Chapter 3
Cancer-Induced Neurogenesis

Tanya Miladinovic and Gurmit Singh

Abstract This chapter explores what we know about the structure and function of neurons, including the identity and location of adult neural stem cells, the proliferation and specification of neural progenitors, and their suspected involvement in cancer. We begin with a brief review of conventional accounts of neurogenesis and progress toward current issues in the field. Finally, we discuss the potential influence of cancer on the formation and innervation of new neural networks, and the effects of this on metastatic tumour progression. The process of neurogenesis was traditionally believed to occur exclusively during embryonic stages, but recent evidence strongly suggests that neurogenesis occurs in discrete regions of the adult mammalian central nervous system (CNS), and that this process may be upregulated in the presence of cancer. A complex network of biochemical pathways and signalling molecules influence metastatic tumour growth. The dysregulation of these signalling pathways by cancer drives tumour growth and leads to significant symptoms, including pain. Tumour cells secrete growth factors, cytokines, and chemokines and are reported to stimulate adjacent nociceptors. Progressive tumour growth is accompanied by escalating pain behaviours in murine models of cancer-induced bone pain. Neurotrophic factors play an important role in the functionality of nociceptive afferents, and represent a probable link between metastatic tumour growth and pain.

Keywords Neurogenesis · Cancer · Cancer-induced neurogenesis · Neurotrophins · Nerve growth factor · Brain-derived neurotrophic factor

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Abbreviations

CNS	Central nervous system
BrdU	Bromodeoxyuridine
IHC	Immunohistochemistry
VEGF	Vascular endothelial growth factor
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
NT	Neurotrophin
NTR	Neurotrophin receptor
CIBP	Cancer-induced bone pain
NSAIDs	Nonsteroidal anti-inflammatory drugs
CGRP	Calcitonin gene-related peptide
NMDA	N-methyl-D-aspartate
AFT-3	Activating transcription factor-3

Introduction

While our understanding of neurogenesis has increased dramatically within the past decade, the field remains relatively elusive, and we are far from a comprehensive understanding. Even more mysterious is the role of neurogenesis in cancer. This is an exciting time to be involved in the field of neurogenesis, as imaging techniques are creating platforms to investigate novel ideas. This chapter will explore what we know about the structure and function of neurons, including the identity and location of adult neural stem cells, the proliferation and specification of neural progenitors, and their suspected involvement in cancer. We begin with a brief review of conventional accounts of neurogenesis and progress toward current issues in the field. Finally, we discuss the potential influence of cancer on the formation and innervation of new neural networks, and the effects of this on metastatic tumour progression.

The Birth of Neurogenesis

Santiago Ramon y Cajal is widely recognized as the father of neuroscience. Traditionally, neurons were believed to be generated exclusively during the prenatal phase of development [79]. “No new neurons after birth” became the central dogma in neuroscience for nearly a century [36]. In the late 1950s, however, a new technique was developed to label dividing cells with [H^3]-thymidine, which incorporates into DNA during the replicative S-phase of the cell cycle and can be detected with autoradiography [95]. In 1961, the generation of new neurons was first reported using this technique on three-day-old mouse brains. Shortly after, Altman and colleagues published a series of reports demonstrating [H^3]-thymidine evidence for new

neurons in the adult rat brain, particularly in the dentate gyrus of the hippocampus [4], neocortex [3], and olfactory bulb [2]. At the time, these studies were seen to lack functional relevance and were not given much attention. In the late 1970s, the issue of adult neurogenesis was revisited when it was demonstrated that newborn neurons in the hippocampus survive for a long period of time [49], receive synaptic inputs [48], and project their axons to target areas [96]. Meanwhile, a series of studies that focused on adult neurogenesis in songbirds provided evidence for functional roles of post-natal neurogenesis in seasonal song learning [72].

Neurogenesis is now a widely studied phenomenon and has known applications that extend beyond simple embryonic proliferation. Research suggests that it is functionally implicated in many mental and physiological illnesses, including cancer.

Neurons

A neuron is an electrically excitable cell that uses chemical signals to transmit information between the brain and body. A typical neuron is composed of a soma (cell body), dendrites, and an axon (Fig. 3.1). Dendrites are thin, branched

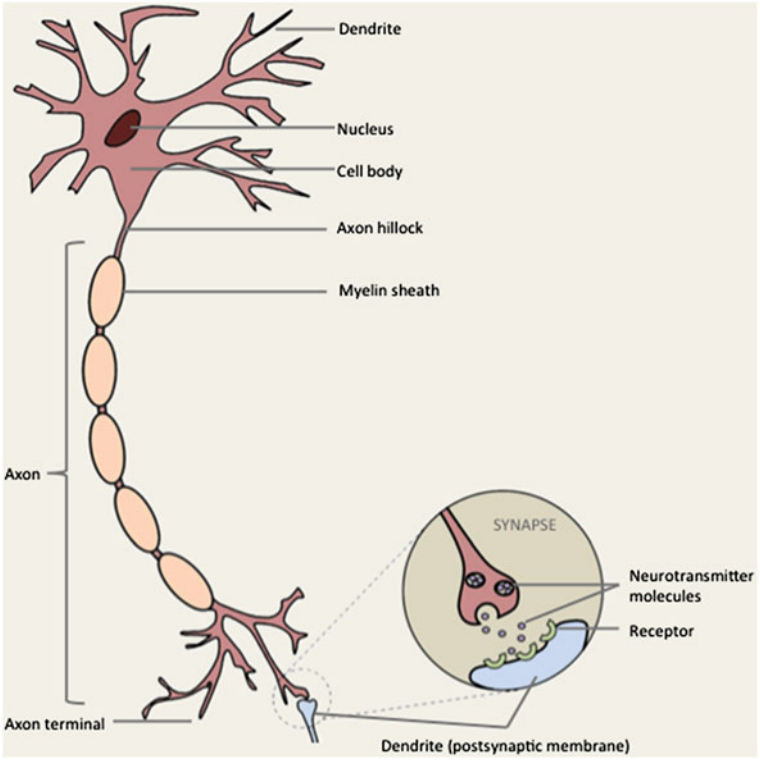

Fig. 3.1 A typical neuron

structures that extend from the cell body and cumulatively form the dendritic tree. Axons also extend from the cell body, but unlike dendrites, each soma gives rise to only a single axon. Axons leave the soma at a swelling called the axon hillock and may be extremely long, extending up to one metre in humans. Electrical signals are transmitted from the cell body, down the axon, to the dendrites of adjacent neurons through a process called saltatory conduction. A layer of electrically insulating material called myelin surrounds axons, creating a myelin sheath that propagates the nerve impulse while preventing the loss of electrical current [103]. At the synapse, the small gap between neurons, signals are transmitted from the axon of one neuron to the dendrites of surrounding neurons via excitatory and inhibitory messengers, called neurotransmitters. Neurons do not undergo cell division, but arise from progenitor cells, or stem cells.

Neurogenesis

Neurogenesis is the process of generating functionally integrated neurons from progenitor cells. Traditionally, this process was believed to occur exclusively during embryonic stages [79], but recent evidence strongly suggests that neurogenesis occurs in discrete regions of the adult mammalian central nervous system (CNS), including two brain regions called the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus in the hippocampus [36, 50, 58]. Beyond these two structures, neurogenesis has appeared to be nonexistent in healthy individuals. However, following trauma and pathological stimulation, non-neurogenic regions in the adult brain appear to support neurogenesis. In an effort to study this phenomenon in adults, neural stem cells were first isolated from the adult CNS of rodents [80] and later from humans [54].

Bromodeoxyuridine (BrdU), a synthetic thymidine analogue and S-phase marker of the cell cycle [34], is detectable by immunohistochemistry (IHC) and can be used for phenotypic analysis and stereological quantification, making IHC the most commonly used technique in the field. Adult neurogenesis has been observed with BrdU incorporation in mammals and human samples [29]. Evidence from combined retroviral-based lineage tracing [76, 84] and electrophysiological studies [12, 20, 101] suggest that newborn neurons in the adult mammalian CNS are functionally and synaptically integrated.

Like angiogenesis, neurogenesis involves the development of intricately branched networks that are regulated by guidance factors and cytokines, including semaphorins and their receptors [111], vascular endothelial growth factor (VEGF), which supports neuronal survival [56], plexins [111], and neuropilins [27], which are involved in tumour vascularization. Multiple clinical observations suggest that angiogenesis and angiogenic factors promote neurogenesis. Seizure- and cerebral ischemia-induced brain injury stimulate both angiogenesis and neurogenesis [37, 74]. Notably, neurogenesis is observed in both human patients and animal

models of neurodegenerative diseases including Huntington's, Alzheimer's, and Parkinson's [35, 47, 104].

Adult neural stem cells are unspecified precursor cells with the ability to proliferate and make new neurons, astrocytes, and oligodendrocytes. Bone marrow-derived $CD34^+$ progenitor cells offer promise for the treatment of various diseases through the repair of damaged tissues. Stem cells differentiate into endothelium, hematopoietic cells, and as reported by some, into neurons, fibroblasts, and muscle [32]. $CD34^+$ and $CD133^+$ differentiate into endothelial cells and thereby participate in neurovascularization, the healing of injured tissues, and promotion of tumour growth and inflammation [7, 21, 40]. In animal models, $CD34^+$ stem cells have been shown to indirectly promote neurogenesis through angiogenesis following stroke, possibly due to a reduction in the G1 phase of the cell cycle [99].

Neurotrophins

Neurotrophins are proteins that regulate neuronal survival, axonal proliferation, synaptic plasticity, and neurotransmission [64, 106]. They are a superfamily of polypeptide growth factors including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins (NTs) 3–6 [16, 25]. They influence cellular function by activating their respective tyrosine kinase receptors TrkA, TrkB, TrkC, and the common neurotrophin receptor (NTR) p75 [23, 98]. Along with their receptors, neurotrophins are key to the survival, development, and function of the vertebrate nervous system [11, 14, 57], and are also present in non-neuronal tissues [92].

Although classically known for their effects on neurons, neurotrophins have been found to be multifunctional and to affect non-neuronal cells as well. Neurotrophins function to stimulate proliferation and differentiation in various cell types, have been implicated in the pain response, and have receptors that are highly expressed in the central and peripheral nervous systems. Although originally thought to function during the developmental stage only, it is now known that their functionality extends to mature stages of life.

Neurotrophins are constitutively expressed at low levels in adult tissues and are upregulated in inflammatory pain states. The p75 NTR binds all the members of the neurotrophin family with low affinity, while NGF, BDNF and NT-4/5, and NT-3 bind preferentially to TrkA, TrkB, and TrkC, respectively (Fig. 3.2). Under normal physiological conditions, neurotrophins regulate the differentiation, growth, and survival of neurons.

The Trk receptors are tyrosine kinase receptors. Activation by their ligands leads to dimerization of the receptor and phosphorylation of residues that promote the

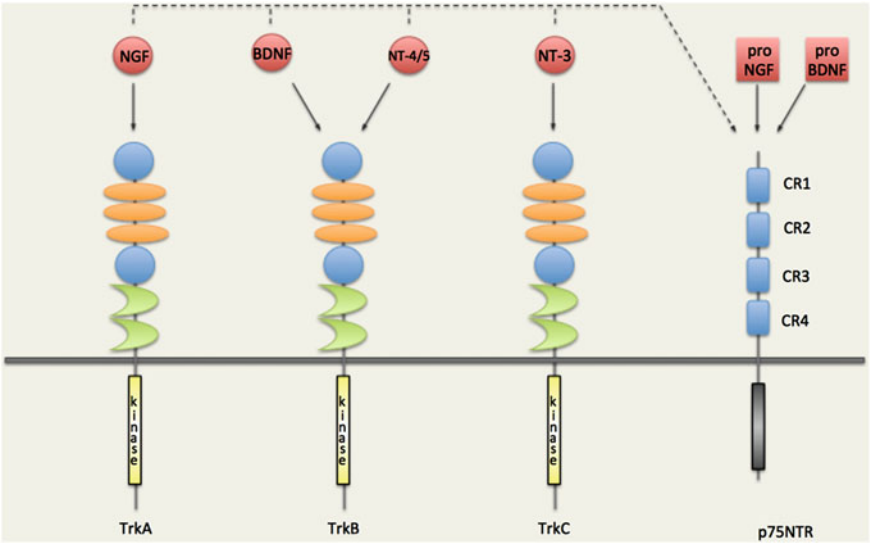

Fig. 3.2 Major neurotrophin–receptor interactions. Proneurotrophins bind p75NTR, but not the Trk receptors. Mature neurotrophins bind and activate p75NTR, and specifically interact with the three Trk receptors. NGF activates TrkA; BDNF and NT4 activate TrkB; NT3 activates TrkC. Ligand-binding specificity is affected by the presence of p75NTR

activation of the Ras-Raf-MAPK, PI3 K-Akt-GSKIII, PLCγ-DAG- PKC, and S6 kinase signalling pathways (Fig. 3.3). The p75 receptor increases the rate of binding of NGF to TrkA, thereby increasing the number of high affinity binding sites [10].

During early development, activation of these pathways blocks apoptosis, thereby promoting cell survival and differentiation. Activation of these pathways in adult neurons regulates neural responsiveness and synaptic function and has important consequences for pain signalling systems.

Regulation of Neurotrophins by System x_C^-

The system x_C^- antiporter exchanges intracellular glutamate for extracellular cysteine at a 1:1 ratio in an effort to protect against oxidative stress. Considerable evidence suggests that glutamate released from system x_C^- is involved in multiple physiological and pathological processes, which may alter neuronal plasticity and can cause cellular toxicity. Glutamate released from activated astrocytes and microglia are capable of killing cortical neurons [30] and granule cells [75], respectively. System x_C^--mediated cystine uptake plays an important role in the regulation of cellular glutathione levels, as glutathione synthesis in the brain is rate-limited by the uptake of cystine [83]. In astrocytes, overexpression of xCT, the functional subunit of system x_C^-, enhances glutathione release and protects

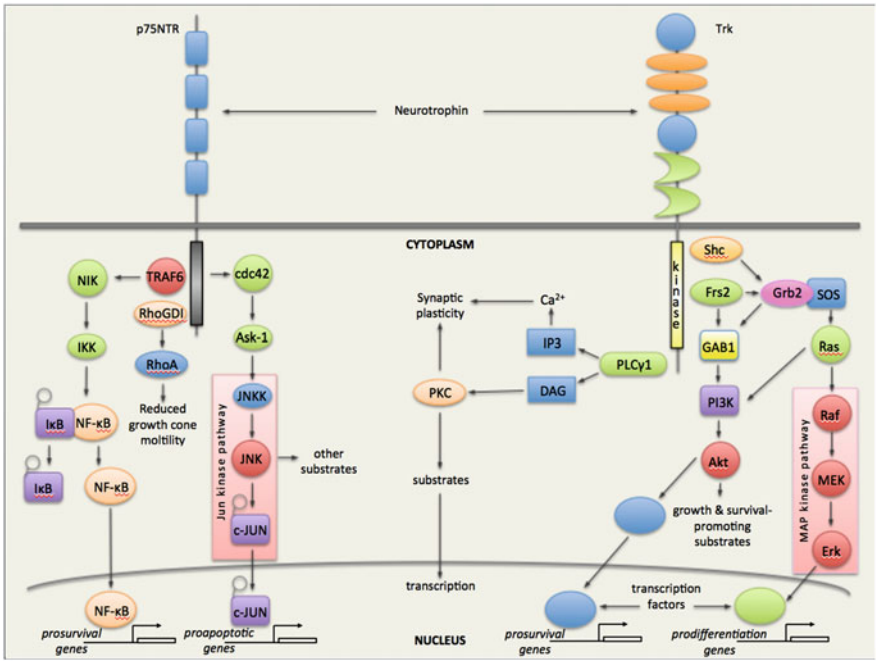

Fig. 3.3 Major intracellular signalling pathways and the interactions of neurotrophins with Trk and p75NTRs. The p75NTR regulates three signalling pathways. NF-kB activation results in transcription of multiple genes that promote neuronal survival. Activation of the Jun kinase pathway regulates activation of several genes, some of which promote neuronal apoptosis. Ligand engagement of p75NTR controls the activity of Rho, which controls growth cone motility. Each Trk receptor controls three major signalling pathways. Activation of Ras activates the MAP kinase signalling cascade, which promotes neuronal differentiation and neurite outgrowth. Activation of PI3 kinase through Ras or Gab1 promotes survival and growth of neurons and other cells. Activation of PLC-g1 results in activation of Ca2C- and PKC-regulated pathways that promote synaptic plasticity. Each of these signalling pathways also regulates gene transcription

neurons from oxidative stress [93]. By overexpressing glutamate, system x_C^- has the potential to cause excitotoxicity.

Cancer cell lines release excess glutamate via system x_C^- [86, 90, 100], and there is considerable evidence for bidirectional signalling between glutamate and neurotrophins. That is, glutamate upregulates neurotrophin expression, while the neurotrophins then upregulate the expression of the system x_C^- transporter [61]. Neurotrophins have many functions within the CNS, including mediating excitotoxicity, oxidative stress, and cellular glutathione levels. Given the high level of overlap between system x_C^- and growth factors, it is perhaps not surprising that some neurotrophic effects may be mediated by the functional regulation of system x_C^-.

Cancer-Induced Bone Pain

Cancer has the propensity to metastasize to the bone microenvironment, causing severe cancer-induced bone pain (CIBP) in patients [69], which is characterized as ongoing or breakthrough pain. Ongoing pain is characteristically dull, persistent, increasing in intensity over time, and is often pharmacologically managed with the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Breakthrough pain is further characterized as "spontaneous pain," without an apparent trigger, or "movement-evoked pain," brought on by movement of the tumour-bearing bone. Exogenous glutamate sensitizes adjacent nociceptors and initiates a pain response in peripheral tissues [18, 19].

CIBP elicits neurochemical changes unique from inflammatory or neuropathic pain states. Bone is innervated with Aβ, Aδ, and C fibres [65]. The acidic tumour environment, along with the secretion of growth factors, cytokines, and chemokines from the tumour cells, are reported to stimulate adjacent nociceptors and evoke pain [71].

Studies have shown that progressive tumour growth is accompanied by pain behaviours in rats [38] and mice [100]. Metastatic tumour growth is influenced by a complex network of biochemical pathways and signalling molecules. The dysregualtion of these signalling pathways by cancer drives tumour growth and leads to significant symptoms, including pain. Neurotrophic factors play an important role in the functionality of nociceptive afferents, and represent a probable link between metastatic tumour growth and pain.

Animal sarcoma models mimic the relative resistance to opioid therapy seen in humans with bone cancer pain, such that 10-fold higher doses of morphine are required to control bone cancer pain as compared to chronic inflammatory pain [63]. Neuropathic pain is also resistant to standard opioid analgesic therapy [108]. Taken together, this information suggests that a potential neuropathic component may be involved in driving bone cancer pain.

Neurotrophins as a Mechanism for Cancer-Induced Bone Pain

Progenitor cells function to repair injured tissues by facilitating new muscle, nerve, and blood vessel formation. Paradoxically, the same progenitors may contribute to tumour growth by promoting angiogenesis and tumour invasiveness.

A tumour is far from an isolated structure within its host organism; it interacts with its environment directly via cell-to-cell contacts. Like native cells, tumours require nutrients and oxygen, as well as a method of excretion for metabolic wastes and carbon dioxide. As an angiogenic response to cancer cells, existing blood vessels are recruited for the host vascular network and new blood vessels form.

It has been hypothesized that neurogenesis significantly contributes to cancer pathology, and that cancer-induced tumours initiate their own innervation by the release of neurotrophic factors in a fashion similar to angiogenesis [28]. That is, tumour cells release neurotrophins, which stimulate adjacent neurons to develop axons that innervate the tumour. These axons then release neurotransmitters, which initiate migratory activity of tumour cells and ultimately foster metastases development [41]. Neural innervation promotes tumour spread along axons, and a major consequence of this innervation is cancer pain.

Neurogenesis clearly has a regulatory mechanism in cancer progression. Notably, neurotrophic activity does not necessarily constitute a sign of malignancy. That is, positive immunostaining for activated neurotrophin receptors in tumour biopsies does not prove that neurotrophins cause metastatic tumours. However, functional experiments provide compelling causative evidence for the involvement of neurotrophins in certain metastatic tumours. Functionally significant neural proliferation has been implicated in neuroblastoma, prostate cancer [8], colorectal cancer [1], esophageal and cardiac carcinoma [62], tumours of the urinary bladder [88], and choroidal melanoma [89]. Together, these studies suggest that the neuroendocrine system plays a major role in metastatic development and cancer progression.

NGF

NGF is important in the modulation of inflammatory [13, 43, 55, 109] and neuropathic [78, 81] pain states, and is expressed by several tumour, inflammatory, and immune cells [26, 102]. Once bound to TrkA, it modulates the expression of the neurotransmitters substance P and calcitonin gene-related peptide (CGRP), receptors, channels, and structural molecules implicated in nociception [39]. It supports nociception through the mechanistic augmentation of afferent neurotransmitter production [5], stimulation of sympathetic fibre ingrowth into dorsal root ganglia [24, 77], and activation of signalling pathways including MAPK [44, 73].

NGF is involved in tumour progression via the generation of a positive microenvironment for cancer cell survival and proliferation [53, 66, 70], and acts as a mediator and modulator of pain in a variety of pain states, including metastatic tumour-induced pain [6, 52, 94]. Humans also report pain at the site of injection after acute administration of NGF [68, 97].

In several malignancies, including breast, prostate, and pancreatic cancers, NGF is implicated in perineural invasion, a process in which cancer cells invade the surrounding nerves [51, 112]. Accordingly, as a potential therapy for cancer pain, researchers have suggested the pharmacological inhibition of NGF and its cognate receptor, TrkA [9].

Early and sustained administration of anti-NGF has been shown to suppress tumour-induced pain and nerve sprouting within tumour-bearing bones [17, 39, 45]. Mouse models of CIBP reveal that nociceptive fibres that innervate bone express TrkA receptors, and treatment with anti-NGF, a selective antagonist, attenuates

behavioural signs of CIBP [17, 39, 45, 46, 67]. Tumour angiogenesis and growth are facilitated by NGF-induced neuronal system development [82]. NGF is a pro-angiogenic factor in breast cancer [82], and neutralization of NGF partially reverses cancer-induced angiogenesis. Together, NGF and its cognate receptor are considered to be major mediator of chronic pain [107].

BDNF

While NGF seemingly has the most prominent influence on CIBP, BDNF also plays a role in tumour pain, although its precise role has not yet been fully elucidated. BDNF is expressed by nociceptors and is upregulated in inflammatory conditions. Increased levels of BDNF are observed in several tumours, including orthotopic hepatocellular carcinoma, multiple myeloma, and neuroblastoma [110]. BDNF released within the spinal cord induces phosphorylation of N-methyl-D-aspartate (NMDA) receptors on adjacent spinal cord neurons, leading to the induction and maintenance of behavioural hypersensitivity following nerve injury [105]. Rats in CIBP groups show microglia and astrocyte activation and upregulation of pro-inflammatory factors, including BDNF, and mechanical allodynia. These phenomena are reversed upon inhibition of the p39 MAPK signalling pathway [60].

Cancer-Induced Neurochemical and Cellular Reorganization

Sarcomas have been shown to induce peripheral changes, including upregulation of activating transcription factor-3 (ATF-3), a marker for injured neurons, and macrophage infiltration of dorsal root ganglion in tumour-bearing femurs [85, 87]. Both mouse and human neoplasms contain few nerve fibres [85, 91], but human studies have revealed abnormal remodelling of adjacent sensory nerve fibres and associated pain in response to tumour growth [15, 22, 59]. Mouse studies show increased periosteal expression of CGRP and substance P, neuropeptides expressed by a subgroup of small neurons that respond to noxious and thermal stimuli.

Spinal cord reorganization is also observed in a fashion similar to central sensitization seen in other pain states, including the upregulation of dynorphin and astrocyte hypertrophy [42, 85, 91]. Interestingly, spinal cord injury patients rarely develop prostate cancer, confirming the significance of nerves in disease progression [31].

NGF stimulates the pathological reorganization of adjacent TrkA sensory nerve fibres. Attempts to systematically prevent the reorganization of sensory nerve fibres reveal the potential mechanisms driving cancer pain [45, 67]. In a mouse model of

prostate CIBP, both preventative and late administration of anti-NGF therapy reduced nociceptive behaviours, sensory and sympathetic nerve sprouting, and neuroma formation [46]. Another study showed that early and sustained inhibition of TrkA markedly attenuated bone cancer pain and significantly blocked the ectopic sprouting of sensory nerve fibres and the formation of neuroma-like structures in the tumour-bearing bone in mice. Late and acute administration of the TrkA inhibitor, however, did not significantly reduce pain or nerve sprouting [33].

Conclusion

Considerable evidence suggests that neurotrophins contribute to tumour growth and cancer pain. NGF acts as a peripheral mediator of pain and is upregulated in inflammatory states. High affinity TrkA receptors are expressed by nociceptors, and NGF sensitizes peripheral nociceptive terminals. Inhibition of this neurotrophin abolishes symptoms characteristic of pain. BDNF is also expressed by nociceptors and is upregulated in inflammatory states. Neutralization of this neurotrophin partially eradicates pain sensitization.

We are in the early stages of understanding the mechanisms that drive metastatic tumour growth, cancer pain, and cancer in general. Neurogenesis appears to contribute to disease progression in a bold way, but more research is needed to elucidate the mechanisms driving sarcomas and to explore treatment options. The use of pharmacological agents to systematically inhibit neurotrophins and their cognate receptors is providing the platform to further investigate promising therapies for controlling tumour proliferation.

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