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Perspective

Cancer hallmarks intersect with neuroscience in the tumor microenvironment

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SUMMARY

The mechanisms underlying the multistep process of tumorigenesis can be distilled into a logical framework involving the acquisition of functional capabilities, the so-called hallmarks of cancer, which are collectively envisaged to be necessary for malignancy. These capabilities, embodied both in transformed cancer cells as well as in the heterotypic accessory cells that together constitute the tumor microenvironment (TME), are conveyed by certain abnormal characteristics of the cancerous phenotype. This perspective discusses the link between the nervous system and the induction of hallmark capabilities, revealing neurons and neuronal projections (axons) as hallmark-inducing constituents of the TME. We also discuss the autocrine and paracrine neuronal regulatory circuits aberrantly activated in cancer cells that may constitute a distinctive "enabling" characteristic contributing to the manifestation of hallmark functions and consequent cancer pathogenesis.

INTRODUCTION

The hallmarks of cancer constitute a theoretical framework that has proved to be of enduring utility for rationalizing the vast complexity of cancer and its underlying mechanisms. The core of the theory involves eight acquired functional capabilities—the hallmarks—and two "enabling characteristics," namely abnormalities of the neoplastic state that contribute to the acquisition of said hallmark capabilities.[1,2] The core hallmark capabilities comprise: sustaining proliferative signaling; evading growth suppressors; resisting cell death; enabling replicative immortality; inducing or accessing vasculature; activating invasion and metastasis; deregulating cellular metabolism; and avoiding immune destruction. The two well-validated aberrant features of the disease state that variously enable their acquisition are (1) genome instability and mutation in the cancer cells and (2) tumor-promoting inflammation, principally by cells of the innate immune system.[1] The bar for inclusion of these ten parameters was that each had broad applicability across the spectrum of human cancer types and subtypes rather than being selectively restricted to one or a few. While the core conceptualization continues to resonate, evidence is growing that other potentially generalizable parameters are important and not easily categorized within the specific scope of the ten core hallmark parameters. Recently, several provisional parameters have been posed to stimulate debate, discussion, and experimental elaboration: phenotypic plasticity, non-mutational epigenetic reprogramming, polymorphic microbiomes, and senescent cells in the tumor microenvironment (TME).[3] Not mentioned is another exciting frontier in biomedicine, and that is the intersection between the nervous system and cancers. Increasing experimental evidence is substantiating this connection and its many facets, ranging from systemic effects of tumors on the functionality of the nervous system (e.g., cachexia, cognitive impairment, sleep disruptions), to local remodeling of tissue innervation by tumors, to modulatory effects of the nervous system on tumor phenotypes, topics that have been extensively reviewed.[4–9] What has not been a specific focus of such perspectives on cancer neuroscience is the growing realization that interconnections between the nervous system and developing cancers at both the cellular and molecular levels can facilitate the acquisition of hallmark capabilities, which is the theme of this perspective.

Impact of neurons and innervation on the acquisition of hallmark capabilities

The nervous system arborizes extensively throughout the body, enabling not only functions like movement and sensation but also innervating tissue stem cell niches to regulate the development, homeostasis, and regeneration of diverse organs and tissues. It is therefore not surprising that the nervous system similarly modulates cancer phenotypes, often through the co-option of neural mechanisms that parallel their roles in healthy tissues. Here, we will consider several examples that illustrate how innervation influences the acquisition of various hallmark capabilities, as summarized in Figure 1.

Neuronal activity promotes proliferative signaling

In certain CNS tumors—glioblastoma, diffuse intrinsic pontine glioma, and optic pathway glioma—glutamatergic neuronal activity drives proliferative signaling through paracrine mitogens released both from neurons and from other stromal cells in a neuronal activity-dependent manner.[10,11] Using optogenetic

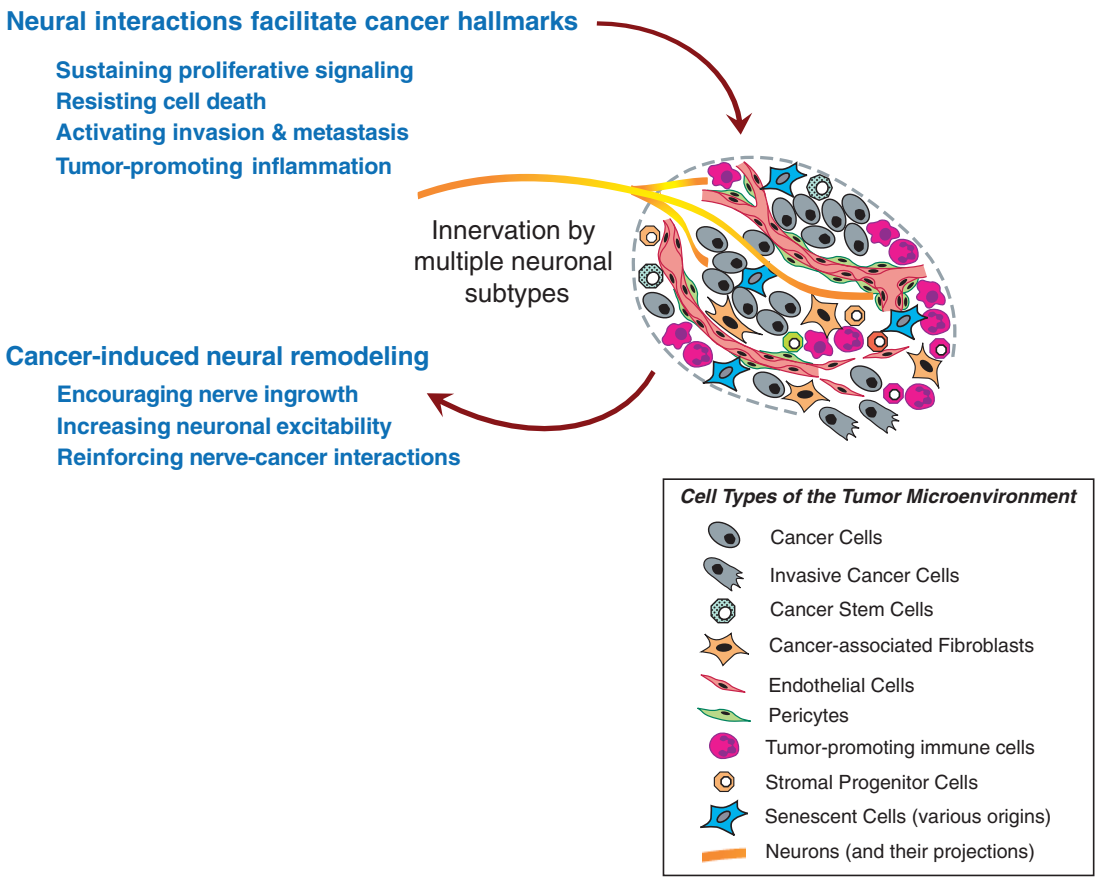

Figure 1. Neurons and their axonal projections are implicated as a common, functionally enabling constituent of the heterotypic cellular microenvironments of tumors

Peripheral innervation involves three principal subtypes: motor, sensory, and autonomic (including sympathetic and parasympathetic) nerves. Signaling between innervation (axonal projections of distant neurons, orange/yellow) and cancer cells enables multiple hallmarks of cancer, while reciprocal effects of cancer cells on the nervous system result in the remodeling of neural form and function that contributes to neurological complications of cancers and amplifies the consequences of neurons on cancer pathophysiology.

stimulation of neuronal activity in patient-derived high-grade glioma models[10] or in genetically engineered mouse models (GEMMs) of neurofibromatosis type 1 (NF1)-associated low-grade optic pathway glioma,[11] neuronal activity has been revealed to robustly promote cancer cell proliferation and tumor growth. Co-culture of neurons and glioma cells causes a marked increase in cancer cell proliferation,[12] which can be partially explained by the effects of paracrine secreted factors: exposing cultured glioma cells to conditioned medium collected from either cortical explants or from retinal plus optic nerve explants causes increased glioma cell proliferation through activity-regulated, mitogenic paracrine factors that include the neurotrophin brain-derived neurotrophic factor (BDNF) and the postsynaptic adhesion protein neuroligin-3 (NLGN3).[10,11] NLGN3 is shed from the cell surface of neurons and oligodendrocyte precursor cells (brain stromal cells) in an activity-regulated manner,[13] thereby stimulating PI3K-mTOR and other oncogenic signaling pathways in glioma cells.[10,13] NLGN3 exposure in the TME also stimulates NLGN3 expression in and shedding by the glioma cells themselves, thereby promoting oncogenic signaling via both autocrine and paracrine mechanisms.[13] Beyond gliomas, NLGN3 has also been implicated in autocrine stimulation of neuroblastoma growth, again in part by stimulating the PI3K-mTOR pathway.[14]

The effects of neuronal activity on glioma proliferation and growth were striking,[12,13] motivating a search for additional mechanisms beyond neuronal activity-regulated paracrine mitogens, which revealed functional synaptic signaling between glutamatergic neurons and glioma cells via calcium-permeable AMPA receptors in both pediatric and adult forms of glioma that resulted in depolarizing currents in the cancer cells.[12,15] This bona fide synaptic communication regulates glioma cell proliferation and growth, as evidenced by genetic blockade (expression of a dominant-negative version of the GluA2 subunit of AMPA receptors in glioma cells) or by pharmacological blockade of AMPA receptors in neuron-glioma co-culture and *in vivo*.[12] In the aforementioned pontine glioma, a second type of neuron-to-glioma synapse has been identified, involving GABAergic interneurons and diffuse midline glioma cells expressing $GABA_A$ receptors.[16] Because of high intracellular chloride concentration in the cancer cells, GABAergic synapses caused membrane depolarization rather than hyper-polarization in diffuse midline gliomas.[16] The electrochemical current consequent to synaptic signaling was key to the proliferation-promoting

mechanism: membrane depolarization alone was sufficient to promote glioma proliferation.[12] This mechanism illustrates a fundamentally neural form of signaling that promotes the acquisition of a key hallmark of cancer—proliferative signaling. The paracrine and synaptic mechanisms of neuronal activity-regulated glioma growth are related—in addition to acting as mitogens, both BDNF and NLGN3 promote neuron-to-glioma synaptogenesis.[12,17] Moreover, such electrochemical signaling is not restricted to primary brain tumors. As discussed in more detail below, breast cancer metastases to the brain engage glutamatergic signaling by mimicking astrocytes in pseudo-tripartite synapses to activate glutamatergic NMDA receptor signaling in the breast cancer cells so as to promote brain metastatic growth.[18]

Neuronal activity in the brain TME thus promotes the hallmark of sustaining proliferative signaling through both paracrine signaling mechanisms that activate oncogenic pathways like PI3K-mTOR, as well as through neuron-to-cancer synaptic signaling, a canonically neuronal mechanism.

Nervous system interactions with cancers outside of the CNS also promote proliferative signaling. For example, in NF1-mutant neurofibromas, which originate from peripheral nerve glial cells called Schwann cells, adjacent sensory nerves promote preneoplastic *NF1*$^{-/-}$ Schwann cell proliferation through activity-regulated secretion of a type I collagen chain (COL1A2),[19] which acts as a paracrine mitogen for Schwann cells. In another example discussed below, parasympathetic nerves drive gastrointestinal cancer proliferation and growth through cholinergic signaling that activates WNT signaling in gastrointestinal cancer cells[20]; numerous additional examples involve various nerve types sustaining cancer cell proliferative signaling through neurotransmitter and nerve-derived growth factor release into the TME.

Neural activity conveys resistance to cell death

Pioneering work from the laboratory of the late Paul Frenette demonstrated that adrenergic signaling from sympathetic nerves recruited into the TME promotes tumorigenesis of prostate cancer through β_2 and β_3 adrenergic receptors expressed on prostatic stromal cells. In xenograft models, sympathetic denervation of the prostate or genetic ablation of the *Adrb2* and *Adrb3* genes encoding β_2 and β_3 adrenergic receptors markedly impaired prostate cancer progression.[21] The effects of chemical sympathectomy of the prostate included impaired engraftment of xenografted prostate cancer cells as well as increased apoptosis of normal prostatic epithelial cells, underscoring a general trophic role for sympathetic innervation in the prostate. Deletion of *Adrb2*, *Adrb3*, or both genes in mice revealed a delay in xenografted tumor development upon loss of a single β-adrenergic receptor and profound inhibition of tumor engraftment in the absence of both β_2 and β_3 adrenergic receptors.[21] Similarly, in a *Myc*-driven GEMM of prostate cancer, chemical or surgical sympathectomy markedly reduced tumorigenesis. Increased numbers of apoptotic epithelial cells were observed within regions of prostatic intraepithelial neoplasia in sympathectomized mice,[21] indicating that sympathetic innervation of the prostate promotes the hallmark of evading cell death, thereby facilitating expansive tumor growth.

In the CNS, neuronal activity can also regulate tumor initiation and maintenance. In the aforementioned GEMM of NF1-associated optic pathway glioma, in which low-grade gliomas form in the optic nerve and chiasm, modulating optic nerve activity by decreasing visual stimulation (by rearing animals in complete darkness) just before the expected onset of tumorigenesis completely prevented tumor formation.[11] In contrast, 100% of littermate control mice reared with normal visual stimulation developed tumors. This blockade of tumorigenesis was durable even when normal visual experience was reintroduced after what appears to be a critical temporal window of tumorigenic susceptibility for this pediatric tumor. NLGN3, which functions as a neuronal activity-regulated growth factor for optic and other gliomas, as discussed above, evidently plays a key role in optic nerve-regulated tumorigenesis since genetic ablation of NLGN3 phenocopies the effect of dark rearing on tumor initiation in this model.[11] Importantly, decreasing optic nerve activity by limiting visual experience (dark rearing) subsequent to the temporal window of tumor initiation also markedly decreased the number of tumors evident at later time points, highlighting a role for optic nerve activity in tumor maintenance. Thus, with normal visual experience, this optic glioma mouse model consistently develops tumors beginning at 9 weeks of age, whereas decreasing optic nerve activity from 12 weeks onward in tumor-bearing mice substantially reduced the number and size of detectable tumors at 16 weeks. This finding can only be explained through tumor regression, thereby associating optic nerve activity with tumor maintenance via the hallmark of resisting cell death[11]; further research will be required to reveal the forms of programmed cell death operative in regressing tumors.

Together, these studies demonstrate the important role of innervation in conveying the hallmark of resisting cell death in multiple tumor types. An interesting corollary for future work will be to determine if such disruption of neuronal contributions to tumor maintenance has the potential to synergize with cytotoxic and other anti-cancer therapies.

Neural activity stimulates invasion and metastasis

The illuminating prostate cancer study discussed above[21] further demonstrated that while sympathetic innervation drives tumor growth, cholinergic innervation by parasympathetic nerves served to regulate tumor invasion and metastasis through muscarinic receptors (*Chrm1*) expressed by prostatic stromal cells.[21] Cholinergic agonists increased prostate cancer cell proliferation and metastatic spread to draining pelvic lymph nodes, whereas cholinergic blockers decreased lymph node dissemination in mouse models.[21] Thus, tumor cell proliferation, invasion, and distant metastasis were markedly reduced by pharmacologically or genetically blocking muscarinic signaling to the prostate TME,[7] illustrating a role for parasympathetic innervation in the hallmark of invasion and metastasis, as well as further sustaining proliferative growth.

Another long-recognized connection involves perineural invasion (PNI) that is evident in pancreas and prostate (and certain other) tumors whereby cancer cells invade along nerves into adjacent tissue. While often envisaged as a path of least resistance for cancer cells to invade through tissues, as opposed to breaking down extracellular matrix and tissue architecture, there are now clues that PNI can be actively facilitated by reciprocal interactions between tissue innervation and cancer cells. One prescient example involves Schwann cells, the glial support cell for peripheral nerves, in pancreatic ductal adenocarcinoma (PDAC). PDAC cancer cells reprogram proximal Schwann cells to become "tumor activated," involving paracrine induction of a c-Jun/AP-1

transcriptional network, similar to that observed in Schwann cells during the repair of wounded nerves.[22] There are two manifestations of the reprogrammed state. First, the Schwann cells assemble into microchannels ("tracks") that envelop and stimulate the motility of the cancer cells along the pathways of tissue innervation rendered aberrant by the PDAC cancer cells.[22] Second, the activated Schwann cells secrete the chemokine CCL2, which recruits inflammatory monocytes that differentiate into macrophages expressing the extracellular protease cathepsin B; both the macrophages and the protease they produce are functionally involved in stimulating PNI.[23] While yet to be generalized, these studies in PDAC functionally implicate the nervous system in a clinically important invasive phenotype.

Finally, considering an example from CNS cancers, the glutamatergic neuron-to-glioma synapses described above also play an important role in glioblastoma invasion. AMPAR-mediated synaptic input to a subpopulation of glioma cells at the tumor margin promoted their invasiveness. Synaptic communication-evoked intracellular calcium signaling was required for this effect on invasiveness, which could be blocked by calcium chelators or CREB inhibition.[24] Live *in vivo* imaging revealed that some of the invasive cells transitioned to a stationary proliferative phenotype over time, while other cancer cells continued to invade further, thereby collectively expanding the area of the brain colonized into the tumor mass.

Accordingly, these examples make a strong case for the nervous system as an enabling component of invasive cancer.

Neurons facilitate tumor-promoting inflammation

In NF1-associated low-grade gliomas (LGGs), CCL5 secreted by microglia and bone marrow-derived myeloid cells was found to promote tumor growth. CCL5 secretion by these cells in turn depended on paracrine signals from tumor-infiltrating $CD8^+$ lymphocytes. Neurons in the TME secreted the paracrine factor midkine, which stimulated the recruitment and activation of $CD8^+$ lymphocytes to secrete the chemokine CCL4, thereby inducing expression in microglial/myeloid cells of CCL5, which triggers the cell cycle (proliferative signaling) and suppresses apoptotic cell death in the cancer cells.[25] Remarkably, therefore, this mechanism conveys, in an unusual fashion, the hallmark capability to evade immune destruction by $CD8^+$ T cells, not by restricting their chemo-attraction and infiltration, but instead by maintaining the recruited ostensibly "activated" $CD8^+$ T cells in a state such that there is no evident T cell attack and killing of cancer cells (in mouse LGGs and inferentially human LGGs). As such, the $CD8^+$ T cells form the basis of an unusual form of tumor-promoting inflammation that conveys the hallmark capabilities of sustaining proliferative signaling, resisting cell death, and evading immune destruction.[25]

As summarized in Figure 1, these examples collectively support a growing appreciation that the innervation of tumors represents an important, hallmark-facilitating constituent of the TME.

Co-option of neuronal regulatory circuits by cancer cells serves to orchestrate hallmark capabilities

Beyond the effects of tumor innervation on the induction of hallmark capabilities, another dimension to the interconnection between neurobiology and cancer biology lies in the expression of neuronal signaling and regulatory circuits in cancer cells of multiple origins, not just ones with ontological relationships to neurons. A variety of signaling receptors are expressed in cancer cells, stimulated by the autocrine and/or paracrine supply of their cognate ligands, the latter often involving "feedforward" interchanges of ligands with various subtypes of innervation. Together with the engagement of signaling mechanisms classically neuronal in nature that are considered herein, cancer cells also can exhibit distinctly neuronal structural features, such as the extension of long, neurite-like processes that facilitate cell-to-cell communication in the TME,[26,27] that, while intriguing, are beyond the scope of this perspective. The following studies illustrate the emerging realization that multiple co-opted neuronal regulatory mechanisms aberrantly operative in cancer cells can make instrumental contributions to the acquired functional capabilities that drive cancer pathogenesis (Figure 2).

Autocrine/paracrine signaling mediated by neurotrophins and neurotransmitters promotes proliferation and vascularization

In a mouse model of PDAC, chronic stress-induced norepinephrine released from sympathetic innervation of tumors was observed to stimulate the β2 adrenergic receptor (ADRB2) expressed in the PDAC cancer cells, thereby upregulating the expression and secretion of the neurotrophic ligands NGF and BDNF, producing both autocrine and paracrine effects that collectively accelerated tumorigenesis and reduced survival.[28] Autocrine NGF signaling via its TRK receptors stimulated cancer cell proliferation and tumor growth, concomitant with paracrine NGF-stimulated hyper-innervation by adrenergic neurons, releasing more norepinephrine into the TME. Functional perturbations involving upregulation vs. inhibition of NGF/TRK and of ADRB2 established that both signaling circuits were instrumental in promoting tumorigenesis and reducing survival. Association studies in human PDAC linked the use of ADRB2 inhibitors (β-blockers) with modestly improved survival, as did the comparatively lower expression of the NGF paralog BDNF, consistent with the concomitant roles of TRK and ADRB2 signaling in the pathogenesis of this human cancer.

In a variation of the theme, another neurotransmitter, acetylcholine (ACh), was shown to similarly induce NGF in a mouse model of gastric cancer via stimulation of its receptor—muscarinic receptor-3 (CHRM3)—in cancer cells, resulting in autocrine stimulation of tumor progression and paracrine amplification of cholinergic hyper-innervation.[20] In this case, ACh bioavailability was mediated both by intestinal tuft cells and cholinergic neurons, which stimulated CHRM3 signaling to induce the expression and secretion of NGF, leading to autocrine TRK receptor signaling in the cancer cells and paracrine-mediated expansion of ACh-expressing tuft cells, as well as the ingrowth and elaboration of cholinergic nerves in the TME. Together, these interactions amplified tumor-promoting signaling in cancer cells, including activation of the WNT and YAP pathways, which are known to enhance cancer cell proliferation.

In both models, the collaborative effects of NGF and neurotransmitters clearly affected the proliferative hallmark; potential effects on other cancer hallmarks were not explored but warrant future investigation.

To that end, a third illustrative study implicated noradrenaline signaling via ADRB2 in another hallmark capability, namely triggering of the angiogenic switch that induces and sustains tumor

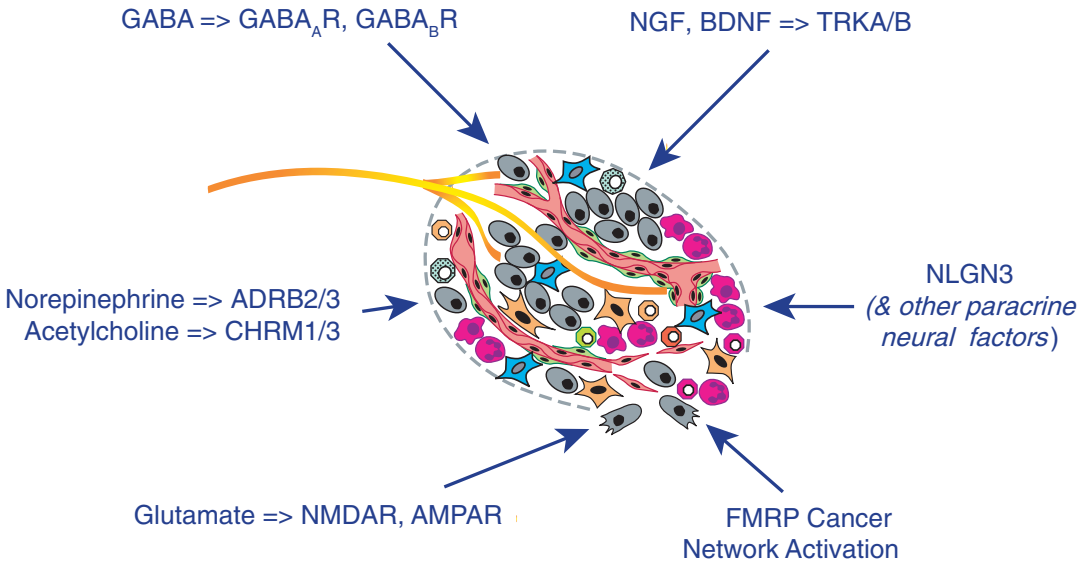

Figure 2. Neuronal regulatory pathways co-opted in cancer cells are implicated in facilitating the acquisition of hallmark capabilities
While evidently activated by non-mutational epigenetic reprogramming and, in some cases, genome instability and mutation, the increasing breadth of co-opted neuronal regulatory circuits in cancer cells suggests that this concept warrants being highlighted as an important hallmark-enabling characteristic that is instrumental in multiple tumor phenotypes.

vascularization to support expansive tumor growth.[29] Mouse models of prostate cancer also have elevated levels of noradrenaline, and when *Adrb2* was ablated genetically or ADRB2 was pharmacologically inhibited, tumor progression was impaired, concomitant with the failure to activate neovascularization. Tissue-specific knockouts of *Adrb2* in endothelial cells, pericytes, and myeloid cells revealed that only endothelial cell expression of ADRB2 was crucial for the angiogenic switch, establishing its importance for activation of this hallmark capability. The underlying physiological mechanism proved to be alterations in endothelial cell metabolism: noradrenaline-stimulated ADRB2 instructed the metabolic state of aerobic glycolysis that supported the angiogenic phenotype. Conversely, its absence switched endothelial cell physiology to depend on oxidative phosphorylation, which thereby inhibited the induction of angiogenesis. As such, the hallmarks of inducing angiogenesis and reprogramming cellular metabolism were affected.

Neurotransmitter and neurotrophin signaling pathways are similarly crucial in cancers within the CNS, such as the glutamatergic signaling mediated by AMPA receptors in gliomas[13,15,24] and NMDA receptors in breast cancer brain metastases[18] discussed below. Such neurotransmitter signaling in glioma is elaborated and reinforced by neurotrophin (BDNF) signaling that promotes increased numbers and strength of glutamatergic neuron-to-glioma synapses.[17]

Collectively, the multifactorial amplification of neurotrophins and neurotransmitters, involving autocrine and paracrine signaling crosstalk between cancer cells and neurites in the TME, concomitant with the expression of their receptors and activation of receptor-mediated signaling in these as well as other cell types—e.g., endothelial cells and intestinal tuft cells—demonstrably contributes to the acquisition of several hallmark capabilities, with more likely to be illuminated in future studies.

GABA-mediated autocrine signaling of proliferation and immune evasion

GABA, converted from intracellular glutamine by glutamic acid decarboxylases (GAD1/2), is secreted to serve as an inhibitory neurotransmitter in the CNS via signaling through two receptors, GABA$_A$R and GABA$_B$R. Levels of GABA are elevated in late-stage human tumors and are inversely correlated with prognosis, as is the expression of GAD1 and GABA$_B$R, which are typically co-expressed in cancer cells, thereby establishing an autocrine signaling loop.[30] Genetic and pharmacological perturbation of GAD1 and GABA$_B$R in tumor cell lines and mouse models has revealed that GABA-mediated signaling contributes to the hallmark capabilities of sustaining cancer cell proliferation and evading immune destruction. Moreover, it modulates tumor-promoting inflammation and hence the balance between these two dichotomous parameters of the immune response to tumors, which was shifted to favor T cell attack when GABA signaling was inhibited. A key component of the molecular mechanism involved suppressing GSK-3β activity so as to stabilize β-catenin levels and thereby enhance its regulatory signaling.[30] While β-catenin has been long recognized as a proliferation-inducing oncogene in certain tumor contexts, the impairment of GABA-stimulated tumor growth was more pronounced in immunocompetent vs. immunodeficient mice, implicating evasion of adaptive immunity. Indeed, functional studies revealed a mechanism underlying this immune evasion: GABA→GABA$_B$R→β-catenin signaling in cancer cells repressed expression of the pro-inflammatory chemokines CCL4/5, which were upregulated in the context of *GAD1* knock down or pharmacological inhibition of GABA$_B$R, thereby recruiting T cells and CD103$^+$ dendritic cells, both of which were necessary for productive tumor immunity when this signaling pathway was suppressed. It will be of interest to investigate the role of GABA signaling in modulating the balance between tumor-promoting inflammation and anti-tumor

Figure 3. Cancer hallmarks meet neuroscience

The examples presented herein demonstrate the functional involvement of innervation as a constituent of the TME, and/or of co-opted neuronal signaling in cancer cells, in modulating six (highlighted) of the ten parameters constituting the core hallmarks' conceptualization. Future research may reveal connections to those that are currently unconnected, as well as to other provisional parameters on the horizon.

immunity in other tumor types, as well as to further illuminate the roles that other neuronal signaling pathways are playing, as exemplified by the discovery of an immunomodulatory role for the fragile X mental retardation protein (FMRP) that is described below.

Glutamate-mediated autocrine/paracrine signaling of the invasive/metastatic hallmark

Another important co-opted neuronal signaling pathway involves the glutamate-stimulated NMDA receptor, normally involved in synaptic transmission. Although paracrine in synapses, in pancreatic tumors—both neuroendocrine and ductal—autocrine signaling activity was induced.[31] Glutamate transporters were up-regulated to secrete glutamate, which activated NMDAR expressed in the same cancer cells. The consequence was an impact on two hallmarks: proliferation and, most notably, invasion.[31,32] By contrast, paracrine activation of NMDAR by glutamate was evident in the aggressive triple-negative form of breast cancer, where NMDAR signaling fostered colonization of brain metastases. In this case, glutamate is not secreted by the breast cancer cells themselves but rather is supplied in a paracrine fashion via the association of triple-negative breast cancer (TNBC) cells with neuronal synapses in the brain, where glutamate is secreted during synaptic transmission.[18] Congruently, as described above, glutamatergic signaling via bona fide synapses promoted tumor invasion and brain colonization in glioma.[24]

FMRP regulates invasion, metastasis, and immunosuppression

The co-option by cancer cells of neuronal regulatory mechanisms extends beyond ligand-receptor signaling, of which a salient example is the aforementioned FMRP, a neuronal regulatory protein normally involved in synaptic transmission. FMRP is an RNA-binding protein that governs protein translation and mRNA stability, affecting the expression and activity of hundreds of genes. FMRP is controlled by glutamate-stimulated NMDAR signaling in neurons, and also in certain cancer cells, wherein this signaling pathway is aberrantly upregulated.[32] Notably, however, FMRP is broadly over-expressed in human solid tumors, many of which do not evidence NMDAR activity, indicative

of additional regulatory mechanisms.[33] FMRP was initially implicated as a driver of invasion and metastasis.[32,34,35] More recently, FMRP has been revealed as a master regulator of the immunosuppressive TME in multiple tumor models and implicated in human cancers.[33] Among its functional effects revealed in tumors via knockout/knockdown in cancer cells of the *Fmr1* gene that encodes it, FMRP stimulated the secretion of cytokines that increase the abundance of regulatory (immunoinhibitory) T cells, and it programmed immunosuppressive macrophages, thereby collectively conveying resistance to tumor immunity and immunotherapy. In the absence of FMRP's induction of these and potentially other immunoregulatory cell types, the TME became immunostimulatory, recruiting and activating T cells that productively attacked the cancer cells, impairing tumor growth. These intriguing findings connect FMRP expression and the network of genes it regulates in cancer cells to the hallmarks governing morbidity and mortality—invasion and metastasis and evasion of immune destruction.

Collectively, as summarized in Figure 2, these examples illuminate a remarkable characteristic of cancer cells of diverse origins, namely the activation of a number of regulatory pathways normally operative in neurons that variously contribute to tumor growth and progression.

CONCLUSION

The integration of functional capabilities—hallmarks of cancer—acquired by cancers during tumorigenesis and malignant progression has proved to be an enduring conceptual framework with which to distill the daunting complexity of the disease. While the eight core hallmark capabilities are well established as having applicability across the spectrum of human cancers, along with a ninth—phenotypic plasticity of cancer cells—that is under consideration,[3] the cellular and molecular mechanisms by which the hallmarks are acquired are increasingly appreciated to be diverse, encompassing more than just the two well-validated enabling characteristics, namely genome instability and mutation, and tumor-promoting inflammation. Herein, we make a case for another fascinating parameter, namely the multifaceted connectivity between cancer hallmarks and the nervous system, a link that has recently been considered by others.[36–38] The exemplary studies presented herein illustrate the emerging realization that the nervous system is profoundly influential, manifested both in tumor innervation and in neuronal regulatory circuits co-opted to be operative in cancer cells. Much as for another emerging parameter, that of polymorphic microbiomes populating tumors and their hosts, there is partial but not complete overlap with tumor-promoting inflammation. Both phenotypic characteristics can elicit tumor-promoting inflammation, and yet each has much broader effects in enabling hallmark capabilities that are not logically categorized as an integral element of inflammation.

In summary, it seems reasonable to postulate that co-opted neuronal signaling circuits in cancer cells constitute a distinctive and instrumental regulatory mechanism that modulates tumor development and malignant progression, at least for the illustrated cancer types and for others we have not discussed. Additionally, the now well-accepted concept that a set of heterotypic cell types populate most tumor environments and thereby contribute to the induction of cancer hallmarks can arguably be expanded to include neuronal innervation as a common and functionally impactful constituent of the TME. Thus, these two distinctive interfaces with the nervous system are implicated as substantive contributors to hallmark cancer phenotypes (Figure 3). It is conceivable that tumor innervation and co-opted neuronal signaling in cancer cells will prove to modulate additional hallmark capabilities and associated parameters, e.g., phenotypic plasticity,[3] which has been recently demonstrated in small-cell lung cancer,[39] above and beyond those highlighted in Figure 3; such possibilities deserve future investigation. Finally, a metric for formal incorporation into the hallmarks of cancer schema has been a consensus for appreciable generality. For example, deregulating cellular metabolism and avoiding immune destruction, initially posited as "emerging" hallmarks in 2011,[1] are now broadly validated and have consequently been incorporated into the core conceptualization.[3] The multifactorial connectivity of neurobiology to hallmark capabilities described herein—currently described variously in cancers of the brain, skin (basal cell, melanoma), head and neck, breast, lung, stomach, colon, pancreas, and prostate—are provocative and warrant continuing elucidation and experimental validation across the landscape of human cancers.

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DECLARATION OF INTERESTS

D.H. is a scientific founder of and stockholder in Opna Bio SA (Lausanne, CH), which has licensed a patent from EPFL, wherein D.H. is a co-inventor, describing FMRP as a cancer cell-intrinsic immunosuppressor and a potential therapeutic target; he serves on the company's board of directors (BoD) and scientific advisory board (SAB). M.M. was on the SAB of Cygnal Therapeutics, and her family holds equity in MapLight Therapeutics.

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