad range of cancers, the neural influences on development of the

corresponding normal tissue and the expression of receptors for neural molecules such as neurotransmitters on the tumor cells suggest that neural signaling promotes growth. However, as lessons from development suggest, neuronal influences could, conversely, promote cancer differentiation and involution. Here we will consider the functions of neuronal activity in tissue development, homeostasis and plasticity, together with the emerging roles for active neurons in tumor initiation and growth.

Since the influence of neuronal activity in development is best studied in the nervous system itself, we will begin there, as many of the mechanisms hold true in the development of non-nervous system organs as well. Recurrent themes of neural influences in development and cancer include the actions of neurotransmitters and neurotrophins in healthy and malignant tissue growth and differentiation; these mechanisms emerge as a common thread throughout the organ systems discussed below. Detailed studies of neuronal activity-dependent growth, facilitated by modern experimental tools, are also uncovering novel and unexpected mechanisms. Our understanding of neural influences on both nervous and non-nervous system organs is in its infancy. We will review what is known (and what remains to be discovered) about the influences of neurons in development and in the cancer microenvironment, organizing the breadth of the currently available information from a developmental perspective by tissue type (germ layer) and organ system.

The role of neurons in ectodermal tissues

Neurodevelopment and cancers of the CNS

The role of neuronal activity in the refinement of nervous system structure and function has long been appreciated [1–3]. Electrical activity shapes the earliest stages of brain organogenesis, as well as the behavior of persistent populations of neural precursor cells in the childhood and adult brain (Text Box 1). While the cells of origin for brain cancers remain an area of intense research and debate, it is generally accepted that the most malignant forms of brain tumors, including **pediatric and adult high-grade gliomas**, **primitive neuroectodermal tumors** (PNETs), **medulloblastomas** and **ependymomas** arise from and phenotypically resemble **neural precursor cell (NPC)** populations (see Glossary). Medulloblastoma subtypes are thought to arise from distinct cerebellar neuronal precursor populations or ventricular zone stem cells [4–9], high-grade gliomas from glial precursors (**gliogenic stem cells**, pre-oligodendroglial progenitor cells (pre-OPCs) or **OPCs**)) [10–14], and ependymomas from radial glial cells [15]. The microenvironmental

factors regulating the proliferation, survival and differentiation of NPC populations thus bears potential relevance to the behavior of their malignant counterparts. Given the putative origins of these tumors, we will focus on the physiological role of neuronal activity on the proliferation, differentiation and survival of neural stem and precursor cells and the recapitulation of these mechanisms in central nervous system malignancies.

Electrical activity profoundly influences central nervous system (CNS) development, including neural induction [16], neural stem and precursor cell proliferation, migration, differentiation, neuronal survival, synaptogenesis, oligodendrogenesis and myelination. Canonical neuronal activity- synapse-mediated depolarization of neurons- triggers calcium influx and subsequent calcium-dependent signaling that can affect a range of cellular functions [17–19]. However, even before neurons have matured or synapses have formed, patterned waves of electrical activity are crucial to neurodevelopment. Prior to maturation and generation of classical **action potentials**, depolarizing neurotransmitters are released non-synaptically and calcium transients are found in all parts of the nervous system [20,21]. During embryonic development, gap junctions composed of connexin-26 (Cx26) and connexin-43 (Cx43) couple ventricular zone stem cells (radial glial cells) and NPCs into adjacent, radially arrayed clusters, particularly during the period of intense cortical neurogenesis [22,23]. These coupled clusters form functional units and microenvironmental cues sensed by one or more cells can thus affect the entire cluster. Depolarization and the resultant calcium transients occur synchronously throughout the cluster. Gap junctions similarly couple postnatal NPCs [24] as well as mature astrocytes [25]. Elegant recent studies showed that, like NPC populations, glioblastoma (GBM) cells form an interconnected network that similarly depends upon Cx43 gap junction protein-mediated communication through which calcium waves can propagate [26]. Activity-regulated growth signaling might be communicated through such interconnected cancer cells as it is in normal NPCs or astrocytes.

In mature neurons, propagation of depolarization down the axon via voltage-gated sodium channels results in synapse-mediated communication between cells. Neurotransmitters released during synaptic transmission are rapidly cleared by perisynaptic astrocytes in the healthy mature brain [27]. Synaptic release of neuropeptides also mediates communication between some classes of neurons. Growth factors such as the neurotrophin BDNF (see Text Box 2) can also be secreted in an activity-dependent fashion. The effects of neuronal activity on development and neural plasticity are also mediated indirectly by activity-dependent changes in other cells of the

microenvironment, such as activity-dependent synaptic pruning by microglia [28]. Below, we will discuss influences of neuronal activity on neuronal and glial development as well as the corresponding malignancies.

Neurogenesis and neuroepithelial tumors

While exposure of neurogenic precursors to depolarizing neurotransmitters generally promotes generation of neurons, the effects of membrane depolarization and exposure to specific neurotransmitters on the proliferation and differentiation of NPCs varies with cell populations and with neurotransmitter types.

Early in embryonic development, paracrine non-synaptic neurotransmitter release causes ventricular zone NSC depolarization via **ionotropic glutamate (AMPA/kainate)** and **GABA_A receptors** [29]. Glutamate and GABA are depolarizing/excitatory neurotransmitters in immature neural stem cells; while GABA is the chief inhibitory neurotransmitter in the mature CNS, GABA results in depolarization in immature NPCs due to a high intracellular chloride concentration [30]. Exposure of NSCs to GABA or glutamate during embryonic neurogenesis results in decreased proliferation [29]. In contrast, glutamate stimulates the proliferation of restricted neuronal progenitors (**neuroblasts**) during prenatal forebrain neurogenesis, either via AMPA/kainate receptors (in cortical neuroblasts) or via **NMDA receptors** (in striatal neuroblasts) [31,32].

Postnatal SVZ neurogenesis is regulated by various neurotransmitters including glutamate, GABA, dopamine, serotonin and acetylcholine. Glutamate promotes SVZ-derived neuroblast survival via NMDA receptor signaling [33]. GABA, released from differentiating neuroblasts, results in depolarization and decreased NSC proliferation [34]. Both serotonin and dopamine promote NPC proliferation in the SVZ [35–37]. A recent study identified a novel population of subventricular zone cholinergic neurons that extensively innervate the postnatal SVZ niche [38]. **Optogenetic** experiments (control of neuronal activity using light and neuronal expression of light-responsive microbial opsins, for review see [39]) demonstrate that activity of these cholinergic subependymal neurons promotes NPC proliferation and neuroblast generation via local acetylcholine release [38].

In the postnatal hippocampus, neuronal activity promotes the differentiation of proliferating NSCs into mature granule cell neurons via a calcium channel-dependent induction of a proneural gene expression program [40]. Postnatal hippocampal NSCs sense circuit activity largely via depolarizing GABAergic inputs [41,42]. In addition, GABAergic inputs from parvalbumin-

positive interneurons also promote neuroblast survival [42]. In the hippocampus, glutamatergic and serotonergic signaling similarly promote neurogenesis [40,43–45].

Like the neurogenic precursor cells of the cerebrum, cerebellar GCPs are also depolarized by glutamate and by GABA via kainate and GABA_A receptors, respectively [46]. The effect of depolarization on GCPs is not yet entirely clear [47]. In contrast, glutamatergic signaling through the **metabotropic glutamate receptor** mGluR4 decreases GCP proliferation and promotes differentiation [48]. Conversely, glutamatergic signaling through mGluR5 stimulates the proliferation of retinal neuronal precursors, emphasizing the differential effects of neurotransmitter-mediated membrane depolarization in various precursor populations [49].

The effects of elevated neuronal activity on tumors that arise from and are similar to neurogenic NPCs, such as medulloblastoma and PNETs, are difficult to predict given the diverse responses of NPC populations to depolarization and neurotransmitter exposure. The role of neuronal activity in the microenvironment of these tumors has also not been explicitly studied to date. Medulloblastoma is a disease that parses into four molecular subtypes, termed SHH, Wnt, Group 3 (Myc-amplified) and Group 4 [50,51]. A majority of human medulloblastoma samples examined express metabotropic glutamate receptors and mGluR4 expression correlates inversely with histological anaplasia, dissemination and clinical outcome [52]. mGluR4 agonism, analogous to its role in normal GCPs, reduces proliferation of human medulloblastoma cell lines *in vitro* and decreases tumor progression *in vivo* in genetic mouse models of SHH subtype [52]. Group 3 tumors exhibit up-regulated expression of the $\alpha 5$ subunit of the GABA receptor [53], but the functional roles of GABAergic signaling in Group 3 and other subtypes of medulloblastoma are not yet clear. Direct modulation of glutamatergic and GABAergic neuronal activity in the medulloblastoma microenvironment is needed to ascertain the role of neurons and to identify possible novel therapeutic strategies.

Gliogenesis and gliomas

Neuronal activity promotes the proliferation and subsequent differentiation of myelin-forming precursor cells. Decreased neuronal activity in the optic nerve, achieved via optic nerve transection or infusion of **tetrodotoxin** (blocker of voltage-gated sodium channels), results in decreased OPC proliferation [54]. Optogenetically elevated neuronal activity in the premotor circuit of awake, behaving animals results in brisk proliferation of OPCs and earlier precursors (Figure 2), followed

by differentiation of the recently divided OPCs into oligodendrocytes and myelin remodeling [55]. In contrast, astroglial cells do not proliferate in response to elevated neuronal activity [55]. Activity-dependent OPC proliferation and subsequent differentiation occurs in both juvenile and adult rodent brains [55], and these activity-dependent changes in myelin-forming cells, termed “myelin plasticity” or “**adaptive myelination**”, positively influences neurological function [55,56], indicating a newly-appreciated dimension of neuroplasticity along which neuronal activity modulates brains structure and function throughout life.

The molecular mechanisms of adaptive myelination that mediate changes in OPCs remain an area of active research. A number of possible mechanisms were suggested by elegant *in vitro* studies, including activity-regulated adenosine and astrocyte-derived LIF [57–59]. Neuregulin-ErbB3 signaling and Bdnf signaling may also play roles in activity-regulated responses of myelin forming cells [60,61]. Additionally, OPCs express a range of neurotransmitter receptors and form *bona fide* synapses with neurons in both grey and white matter [62–65]. GABAergic interneurons synapse onto OPCs and, like other precursor cell populations, GABA is depolarizing in OPCs due to high intracellular Cl⁻ concentrations [66]. The precise role of glutamatergic or GABAergic axoglial synapses in OPC proliferation, differentiation or migration during innate and during activity-dependent myelination remains to be elucidated. Recently, it was shown that GABA decreases perinatal OPC proliferation and promotes oligodendrocyte differentiation, indicating a key functional role in development [67].

The central role that neuronal activity plays in promoting the growth, survival and functional refinement of normal brain development suggests that parallel roles in the biology of brain cancer would be advantageous to tumor growth and progression. This may be particularly true for gliomas given their similarity to gliogenic NPCs [55]. To test the role of neuronal activity in the glioma microenvironment, we recently used *in vivo* optogenetic stimulation of the premotor cortex in the context of a patient-derived orthotopic xenograft derived from a pediatric cortical GBM. Elevated premotor cortical projection neuronal activity resulted in a circuit-specific increase in glioma cell proliferation and overall increase in tumor growth *in vivo* (Figure 2) [68]. To test the role of activity-regulated secreted factors in activity-dependent glioma growth, we collected the conditioned media from acute cortical slices of varying activity levels (optogenetically stimulated slices with 20 Hz neuronal firing rate; slices exhibiting spontaneous neuronal activity; and slices electrically silenced by tetrodotoxin) and exposed a range of patient-derived cultures derived from

clinically and molecularly distinct high-grade glioma types to these conditioned media. Secreted factors regulated by neuronal activity increased the proliferation and growth of 9 out of 10 patient-derived high-grade gliomas, including pediatric HGGs (diffuse intrinsic pontine glioma (DIPG), pediatric cortical GBM) and adult HGGs (GBM and anaplastic oligodendroglioma). Further biochemical, proteomic and functional analyses revealed that activity-regulated secretion of Bdnf and, unexpectedly, a synaptic adhesion protein called **neuroligin-3** (Nlgn3) mediates this growth-promoting effects [68]. While Bdnf is known to promote OPC proliferation and myelination [69], the role of Nlgn3 in normal myelination is not yet clear, but these findings in glioma may help to elucidate molecular mechanisms of healthy myelin development and plasticity. Secreted Nlgn3 binds to an unidentified glioma cell binding partner and subsequently activates PI3K-mTOR signaling and proliferation [68]. Additionally, exposure to Nlgn3 results in a positive feed-forward loop that upregulates Nlgn3 expression, which may promote autocrine/paracrine effects. Accordingly, NLGN3 expression in human GBM correlates inversely with overall survival [68]. Subsequent work is needed to understand the potential roles that other neuronal subtypes may have on glioma growth. Further, it should be noted that the slice experimental platform described did not allow for evaluation of local effects of neurotransmitters or other molecules that are rapidly cleared from the microenvironment and cannot be detected in conditioned media but may play a key role *in vivo*.

Responsiveness of glioma cells to neurotransmitters has been appreciated for some time and has been considered to date largely in the context of non-synaptic paracrine/autocrine secretion of glutamate from cancer cells. Adult GBM cells secrete copious amounts of glutamate *in vitro* [70] and glutamate levels are increased in the tumor tissue and brain parenchyma surrounding GBM tumors *in vivo* [71]. Glutamate release from tumor cells can be excitotoxic to neurons in the tumor vicinity [70,72] and gliomas that secrete higher levels of glutamate exhibit a growth advantage in rodent models [72]. Glutamate effects on cell proliferation, survival and migration/invasion can be mediated by calcium-permeable AMPA receptor stimulation [73,74]. Further, gliomas increase the excitability of the neural circuit in which they are growing (Figure 1), an effect that is mediated in part by glutamate secretion [75,76]. Additional mechanisms by which gliomas promote increased neuronal activity and stimulate further growth, remain to be elucidated.

The direct effects of GABA on glioma cells are less clear. Examining a panel of patient-derived glioma cultures, one study found that functional GABA_A receptors were variably expressed and

tended to be present in lower grade gliomas (grade II and III astrocytomas) and in oligodendrogliomas rather than GBM (grade IV) [77]. In some patient-derived tumor cells, GABA was depolarizing while in others it was hyperpolarizing; intratumoral heterogeneity also dictates that some cells within a given tumor are depolarized and others are hyperpolarized [77]. Other studies examining a patient-derived GBM culture found evidence of functional GABA_A receptors in a subset of cells (~12%), for which GABA and GABA agonists, such as diazepam and etomidate, were depolarizing [78]. The functional effects of GABAergic signaling in glioma are yet to be clarified, but given the effects of GABA on glial precursor cells development [67], it is conceivable that GABA could promote glioma differentiation.

Adult GBMs also express serotonin (5-HT(7)) [79] and dopamine receptors [80,81]. A recent screen of patient-derived GBM cultures against a library of neurochemical compounds identified agents modulating serotonergic, dopaminergic and cholinergic signaling, with agents blocking the dopamine receptor DRD4 particularly effective at reducing GBM cell proliferation and survival via disruption of autophagy [81]. Further supporting the concept that dopamine receptors may be a useful therapeutic target, an earlier study found that knock-down or pharmacological inhibition of D2DR dopamine receptor reduced tumor growth in patient-derived GBM cell cultures [80]. While more work is needed to elucidate the role of serotonergic signaling in glioma, a reassuring retrospective analysis of selective serotonin re-uptake inhibitor (SSRI) use in patients with both GBM and depression found no indication that SSRI and subsequent increase in microenvironmental serotonin levels affects survival [82]. Targeting neurotransmitters receptors in glioma may prove a useful therapeutic strategy.

Neuronal growth factors in neurodevelopment and cancer progression

The neurotrophins (Text Box 2) are secreted proteins that promote survival, growth, development and plasticity in the central and peripheral nervous systems. The survival of embryonic cortical neurons depends on expression of BDNF [83]. Activity-regulated Ca⁺⁺ influx through voltage-gated channels promotes the binding of calcium-responsive transcription factors CREB and CaRF to regulatory elements in *BDNF* and increased BDNF expression [84,85]. Neuronal activity also regulates BDNF secretion [86,87]. Neuronal expression and secretion of BDNF influences diverse processes, from increased NPC proliferation and survival [88], to neural circuit development [89] or to long term potentiation (LTP) [90]. In the developing cerebellum, BDNF promotes survival of

immature GCPs in the external granule layer, while NT-3 acts on more mature cells in the internal granule layer [91]. In the oligodendroglial lineage, BDNF regulates OPC proliferation and myelination [69] as well as regeneration after demyelinating injury [92–94]. It should be noted that multiple cell types express and secrete neurotrophins, so neurotrophin signaling does not necessarily indicate involvement of a neuron.

The relevance of the NGF and GDNF families of neurotrophins to cancer and the potential growth advantage of maintaining functional neurons in the cancer microenvironment was suggested by the initial isolation of NGF from mouse sarcomas and GDNF from a rat glioma [95,96]. In glioma, BDNF promotes proliferation and survival through TrkB (NTRK2)-mediated signaling [68,97,98]. Consistent with this, genomic aberrations affecting the Trk genes (*NTRK1*, 2 and 3) are well described in human gliomas. Activating fusions of *NTRK1*, 2 or 3 are found in ~40% of infant HGG [99]; *NTRK2* fusions in ~3% of pediatric pilocytic astrocytomas [100]; infrequent *NTRK1* fusions in ~0.5% of adult GBMs [101]; and amplification of *NTRK1* or *NTRK2* in ~50% of DIPGs [102]. The therapeutic utility of targeting BDNF signaling in gliomas remains to be tested, but the frequency of activating mutations in pediatric HGGs and its proliferative effect indicate that it may be an important target.

The roles of the neurotrophins in medulloblastomas and other neuroectodermal brain tumors are complex and incompletely understood. Medulloblastomas express both NT-3 and TrkC; high TrkC expression levels correlate favorably with prognosis [103]. This is consistent with the role of NT-3 in differentiating granule cells [91] and reflects either a biological activity of TrkC signaling in medulloblastoma or differential expression of TrkC attributable to a different cell of origin or molecular subtype. Higher expression levels of TrkA in neuroblastoma, a primitive neuroectodermal tumor of the peripheral autonomic nervous system, also corresponds to favorable prognosis [104,105]. In the developing human cerebellum, p75NTR is expressed in the EGL and co-localizes with the proliferation marker Ki67; a subset of medulloblastoma tissue samples also exhibit p75NTR expression [106]. Recent studies showed that p75NTR is expressed predominantly in the SHH subgroup [107], but the functional consequence of this remains to be determined. BDNF and TrkB signaling effects in medulloblastoma are even less clear, with one study reporting exposure to BDNF and reduced viability in human medulloblastoma cell lines [108]; but another study reporting a similar effect with TrkB inhibition [109]. The function of neurotrophin signaling in ependymomas is also an area that requires investigation. In a cohort of

pediatric ependymomas and low-grade astrocytomas, CSF levels of BDNF were increased [110]. The p75NTR receptor is more highly expressed in supratentorial ependymoma compared to infratentorial ependymoma [111]. A comprehensive evaluation of neurotrophin receptor expression across a range of human brain tumors using modern gene expression databases, followed by functional testing of neurotrophin signaling in faithful experimental models, would shed considerable light on this promising set of possible therapeutic targets.

Beyond neurotrophins, other growth factors are also secreted by neurons. For example, ventral forebrain neurons secrete sonic hedgehog (Shh) ligand, which signals to postnatal NPCs in the ventral SVZ and regulates neurogenesis of ventrally-derived olfactory bulb neuronal subtypes [112]. Similarly, Purkinje neurons in the developing cerebellum are the source of SHH ligand which drives the proliferation of GCPs in the EGL [113], instructs cerebellar astrocyte subtype specification [114], and stimulates the proliferation of SHH subtype medulloblastoma. Thus, neurons secrete a variety of neurotransmitters, neuropeptides and protein signaling ligands that affect the behaviour of normal and malignant cells.

Outside the CNS: the role of peripheral nerves in development and cancer

Skin

The peripheral nervous system (Text Box 3) elaborates extensions into every tissue in the body. Innervation occurs very early in cutaneous morphogenesis and correlates closely with the development of epidermal structures [115,116]. For example, sensory innervation precedes hair follicle development [116]; with sensory nerves innervating the follicle secreting Shh ligand [117] and follicular stem cells responding to Hh signaling. Similarly, the development and maintenance of Merkel cells, receptor cells of epithelial origin in the skin, are directly supported by sensory innervation [118,119]. Peripheral nerves also instruct cutaneous arterial patterning via nerve-derived VEGF-A and CXCL12 [120,121]. Tissue homeostasis in the epidermis is largely regulated by maintenance and proliferation of local stem cells and shares signaling mechanisms with epidermal morphogenesis [122]. Accordingly, wound healing is influenced by nerve-derived Shh ligand secretion, which supports the stem cell response [117].

Innervation appears to promote the growth of basal cell carcinoma (BCC), a cancer driven by dysregulation of Hh signaling. Peterson et al. reported that stem cells of the follicular and touch dome epithelium are the cells of origin of BCC, and that Shh-ligand expressing sensory nerves

innervating the touch dome epithelium drive BCC progression. Ablation of these Hh-secreting nerves suppresses BCC tumor growth [123]. Thus, in skin, neuronal regulation of Hh signaling links neuron-mediated stem cell mechanisms of homeostasis to those of tumor cell proliferation.

The contributions of innervation to other forms of skin cancers are not well studied. Melanoma, the most aggressive form of skin cancer with a particular propensity to metastasize to brain, expresses a range of both NGF family neurotrophin receptors and ligands, and autocrine/paracrine neurotrophin signaling promotes the proliferation and migration of melanoma cells [124–126]. The role of epithelial innervation in primary melanoma and in CNS metastatic lesions remains to be explored experimentally.

The role of neurons in mesodermal tissues

Bone Marrow and Blood

The bone marrow (BM) niche is a classic example of local microenvironmental regulation of stem cell survival, proliferation, and differentiation. This niche includes blood vessels, stromal cells and sympathetic nerve fibers that work cooperatively to regulate hematopoiesis. Careful regulatory control is exerted on hematopoietic stem cell (HSC) quiescence and activation in response to stress or damage [127]. Innervation regulates quiescence and maintenance of the stem cell pool via TGF- β secreted by non-myelinating Schwann cells (peripheral glial cells) that ensheath sympathetic nerves in the BM. Sympathetic denervation results in loss of TGF- β signaling and depletion of the BM HSC population [128]. Migration of newly generated blood cells out of the BM niche is another key step in blood cell production and can be stimulated by granulocyte-colony stimulating factor (G-CSF) [129]. This migratory response to G-CSF is linked to the chemotactic interaction of CXCR4/CXCL12 within the niche [130]. An important study by Katayama et al. demonstrated that genetically-engineered mice with poor nerve conduction have a dramatic reduction in the egress of the HSCs in response to G-CSF and other known migration-stimulating cytokines.. The migratory response of HSCs appears to be directly regulated by the sympathetic nervous system, since it is impaired by pharmacological or genetic ablation of adrenergic signaling and can be rescued with β_2 adrenergic agonists [131].

The nervous system further regulates blood homeostasis via circadian oscillations of adrenergic activity and oscillatory release of HSCs. Norepinephrine and dopamine levels vary with circadian rhythms in the BM niche, with peak levels observed at night, a pattern disrupted by

sympathectomy [132]. Further linking circadian secretion of norepinephrine to the cyclic release of HSCs, sympathetic nervous system (SNS) signaling via β_3 -receptors on BM stromal cells down-regulates CXCL12 and results in HSC release [133]. The role of SNS in the retention of HSCs suggests a role in blood homeostasis and regeneration by promoting HSC circulation during periods of rest. Supporting a role of the nervous system in regeneration, chemotherapy-induced bone marrow nerve injury hinders hematopoietic regeneration, while nerve regeneration promotes recovery [134].

Perivascular mesenchymal stem and progenitor cells (MSPCs) are also a key component of the BM niche and maintain the HSC population through expression of growth and retention factors including **CXCL12**, **SCF**, and **ANGPT1** [135,136]. These MPSCs associate with sympathetic nerve fibers near blood vessels to form what is termed the neuroreticular complex [137,138]. Innervation regulates proliferation and differentiation of MSPCs, as proliferation of quiescent MSPCs increases after sympathectomy, and osteoblastic differentiation decreases in response to β_3 -adrenergic receptor signaling [135]. Together, these findings emphasize the importance of the nervous system in stem cell behavior within the BM niche.

While role of the nervous system in hematological cancer progression remains to be elucidated, neurotrophin signaling does have an established role. A recently described neurotrophin - **neurotrophin-1 (NNT-1)/B cell- stimulating factor-3 (BSF-3)** - was linked to growth and survival of multiple myeloma (MM) in vitro [139–141]. Neurotrophins have also been implicated in MM progression. MM cells secrete BDNF and express high levels of its receptor TrkB; and BDNF triggers activation of survival pathways including Akt and MAPK [142]. In the BM niche, BDNF is expressed by osteoblasts, megakaryocytes, and endothelial cells [143], suggesting that non-neuronal cells may also contribute to MM through neurotrophin signaling. This reciprocal interaction between MM and the BM microenvironment may also lead to worsened bone disease by stimulating osteoclasts and enhancing bone resorption through the BDNF/TRKB axis [144].

Bone

Bone homeostasis is regulated by a balance of formation by osteoblasts and resorption by osteoclasts. The bone is extensively innervated and tissue turnover is heavily influenced by neural signals. The sympathetic nervous system influences both osteoblast and osteoclast activity. Chemically sympathectomized mice demonstrate a decrease in osteoblastic activity, with reduced

uptake of collagen precursors and deposition of the bone matrix [145,146]. Both β -adrenergic and glutamatergic signaling influences osteoblast function and bone remodeling. **Leptin** (anti-osteogenic peptide hormone) plays a crucial role in bone homeostasis, and leptin-deficient mice have an increased rate of bone formation [147]. Takeda et al. demonstrated that β -adrenergic receptors modulate osteoblast differentiation downstream of leptin signaling. Correspondingly, β -adrenergic agonists decrease bone mass, while β -adrenergic antagonists increase bone mass [148], underscoring a role for the nervous system in bone homeostasis. Other neurotransmitters also influence bone precursor cell function and bone growth. Osteoblasts secrete glutamate in an autocrine/paracrine manner [149] and express both ionotropic and metabotropic glutamate receptors. Glutamatergic signaling through ionotropic glutamate receptors (AMPA and NMDA receptors) stimulates osteoblast ERK signaling, osteoblast maturation, and promotes bone formation [150]. NMDA receptor stimulation in osteoblasts induces *c-fos* and *jun* expression, both of which promote osteoblast proliferation and have been linked to osteosarcoma initiation [151].

The role that innervation plays in mediating bone growth and homeostasis suggests that it could play a similarly prominent role in bone cancer pathogenesis. Present evidence indicates that a variety of receptors for neural molecules are expressed by a range of bone cancer cells. Ewing sarcoma and osteosarcoma cells express β -adrenergic receptors [152]. Both normal osteoblasts and osteosarcoma cells also express several neuropeptide receptors including VIP receptor, substance P receptor, Y receptor, and calcitonin gene-related peptide receptor [153]. A variety of glutamate receptors are also expressed in osteosarcoma cells [154]. The gene encoding mGluR4 was identified in a genome-wide association (GWAS) study of osteosarcoma [155]. mGluR4 was found to be expressed in a significant percentage of both osteosarcoma (~20%) and benign giant cell tumors of bone (~40%), and expression correlated positively with overall survival in osteosarcoma [156]. The function of NMDA-mediated or AMPA-mediated glutamatergic signaling in bone cancers remains to be determined. Adrenergic, dopaminergic and VIP-mediated signaling all promote glycogenolytic metabolism in Ewing sarcoma cells, suggesting a role in cancer metabolism [157,158]. Adrenergic signaling induces expression of IL-6 and IL-11 in osteoblasts and osteosarcoma cells that stimulates osteoclast production [159] and may thereby promote bone remodeling and facilitate tumor mass expansion.

Muscle

Motor neuron innervation regulates skeletal muscle development and homeostasis. In *Drosophila*, innervation is critical for muscle patterning and myoblast proliferation [160]. During embryonic development, innervation is also necessary for myoblast differentiation into mature myofibers; and comparisons of denervated and toxin-inactivated muscle indicate that innervation provides both electrical signals and trophic support [161]. Spinal cord explants promote the growth and maintenance of skeletal myofibers *in vitro* [162], indicating that trophic support is also important for homeostasis. Muscle atrophy that occurs following denervation is thus due not only to disuse, but also to loss of these trophic signals. is one motor neuron-derived secreted signal that promotes neuro-muscular junction (NMJ) synaptogenesis [163,164]. NT-3 is also expressed by motor neurons during development and its TrkC receptor is expressed by skeletal muscle fibers [165]. Little is known about the role of innervation in **rhabdomyosarcomas**. In studies using models of murine sarcoma, in which tumor cells are implanted into thigh muscles, dopamine injections inhibited endothelial progenitor cell mobilization and slowed tumor growth. [166,167]. The possible roles of motor neuron innervation in rhabdomyosarcoma remain to be explored.

The role of neurons in endodermal tissues

Submandibular salivary gland

Like the systems discussed above, glandular development also depends on the activity of neurons (Figure 2). The role of innervation in glandular organogenesis is best studied in salivary glands. Knox et al. showed that during organogenesis, epithelial cell progenitor populations are maintained by direct parasympathetic innervation. Using explant cultures of submandibular gland, they demonstrated that removal of the parasympathetic ganglion (PSG) reduced growth of the normal salivary gland epithelium, as evidenced by a decrease in the number of end buds (acini). Antagonism of acetylcholine production or the associated muscarinic receptor also had an inhibitory effect. Markers of epithelial progenitor cells were decreased after removal of the PSG, suggesting that cholinergic innervation is necessary to the maintenance of the glandular epithelium by maintaining the progenitor cell population [168]. In a different study, the same group identified the neuronal-derived **vasoactive intestinal peptide** (VIP)/cAMP/PKA pathway and **CFTR** as key players during salivary gland tubulogenesis [169]. Thus, parasympathetic innervation coordinates multiple aspects of organogenesis throughout gland development. Illustrating the reciprocal developmental roles of peripheral nerves and their target organs, nerve outgrowth from mouse

submandibular ganglion is stimulated by the local epithelium ([170], Figure 2A), and it has been suggested that salivary epithelial cells may recruit the growth promoting effects of the PSG by secreting neurturin to promote nerve outgrowth [169].

Salivary gland growth, maintenance, and function are also regulated by autonomic nerves [171]. Both parasympathetic cholinergic signaling through muscarinic receptors, and sympathetic signaling through adrenergic receptors modulates saliva secretion. Parasympathetic nerve-derived VIP may also play a role [172]. Though all mechanisms involved have not yet been elucidated, it is known that after denervation the gland undergoes degeneration and atrophy, suggesting that innervation is also crucial for maintenance [171,173–175]. While the role of innervation in salivary gland tumors is unexplored, innervation has been clearly demonstrated to play a key role in cancers of other glandular organs such as the prostate and pancreas, as discussed below.

Prostate

Prostate cancer is among the malignancies for which a growth-promoting role of innervation is best established. The microanatomical relationship of cancer cells with neural elements in the microenvironment first suggested an important role for nerves. A variety of cancers, including prostate cancer, exhibit **perineural invasion (PNI)**, first described by Bastsakis as the invasion of tumor cells in, around, and through the nerves [176]; for review, see [177]. Perineural space invasion and diameter are linked to prostate adenocarcinoma aggressiveness [178–180]. Analysis of gene expression in reactive stroma from grade 3 prostate cancer samples showed high expression of genes involved in the neurogenesis, axonogenesis, and synaptogenesis, including *GDNF*, *NOTCH1*, *FGF19*, *GLI2*, and *CDK5* [181]. More recently, evidence of prostate-cancer related axonogenesis has come to light and correlates clinically with recurrence and aggressiveness [182]. **Axonogenesis**, the process of sending out axons to reach new targets based on microenvironmental axon guidance molecules (semaphorins, netrins, ephrins, cell adhesion molecules, neurotransmitters, and other growth factors), is normal in neural development and plasticity (for a review see [183]). Cancer-related axonogenesis is a more recently described phenomenon, best illustrated by work from Ayala's group. An instructive role of prostate cancer in this exuberant axonal ingrowth into the tumor microenvironment was demonstrated in co-culture models of prostate cancer cell lines and dorsal root ganglia neurons. In this model, cancer cells promoted DRG neurite outgrowth and then proceeded to migrate along these nascent neurites,

mimicking perineural invasion. Compared to controls of tumor cells alone or DRG neurons alone, when cultured together more tumor cell colonies formed and a higher degree of neurite outgrowth was observed, suggesting that DRG neurons and prostate cancer cells promote reciprocal growth ([184], Figure 2). Prostate cancer-induced axonogenesis has since been demonstrated *in vivo* [185], and, as discussed below, has also been described in other malignancies such as pancreatic cancer. Nerve density correlates with prostate cancer cell proliferation and with the expression of proteins involved in survival and hormonal regulation pathways in human radical prostatectomy specimens [186]. Another group has shown that proNGF (precursor to nerve growth factor and known to induce axonogenesis) was overexpressed in prostate cancer specimens in comparison to BPH samples. Levels of proNGF correlated positively with the Gleason score (a measure of prostate cancer aggressiveness). Additionally, co-culture of PC12 neuron-like cells or 50B11 neurons with PC-3 cells showed increased neurite outgrowth *in vitro*, which could be blocked with an antibody against proNGF [187]. The observation that cancers promote axonal sprouting in the tumor microenvironment supports the overarching concept that increased innervation is advantageous to cancer progression (Figure 3B).

The prostate gland is densely innervated and regulated by the autonomic nervous system. Parasympathetic input (via the pelvic nerve) and sympathetic input (via the hypogastric nerve) regulate growth of the prostate [188,189]. [190]. Adrenergic innervation is a key regulator, as rodent sympathectomy of the hypogastric nerve causes a reduction in overall weight of the prostate [189]. Adrenergic agonism with phenylephrine results in benign prostatic hyperplasia (BPH), providing additional evidence that sympathetic signaling contributes to prostate growth [191,192]. Cholinergic nerve fibers are present in both stromal and glandular epithelial compartments of the prostate [193–195]. While the role of cholinergic signaling in normal prostate growth remains to be elucidated [195,196], muscarinic acetylcholine receptors are highly expressed in both the normal prostate as well as in some prostate cancer cells, suggesting that cancer cells may take advantage of the same growth signals.

Indeed, autonomic neurotransmitters are the best-understood mechanism of innervation-induced prostate cancer progression. Cholinergic signaling through muscarinic receptors causes prostate cancer cell proliferation *in vitro* [197,198]. Adrenergic signaling promotes prostate tumor migration *in vitro* and *in vivo* [199]. In prostate xenograft models, lumbar lymph node metastases increased with exposure to norepinephrine, which was blocked with propranolol (B-blocker). Both

treatments had no effect on primary tumor growth [200]. Elegant recent work by the Frenette group elucidated multiple facets of autonomic innervation in prostate tumors. Using xenograft and genetic models, they demonstrated that sympathetic and parasympathetic innervation contribute to distinct aspects of cancer progression [185]. Adrenergic signaling via sympathetic innervation is important during initial stages of cancer development, and surgical or chemical sympathectomy or genetic deletion of stromal β 2- and β 3-adrenergic receptors results in poor tumor engraftment and growth. In contrast, cholinergic signaling via parasympathetic innervation promotes cancer spread. Disruption of parasympathetic signaling by pharmacological inhibition or genetic deletion of stromal type 1 muscarinic receptor reduced tumor invasion and metastasis. Similarly, in stratified human radical prostatectomy specimens; adrenergic fibers are present in surrounding normal prostate tissues, while cholinergic fibers infiltrate the tumor. Higher density of nerve fibers is associated with poor clinical outcome [185]. Taken together, these data indicate a crucial role for innervation in prostate cancer growth and progression and identify both adrenergic and muscarinic signaling as promising therapeutic targets.

Pancreas

Neurons in the microenvironment of pancreatic adenocarcinoma have been widely studied in the context of perineural invasion and histopathologically-apparent alterations in local nerves. Like prostate cancer, pancreatic adenocarcinoma exhibits increased overall nerve density [201], suggesting cancer-induced axonogenesis. While 90% of patients show intrapancreatic perineural invasion, those with additional extrapancreatic nerve plexus involvement have decreased survival [202]. Intrapancreatic, extratumoral perineural invasion also correlates negatively with post-operative survival [202,203]. Nerve plexus invasion is now regarded as one of the most important prognostic factors in invasive ductal carcinoma of the pancreas [204].

Invasion promoted by peripheral nerves was demonstrated in systems of isolated myenteric plexus neurons (MP) or dorsal root ganglia (DRG) neurons co-cultured with pancreatic cancer cells. Both neuron types increased cell invasion through a 3D extracellular matrix gel [205]. The molecular mediators of perineural invasion are coming to light, although it remains to be determined whether and which factors are derived directly from the nerves. Neurotrophins, including NGF and artemin (a GFL neurotrophic factor), are increased in pancreatic adenocarcinoma compared to the normal pancreas [206]. Artemin acts through GFR α 3/RET receptors to influence cancer spread along

pancreatic nerves [207]. Clinical studies examining the expression of GDNF and its receptor tyrosine kinase RET, linked both to pancreatic cancer proliferation and metastasis, invasion, and survival [5,208]. Secretion of GDNF and downstream activation of RET-Ras-MAPK pathway induces migration along nerve fibers [209]. The NGF/TrkA pathway has also been implicated in perineural invasion by prompting nerve hyperplasia [210]. Although both receptors of NGF, TrkA and p75NTR, are associated with perineural invasion, only TrkA is associated with poor prognosis [211]. Consistent with its known function in pro-apoptotic signaling in some molecular contexts, p75NTR is associated with a favorable prognosis [211]. NGF is thus thought to exert dual effects in pancreatic cancer, dependent on patterns of receptor expression.

Importantly, cancer-associated nerve alterations begin in the pre-malignant stages of pancreatic cancer. Stopczynski et al. monitored changes in innervation, neurotrophin expression and perineural invasion in genetically-engineered mouse models of pancreatic ductal adenocarcinoma and observed increased nerve density quite early in the disease course, when only pancreatic intraepithelial neoplasia was present. Increases in neurotrophic factor ligand and receptor expression were also observed from the earliest stages of malignancy. These dramatic changes in intrapancreatic nerve density and neurotrophin mRNA expression correlate with the early onset of pain, evidenced by pain-related decreases in exploratory behavior and nociceptive gene expression in sensory ganglia [212]. Work correlating neuroplastic changes in pancreatic nerves with the onset of pancreatic cancer suggest a critical role of innervation in cancer initiation and progression and helps explaining the early and prominent pain experienced by pancreatic cancer patients.

Gastrointestinal system

The extensive innervation of the gastrointestinal tract by the **enteric nervous system** (ENS), the **vagus nerve** and sympathetic nerves, controls organ function. After initial colonization, the neural crest-derived ENS precursor cells migrate, proliferate, and differentiate to form two layers of ganglia comprising the submucosal and myenteric plexi [213,214]. These processes are regulated through a variety of signaling pathways, including but not limited to GDNF/RET, endothelin-3/EDNRB, as well as classical axon path-finding molecules like netrins and semaphorins [215–223]. Development of the ENS, as well as autonomic innervation in other organs, is particularly dependent on GFL neurotrophin signaling, as loss of *Gdnf*, neurturin (*Nrtn*), RET or GFR α RET

co-receptor (*Gfra1* or *Gfra2*) genes results in insufficient development and severe GI dysfunction [222,224–228].

In the gastric epithelium, homeostasis is regulated by a balance of Wnt and Notch signaling, which promote proliferation of gastric epithelial stem cells, and bone morphogenetic protein BMP4 signaling, which influences differentiation [229]. Innervation promotes the growth of gastric stem cells via cholinergic stimulation of Wnt signaling, as illustrated by co-culture of gastric stem cells with enteric neurons. Pharmacological inhibition of muscarinic signaling suppressed Wnt signaling and gastric organoid growth in a manner dependent on **muscarinic receptor subtype 3** (Chrm3) expression [230]. Recent elegant work further demonstrated that acetylcholine induces LGR5+ enteric stem cell proliferation via YAP-dependent modulation of Wnt signaling [231]. These findings, together with additional data supporting a trophic effect of muscarinic signaling on gastric stem cell proliferation [232] help to explain the long-standing observation that vagotomy results in severe gastric atrophy after denervation [233]. Similarly, cholinergic nerves regulate the maintenance and proliferation of progenitor cells in the intestinal epithelium [234]. Non-synaptic paracrine secretion of serotonin from ENS neurons promotes growth and turnover of intestinal mucosal epithelium through the activation of **5-HT(2A) receptors** on cholinergic submucosal neurons, which then provide muscarinic innervation to intestinal epithelium [235]. Likewise in the liver, proliferation of hepatic progenitor cells is regulated by the vagus nerve, most likely via binding of acetylcholine to the Chrm3 receptor [236].

Like the effects on normal enteric stem cells, autonomic innervation also plays a role in enteric tumor growth. Chemical denervation with benzalkonium chloride (BAC) was found to block development of gastric tumors in a rodent model [237], a finding in contrast to early studies indicating that vagotomy promotes the development of gastric cancers [238,239]. Zhao et al. subsequently demonstrated that denervation at a preneoplastic stage inhibits tumorigenesis in a genetic mouse model of gastric cancer, and that denervation after onset slowed tumor growth [230]. Gene profiling analyses in the denervated gastric tumors identified Wnt and Notch signaling as regulators of tumor growth [230], both of which are known to be key players in normal gastric stem cell development and regeneration [240]. Similarly in the intestine, knock out of Chrm3 in a genetic model of small intestinal neoplasia reduced tumor number, size and β -catenin expression [241], consistent with the effects of muscarinic signaling on normal intestinal stem cell

proliferation. Enteric tumors encourage axonogenesis and increase innervation of the tumor microenvironment by secreting NGF [231]. Acetylcholine released by the recruited nerves drives cancer growth through increased Wnt signaling and further stimulates NGF release in a feed-forward cycle [231]. Strikingly, upregulated NGF secretion and subsequently increased enteric innervation is sufficient to initiate tumorigenesis [231]. Like neuronal regulation of Hedgehog signaling discussed above in brain and skin, neuronal regulation of Wnt signaling via muscarinic innervation in both the stomach and intestine represents an unexpected mechanism of neural regulation of signaling pathways widely implicated in both development and cancer. These shared neural signaling mechanisms suggest the cancer is commandeering normal trophic mechanisms of tissue homeostasis and regeneration. Enteric innervation emerges as a therapeutic target and anti-cholinergic agents as potentially useful drugs.

Concluding Remarks

Key insights pointing towards the importance of neural elements in the cancer microenvironment have come from histopathological observations. The importance of neuron-glioma interactions has long been suggested by a classic histopathological feature of high-grade gliomas called “perineuronal satellitosis”, characterized by microanatomical clustering of glioma cells around neuronal somata [242]. Similarly, the clinical significance of perineural invasion and increased axonal density within the tumor microenvironment of a number of malignancies has highlighted the potential roles of innervation in cancer progression. Advanced microscopy techniques are now shedding new light on further microanatomical relationships central to cancer biology, for example the interconnected network formed by Cx43-dependent glioma “microtubes” revealed by multiphoton laser-scanning microscopy [26]. Application of exciting new imaging techniques such as EM tomography, CLARITY [243,244] and super-resolution microscopy may reveal further structural aspects of neuron-cancer cell interactions in various tissue types.

Nervous system activity strongly modulates the function of stem and precursor cells, influencing organ development, maintenance, plasticity and regeneration in a diverse range of tissues. These neuron-mediated developmental mechanisms are recapitulated or hijacked in malignancy. While the roles of neural elements in cancer growth are becoming increasingly clear for a number of malignancies, considerable work remains to be done in others. In all cases, the mechanisms

mediating neuronal influences on cancer growth and progression are likely incompletely understood (see Outstanding Questions). Presently unexplored are the roles neurons may play not only in cancer growth, but also initiation. Use of modern neuroscience techniques for modulating neuronal activity in both the central and peripheral nervous systems, such as *in vivo* optogenetics or chemogenetics, in the context of cancer models that faithfully represent the human disease will be enormously informative. Already, our emerging appreciation of neural contributions to cancer growth and progression has elucidated novel therapeutic avenues for cancers of the brain, prostate, stomach, pancreas and skin. As our mechanistic understanding grows, neural targets in the cancer microenvironment may prove crucial for effective disease control.

Acknowledgements: We thank Chamelli Jhappan, Paul Frenette and Sam Pfaff for organizing the Nerves in Cancer meeting held at the National Cancer Institute in March 2015 and to all participants for galvanizing and thought-provoking discussions, and to Sarah Knox for critical reading of this manuscript. We gratefully acknowledge support from the National Institutes of Neurological Disease and Stroke (R01NS092597 to M.M.), the National Cancer Institute (1F31CA200273 to H.V.), the V Foundation (to M.M.), the California Institute for Regenerative Medicine (RN3-06510 to M.M.) and the Department of Defense (NF140075 to M.M.). We thank Sarah Chen for the figure illustrations.

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Figure 1: Neuronal activity promotes the proliferation of normal and malignant glial cells.

Active cortical projection neurons (mauve) promote the proliferation of normal oligodendrocyte precursor cells (OPCs, green) and earlier neural precursor cells (NPCs) in the oligodendrocyte lineage. This activity-regulated response of myelin-forming precursor cells contributes to ongoing neuroplasticity during development and into adulthood [55]. While the molecular mediators of activity-regulated OPC proliferation remain to be elucidated, BDNF and glutamate are hypothesized to each play a role. Similar to the effects on healthy OPCs, active neurons drive high-grade glioma cell (red) proliferation in a range of glioma subtypes, including adult glioblastoma, oligodendroglioma, pediatric glioblastoma and pediatric diffuse intrinsic pontine glioma [68]. Neuronal activity-regulated glioma cell proliferation is mediated by a secreted form of neuroligin-3 (sNLGN3), BDNF and glutamate [68,73,74]. Glioma cells in turn act on neurons to increase neuronal excitability and thus increase neuronal activity, an effect that is at least in part mediated by glutamate secretion by the glioma cells [76].

Figure 2: Peripheral innervation promotes glandular organ development and cancer progression. A) Glandular organogenesis depends on autonomic nerve innervation of the stem cell niche, and developing glands instruct proper nerve ingrowth (axon guidance) as illustrated in the submandibular salivary gland [168,169]. B) In pathological processes that mirror development, autonomic nerve innervation promotes prostate cancer progression [185] and prostate tumors instruct exuberant axonal sprouting and increased nerve density in the tumor microenvironment (axonogenesis; [184]). Ach = acetylcholine; Epi = epinephrine.

Text Box 1: Development of the Central Nervous System (CNS)

Development of the human CNS is a protracted process that spans decades. The brain and spinal cord form from neural stem cells (NSCs) of the **germinal zone** subjacent to the ventricles. During prenatal development, these stem cells (also called radial glial cells) first give rise to neurons, and after a “neurogenic to gliogenic switch” in mid-gestation, they begin to give rise to glial cells [245]. Establishment of the 6-layered cerebral cortex requires orderly migration of precursor cells along radial glial vertical fibers [246] and differentiation of layer-specific neurons in an “inside out” progression, with deep layers of cerebral cortex forming prior to superficial layers [245,247,248]. Neurogenesis involves neural stem cell proliferation, migration, and differentiation, with maturation of electrical excitability, outgrowth of axons and dendrites, and neurotransmitter specification. Following prenatal neurogenesis, **astrogliogenesis** and **oligodendroglialogenesis** occur in sequential but overlapping waves that extend well into postnatal life. Functional differentiation of oligodendrocytes to form myelin, or concentric wraps of oligodendrocyte membrane that provide electrical insulation of and metabolic support to axons, are predominantly postnatal processes that span at least the first three decades of human life [249,250]. In contrast to the cerebrum, brainstem and spinal cord, a great deal of cerebellar neuronal development occurs in the early postnatal period, with massive neurogenesis (~38 billion cerebellar granule cells) generated in the first year of human life [251]. Accordingly, cerebellar primitive neuroectodermal tumors, medulloblastomas, are among the most common solid cancers of infancy and early childhood. Cerebellar granule cell neurogenesis depends upon the proliferation, differentiation and survival of **cerebellar granule precursor cells (GCPs)**, which emerge prenatally from the rhombic lip of the midbrain/hindbrain junction to the cerebellar anlage.

Well into the postnatal period, GCPs proliferate in the external granule layer (EGL) of the developing cerebellum, then exit the cell cycle and migrate to the mature inner granule layer (IGL) via specialized radial fibers of Bergmann glia [252]; differentiating granule cell neurons in the IGL then project to and synapse on Purkinje cell dendrites in the molecular layer (Figure 1, Text Box 1). Additional cell types of the cerebellum arise from NPCs of the fourth ventricular zone.

Throughout life, new neuron generation continues in restricted regions of the central nervous system. Neurogenic NPCs may be a cell of origin for PNETs, ependymomas, and/or certain forms of glioma. Postnatal NSCs persist in the subventricular zone (SVZ) and in the dentate gyrus of the hippocampus (Figure 1, Text Box 1). SVZ NSCs give rise to neuroblasts that migrate along the rostral migratory stream [253–255] and differentiate into new neurons of the olfactory bulb and, in the human infant, of the prefrontal cortex [254]. SVZ precursors also give rise to OPCs in the mature brain [256], particularly in the context of injury [257]. Hippocampal stem cells residing in the subgranular zone (SGZ) of the dentate gyrus generate newborn neurons throughout life [258]. In adult humans, dentate gyrus neurogenesis results in ~1400 new hippocampal neurons daily [259].

OPCs are the most actively proliferative cell population in the adult brain of both rodents and humans (Figure 1, Text Box 1) [260,261] and precursors in the oligodendroglial lineage are thought to be the cell of origin for multiple subtypes of gliomas [10–14]. Robust new oligodendrocyte generation continues throughout life in rodents and non-human primates [262,263], and in humans continues at least into childhood [264]. In humans, non-human primates and rodents, myelination and myelin remodeling continues throughout life [262,264,265].

Figure 1, Text Box 1: Germinal zones of the postnatal human brain. After birth, active cell generation continues in several regions of the brain. The cerebellum continues robust development in infancy and early childhood (inset on right), with cerebellar granule cell precursors proliferating in the external granule layer (EGL, green) before migrating through the Purkinje cell (PC) layer to and differentiating in the internal granule layer (IGL). The cerebellum is a major site of pediatric brain tumors such as medulloblastoma, particularly in infancy and early childhood. In the subventricular zone (SVZ) of the lateral ventricles and the subgranule zone (SGZ) of the dentate gyrus in the hippocampus, neural stem and progenitor cells (green) persist throughout life. SVZ neural precursor cells give rise to new neurons of the olfactory bulb and (in infancy) frontal cortex as well as to new glial cells throughout life, particularly

oligodendrocyte precursor cells (OPCs, green process-bearing cells). Hippocampal neural precursor cells continue to give rise chiefly to new dentate gyrus neurons throughout life. Throughout the brain, OPCs continue to proliferate and generate new oligodendrocytes. OPCs are shown in the white matter (WM) of the corpus callosum; OPCs exist throughout both grey matter and white matter of the mature human brain. Both SVZ neural precursor cells and OPCs are putative cells of origin for multiple forms of glioma.

Text Box 2: Neurotrophins

The classical neurotrophin family, of which nerve growth factor (NGF) is the prototype, also includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4). These neurotrophins bind to p75^{NTR}, a Fas tumor necrosis factor receptor family member, and to one of the Trk tyrosine kinase receptors- either TrkA (NGF), TrkB (BDNF and NT4) or TrkC (NT3). Trk receptor tyrosine kinases signal to PKC, RAS/MAPK and PI3K pathways and are trophic in nature (for recent review, see [266]. In contrast, p75-mediated signaling is complex and can be either pro-apoptotic or trophic, depending on molecular context (for recent review, see [267]. NGF was the first neurotrophin discovered as responsible for sympathetic and sensory nerve growth and survival [95]. Subsequently, BDNF, NT-3 and NT-4 were also identified and implicated in a wide range of growth, maturation and plasticity-inducing effects throughout the nervous system and beyond.

A second family of neurotrophic factors belonging to the TGF β superfamily, the GDNF family of ligands (GFLs), includes glial-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN) and persephin (PSPN). Homodimeric ligands bind to receptor complexes composed of RET receptor tyrosine kinase and a GFR α family co-receptor; alternative receptors also exist [268]. GDNF was the first ligand discovered to promote the survival of midbrain dopaminergic neurons [96]. GDNF is expressed by striatal neurons, suggesting that in the healthy brain it is also a **target-derived survival factor** for midbrain DA neurons [269]. GDNF induces BDNF expression in midbrain dopaminergic neurons, which appears to contribute to its pro-survival effects [270]. The GFLs also play key roles in peripheral nervous system development.

Text Box 3: Peripheral Nervous System

The peripheral nervous system (PNS), derived largely from neural crest, is comprised of motor and autonomic efferent fibers and sensory afferent fibers. Most studies to date focused on the role of

the autonomic nervous system (ANS), divided into adrenergic **sympathetic** and **cholinergic** parasympathetic arms, in development and cancer. Innervation of visceral organs, particularly by autonomic nerves, is extensive. Gastrointestinal system innervation, including 500 million enteric neurons located in myenteric and submucosal plexi, is so extensive that it is classified as its own division of the nervous system called the enteric nervous system (ENS). A number of recent studies demonstrated a direct role of peripheral innervation in numerous cancers, including prostate, gastric, pancreatic and skin tumors. In a broader range of cancers, there is evidence for the influence of neurotransmitters and neurotrophins in cancer progression, although the origin of these ligands may be from neural structures, normal stromal cells or the cancer cells themselves. The nervous system also influences cancers indirectly through regulation of hormone secretion via the hypothalamic-pituitary-adrenal axis; for example via elevation of circulating epinephrine and cortisol. This expansive and important topic is outside the scope of this review.

Table summarizing neural molecules in cancer_122816

Neural Signal	Tumor Type	Demonstrated Function	Descriptive Observations	Refs
Glutamatergic signaling	Medulloblastoma	mGluR4 agonism reduces proliferation in vitro; decreases progression in vivo	mGluR4 correlates inversely with histological anaplasia, dissemination and clinical outcome	52
	Osteosarcoma		mGluR4 was found to be expressed in osteosarcoma (~20%) and benign giant cell tumors of bone (~40%), and expression correlated positively with overall survival in osteosarcoma	154-156
	Glioma	Secretion of glutamate by tumor cells increases cell proliferation, survival and migration/invasion can be mediated by calcium-permeable AMPA receptor stimulation		70-76
GABAergic signaling	Medulloblastoma		Group 3 medulloblastomas exhibit increased expression of GABA receptor $\alpha 5$	53
	Glioma	Activation of GABA _A receptors can be either depolarizing or hyperpolarizing	Tends to be present in lower grade gliomas (grade II and III astrocytomas) and in oligodendrogliomas rather than GBM (grade IV)	77, 78
Adrenergic signaling receptors	Ewing sarcoma and osteosarcoma		β -adrenergic receptors are expressed	152
	Prostate cancer	Chemical sympathectomy or genetic deletion of stromal $\beta 2$ - and $\beta 3$ -adrenergic receptors results in poor tumor engraftment and growth		185

Cholinergic signaling	Prostate cancer	Signaling through muscarinic receptors promotes prostate cancer cell proliferation <i>in vitro</i>	muscarinic acetylcholine receptors are highly expressed	197, 198
		Cholinergic signaling via parasympathetic innervation promotes cancer spread. Disruption of signaling reduced tumor invasion and metastasis.		185
	Intestinal adenoma	Knock out of Chrm3 in a genetic model of small intestinal neoplasia reduces tumor number and volume		241
Dopaminergic signaling	glioma	Antagonism of DRD4 particularly effective at reducing proliferation and survival via disruption of autophagy knock-down or pharmacological inhibition of D2DR dopamine receptor reduced tumor growth		80, 81
Bdnf / TrkB signaling	Glioma	Promotes proliferation and survival through TrkB (NTRK2)-mediated signaling	genomic aberrations affecting the TrK genes	95,129,130 131-134
	Medulloblastoma	Both BDNF exposure and TrkB inhibition reduce viability in different studies (conflicting data)	high p75NTR expression (mostly in SHH group)	103
	Pediatric ependymomas and low-grade astrocytomas		CSF levels of BDNF are increased	110
			p75NTR receptor is more highly expressed in supratentorial ependymoma compared to infratentorial ependymoma	111
	Multiple myeloma	BDNF triggers activation of survival pathways including Akt and MAPK	MM cells secrete BDNF and express high levels TrkB	142, 144
GDNF	Pancreatic cancer	Secretion of GDNF and downstream activation of RET-Ras-MAPK pathway induces migration	Expression of GDNF and its receptor tyrosine kinase RET, linked both to proliferation and metastasis, invasion, and survival	207 - 209
NT3	Medulloblastomas		Expression of both NT-3 and TrkC ; high TrkC expression levels correlate favorably with prognosis	103
	Neuroblastoma		Higher expression levels of TrkA corresponds to favorable prognosis	104, 105

NGF/ Pro-NGF	Melanoma	Autocrine/paracrine neurotrophin signaling promotes the proliferation and migration of melanoma cells	Expression of a range NGF family neurotrophin receptors and ligands	124-126
	Prostate cancer		NGF correlated positively with the Gleason score	187
	Pancreatic cancer		NGF increased in pancreatic adenocarcinoma	206
			Both receptors of NGF, TrkA and p75NTR, are associated with perineural invasion. p75NTR is associated with a favorable prognosis, while TrkA is associated with poor prognosis.	210, 211
Nlgn3	High-grade glioma	Secreted NLGN3 promotes growth	Genomic aberrations across multiple cancer type	68
Novel neurotrophin-1/NNT1/BSF3	Multiple myeloma	Linked to growth and survival of MM <i>in vitro</i>		140-142