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REVIEW

The Role of Neuromodulation and Potential Mechanism in Regulating Heterotopic Ossification

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Abstract

Heterotopic ossification (HO) is a pathological process characterized by the aberrant formation of bone in muscles and soft tissues. It is commonly triggered by traumatic brain injury, spinal cord injury, and burns. Despite a wide range of evidence underscoring the significance of neurogenic signals in proper bone remodeling, a clear understanding of HO induced by nerve injury remains rudimentary. Recent studies suggest that injury to the nervous system can activate various signaling pathways, such as TGF-β, leading to neurogenic HO through the release of neurotrophins. These pathophysiological changes lay a robust groundwork for the prevention and treatment of HO. In this review, we collected evidence to elucidate the mechanisms underlying the pathogenesis of HO related to nerve injury, aiming to enhance our understanding of how neurological repair processes can culminate in HO.

Keywords Heterotopic ossification · Nerve injury · Neuromodulation · Neurotrophic factors · TGF-β

Introduction

Heterotopic ossification (HO) is characterized by the abnormal formation of bone in soft tissues where bone typically does not form, which can be induced by multiple factors, and nerve injury is commonly considered the predominant factor [1–3]. Approximately 10% to 53% of HO patients suffer from postcentral nervous system injury [2]. Injuries to the spinal cord or peripheral nerves can also lead to HO [3, 4]. HO is most commonly observed in the hip joint in patients with spinal cord injury, but within patients with traumatic brain injury, HO may occur throughout the body such as in the shoulder and knee joints [2]. However, the structure and characteristics of HO induced by different forms of trauma exhibit significant similarities. The cellular origins of HO formation are relatively complex [5]. Cells involved in pathological ossification may originate from the osteogenic differentiation of various stem cells under specific stimuli [6]. The formation of cartilage occurs first followed by cartilage ossification in traumatic HO. In addition, some mesenchymal stem cells (MSCs) at the site of traumatic HO injury accompanied by nerve damage can also affect cartilage ossification and the differentiation into osteoblasts.

Both HO and the nervous system have been shown to play significant roles in bone development and growth [7–9]. The surface of the periosteal bone is covered by primary sensory and sympathetic nerve axons [5]. The mechanism driving HO is intricate and not entirely elucidated. At the injury site, sensory neurons receive and convey local information to the brain. This results in the brain sending neuroendocrine signals to the hypothalamus, initiating repair [9]. Various neural factors have been identified as key players in bone formation, such as nerve growth factor (NGF) [10] brain-derived neurotrophic factor (BDNF) [11] and neurotrophic factor (NT) [12, 13]. These neurotrophins and their receptors are diffusely expressed within the skeletal tissue promoting bone formation [14]. Localization of neurotransmitter receptors in the skeletal microenvironment plays a crucial role within the bone metabolism. These neurotrophins can signal through tyrosine kinase receptor (TrKs) and neurotrophins receptor

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(p75NTR) to regulate downstream signaling pathways contributing to the formation of HO [15].

The cerebrospinal fluid of patients with brain injuries exhibits osteogenic induction properties. The site of brain injury can promote the release of osteogenic inducers and neurotrophins [16]. These neurotrophins are extensively involved in neural development. Neurotrophins play a crucial role in promoting the development and survival of sensory and sympathetic neurons, as well as in regulating the differentiation of neural precursors [17]. In addition, neurotrophins can promote the formation of granulocytes and monocytes, reduce cardiomyocyte apoptosis, regulate blood glucose levels, induce the differentiation of bone marrow mesenchymal stromal cells (BMSCs), enhance memory and contribute to the treatment of Parkinson's disease [18].

While the exact methods by which these nervous factors stimulate HO remain enigmatic, it is evident that some nerve fibers located near skeletal cells release neurotransmitters and neuropeptides. It has demonstrated that abundant neurite-like protrusions are in contact with the trabecular bone and nearby blood vessels through electron microscopy [19]. Neural fibers observed in the callus formed post-fracture can interact with osteoblasts and its precursors [7]. This interaction regulates bone metabolism through the secretion of neuropeptides and neurotrophic factors. These proteins regulate bone metabolism via multiple signaling pathways. The differentiation of MSCs is steered by both the transforming growth factor-beta (TGF-β) and bone morphogenic protein (BMP) pathways, which are integral to skeletal development and bone formation [20]. Notably, the BMP pathway is vital for regular bone formation and is also implicated in HO development [21–23]. Specific inflammatory factors such as TNF-α have also been identified as contributors to HO except the TGF-β pathway [1, 20, 24].

Given this complexity, understanding the pathogenesis of HO in relation to the nervous system becomes paramount. This review aims to synthesize current knowledge on how the nervous system fosters bone formation and endochondral ossification. It will further explore the roles of neural factors and TGF-β in HO development.

Nerve Injury and HO

HO arises following nerve injury and represents a complex dysfunction rooted in bone formation and remodeling [25]. In vitro research shows that cerebrospinal fluid from patients can stimulate bone growth after nerve injuries [16]. Serum obtained shortly after traumatic brain injury (TBI) contains humoral factors that encourage osteoblast differentiation within skeletal muscles and enhance the growth of skeletal muscle mesenchymal cells, both pivotal in advancing ossification and HO [26, 27]. In a model of polytrauma involving TBI and fracture, it was observed that TBI can alter the local neuroinflammatory state during the fracture healing process, stimulating endochondral ossification and early fracture healing in the contralateral limb [28]. Interestingly, serum from TBI model mice intensifies the proliferation and differentiation of pericytes, exacerbating endochondral bone formation within this model [29]. Neuroendocrine modulation advances bone formation post TBI, and TBI patients exhibit expedited fracture healing [30].

Patients with combined trauma and nerve injury face a substantially higher HO risk than those without combined trauma [31]. Nerve injuries can compromise the blood–brain barrier, prompting the release of cytokines that promote osteogenic factors in the bloodstream [31, 32]. BMPs, part of the transforming growth factor family, which are crucial in endochondral osteogenesis and fracture healing, are often employed to induce HO in vivo [25, 33]. Utilizing bone morphogenetic protein-2 (BMP-2) in spine fusion surgeries has been linked with possible complications of HO [34]. Postbrain injury, elevated BMP gene and protein expression levels have been reported [27].

Evidence has shown enhanced fracture callus formation in TBI patients [35–38]. Osteoinductive factors are released in areas where HO is induced by brain damage [16]. Slc33a1wt/mut mice in dorsal root ganglion cultures and sciatic nerve crush injury models have demonstrated injury-induced axonal regeneration through heightened BMP signaling [39]. There is a notable increase in the downstream BMP signaling pathway components pSmad1/5/8 and Bmpr1a, as well as in the number of myelinated axons within cortical neurons of mice. Conversely, injuries from BMP signaling could be mitigated with therapeutic Noggin, a BMP signaling antagonist [39]. Both BMP-2 and neurotrauma induce the growth of nerve-derived adult pluripotent cells (NEDAPS) to facilitate bone formation [40, 41]. Neural injury-induced HO in mouse models has shown effective BMP-2 suppression using pyrophosphate [41]. BMP-2 directly influences sensory neurons, initiating a neurogenic inflammatory response, which promotes nervous system remodeling and osteogenic stem cell release, culminating in bone formation [3, 42].

Chemically modified mRNA encoding BMP-2, when optimally delivered to osteotomies in rat femurs, aids in bone defect healing [43]. Increased BMP expression might enhance HO occurrence in rat tendons [44]. Overexpressing BMP-2 in the Tie2 + lineage produces HO in mice, linking abnormal BMP-2 signaling to skeletal muscle fiber injuries and increased Tie2 + lineage fibro-adipogenic precursor cells [45]. Around bone injury sites, BMPs may extend into surrounding muscle tissue, potentially inducing HO [46, 47]. Tfr2 deletion in osteoblasts attenuates the BMP-MAPK signaling pathway, inhibiting HO formation in mice [48]. Despite the involvement of the central nervous system

in BMP signaling, the role of BMP in synapse formation in neurons has been proposed [49]. Additionally, BMP-2 appears to stimulate human peripheral neurogenic pluripotent cell differentiation into fibroblasts [50]. Targeting fibrinogen or the BMP signaling pathway might aid central nervous system repair [51]. Regarding bone repair, neurotrophin-3 (NT-3) might act as an osteogenic factor preceding BMP-2, inducing BMP-2 mRNA expression in injured growth plates [52]. Peripheral nerve progenitors can differentiate into osteoblasts and chondrocytes, leading to HO [4]. Trauma-induced neuroinflammation induces early osteogenic differentiation in endoneurial cells and activates HO-essential factors [53]. Osteoblast-specific transcription factors in endoneurial cells advance HO as blood enters the new bone formation site [8].

Studies suggest that inflammatory factors are released, such as TBI. These factors, combined with neural elements, accumulate within the nervous system. They then traverse the blood–brain barrier, entering peripheral circulation, which subsequently drives HO formation [26, 27, 29]. Moreover, trauma-induced HO predominantly unfolds via endochondral ossification [5, 54, 55]. Recent findings emphasize the critical role of osteogenic progenitors residing within the endoneurium, positing them as the chief osteogenic precursors in HO development [8]. Importantly, the regulation of endochondral ossification is deeply influenced by both the BMP pathway and inflammatory mediators. These agents synergistically activate osteogenic progenitors, bolstering the onset of HO [23, 56]. Tendon and muscle-resident interstitial cells also activate chondrocytes and osteoblasts within HO [56].

Furthermore, osteoporosis is another bone metabolic disorder following central nervous system trauma. Patients with TBI may experience an increased risk of bone loss and osteoporosis [57]. Following injury to the central nervous system, there is an increase in sympathetic outflow which activates bone resorption [58]. TBI significantly reduces the bone density of cortical bone with in the mouse model [59]. TBI can disrupt the function of the hypothalamic–pituitary–adrenal axis leading to a deficiency in pituitary-secreted growth hormone, which in turn induces catabolic effects resulting in decreased bone mass and bone density [60]. Furthermore, TBI has been also associated with vitamin D deficiency which is able to lead to osteoporosis [61]. Osteoprotegerin (OPG) plays a crucial role in the negative regulation of osteoclast-mediated bone resorption. The inhibition of stimulated osteoclast formation by OPG can lead to an increase in the volume of ectopic bone [62]. The insertion of metallic particles targeting osteoclasts to stimulate bone resorption around ectopic bone may represent a novel therapeutic strategy for HO [63].

In the context of HO formation following the neurological injury, a multitude of endocrine hormones and paracrine mechanisms are intricately involved in the regulation of bone metabolism. HO induced by spinal cord injury is more common in patients with hyperparathyroidism [64]. Parathyroid hormone (PTH) can stimulate the formation of trabecular bone and participate in bone remodeling, enhancing osteoblast activity and promoting the production of ALP [65, 66]. Parathyroid hormone-related protein (PTHrP) is essential for the production of trabecular bone mass and cortical bone [67]. PTHrP, originating from osteocytes, is transported through the network of lacunar-canalicular. It influences nearby osteoblasts via the PTHR1/cAMP signaling pathway to stimulate bone formation and regulate the expression of genes related to matrix mineralization [68]. PTHrP enhances the production of ephrinB2 and ephrinB2 signaling regulates osteoblast differentiation and the expression of osteoblast genes through a paracrine mechanism within the osteoblast lineage [69]. A reduction in osteocalcin (OCN) can enhance bone formation in mice model [70]. Lower levels of osteocalcin may play a significant role in the development of HO in patients with nerve injuries [71]. Insulin can inhibit BMP2-induced HO in muscle by suppressing the expression of Osterix [46]. Leptin is involved in functional recovery following the neural injury [72]. Leptin participates in the formation of HO by promoting the mineralization of the extracellular matrix and regulating osteoblast function [73]. The expression of SOX9 is reduced and results in the suppression of trauma-induced HO within the leptin-deficient diabetic mice [74]. Fibroblast growth factor receptor 3 (FGFR3) is closely associated with the signaling pathways formation and cartilage development involved in HO [75, 76]. FGFR3 is highly expressed in chondrocytes and osteoblasts and the FGFR3 signaling pathway influences the formation of trabecular bone through a paracrine mechanism [77].

Neural Factors Regulate Bone Formation

Neurotrophic factors, including NGF, BDNF, and NT, play crucial roles in maintaining the functionality of the neural system and promoting neuronal survival and maturation [78–81]. In response to inflammatory stimuli, mast cells and macrophages coordinate the production of these neurotrophic factors [82–84]. Notably, the majority of bone-forming cells show localization of NGF. Additionally, BDNF is present in osteoblast-like cells, and NT-3 is identified in both osteoblast-like cells and hypertrophic chondrocytes within the fracture callus [15].

These neurotrophins activate the p75NTR and TrKs thereby promoting bone formation [83, 85]. Studies have revealed that p75NTR aids in the differentiation of ectomesenchymal stem cells into osteoblasts [12]. After bone injury, there is a significant upregulation of p75NTR expression. This receptor not only influences bone repair but is also vital

for stromal cell migration and subsequent bone regeneration [86]. Activation of p75NTR enhances the proliferation of MSCs, boosts neurological recovery, and activates glial cells [13].

In fracture models, NGF stimulates osteoblast maturation, increases innervation, and speeds up bone repair. Moreover, local administration of NGF accelerates callus maturation [87]. NGF-responsive TrkA-expressing nerves have been linked to enhanced cartilage antigen expression and increased TGF-β signaling at injury sites, leading to HO. NGF and TrkA are expressed within the process of endochondral fracture repair, and local injection of recombinant human β-NGF (β-NGF) within the cartilage promotes the expression of genes associated with endochondral ossification, such as Ihh and Alpl [88]. Consequently, inhibiting NGF signaling could prevent HO [89]. NGF distribution is notably dense at the subchondral bone or articular cartilage interface, with both p75NTR and TrkA being expressed in bone and articular cartilage [44]. Relatedly, MSC treatment has been shown to reduce apoptosis of oligodendrocytes caused by p75NTR [90].

Skeletal neurons release NGF, which promotes bone growth by stimulating the proliferation and differentiation of BMSCs [91]. After rib fractures in male rats, applying NGF to the fracture site significantly increased bone tissue, leading to a higher cartilage proportion [92]. Post bone injury, there is a significant rise in nerve fibers in bone tissue. Dendrites shift within the periosteum, and there is an increase in NGF and TrkA expression [93–95].

In mice, the NGF and TrkA signaling pathways in sensory nerves enhance bone formation in response to mechanical stresses [96]. This signaling pathway also stimulates the mineralization process in human chondrocytes [97]. One study found that following skull injury, macrophage-derived NGF levels rose, promoting sensory axon growth and skull regeneration. However, inhibiting TrkA activity delayed nerve growth and skull repair [98]. TrkA improves the survival and regenerative abilities of BMSCs by amplifying the Erk/Bcl-2 pathway [99]. There is also evidence suggesting that NGF inhibition can reduce bone destruction caused by tumors [100]. Interestingly, NGF has been found to activate the NF-κB signaling pathway by binding to p75NTR [101]. The role of NF-κB in bone metabolism and bone formation is well documented [102–104] and its activation is a key mechanism in the development of HO [105]. Inhibition of the NF-κB signaling pathway can suppress the function and differentiation of osteoclasts, effectively ameliorating bone loss in OVX mice model [104]. NF-κB signaling pathway is involved in the regulation of growth plate cartilage formation and osteoblasts, there is an increase in osteoblast differentiation and BMP-2 activation when the NF-κB signaling pathway is blocked.

NGF may alleviate neuropathic pain by inhibiting TAK1, subsequently suppressing the downstream MAPK and NF-κB signaling pathways. However, it remains uncertain whether this pathway influences HO [106]. In addition, astrocytes can activate the NF-κB pathway, and both TGF-β1 and β-NGF have been noted to upregulate this pathway [107, 108]. BDNF has been shown to promote osteogenesis and HO in BMSCs via the Erk/Runx2 pathway while also enhancing neurogenesis [8, 37]. BDNF stimulates the proliferation and differentiation of mesenchymal stem cells into osteoblasts [109, 110]. Furthermore, BDNF exhibits a high affinity for TrkB [15] and promotes neuronal cell survival through its interaction with TrkB [111].

BDNF and TrkB are present at different stages of bone formation [112]. The BDNF/TrkB signaling activates Akt stimulating the expression of ALP and BMP-2 [113]. TrkB is abundantly expressed in osteoblasts [94]. BDNF can suppress the differentiation of RAW264.7 cells into osteoclasts, and TrkB inhibition further hampers osteoblast proliferation [114]. K252a, BDNF receptor Trk inhibitor, significantly inhibits the formation of peripheral blood mononuclear cells induced osteoclasts stimulated by BDNF [115]. Through the TrkB-Erk1/2 signaling pathway, BDNF modulates the balance of RANKL/OPG expression in osteoblasts. BDNF depletion markedly reinstates RANKL/OPG homeostasis, curbing osteolytic bone destruction [116]. The molecule 7,8-dihydroxyflavone, a BDNF and TrkB agonist mimic, can inhibit BDNF/TrkB signaling, thereby thwarting RANKL-induced osteoclastogenesis and preventing bone loss [112].

Glial cell line-derived neurotrophic factor (GDNF) elevates Nr4a1 expression in BMSCs and activates the PI3K/Akt signaling pathway, enhancing the proliferation and osteogenic differentiation of BMSCs after promoting the presence of activated astrocytes and GDNF in the hippocampus [117]. This factor also fosters the migration and osteogenic differentiation of MSCs94. The neurological system has an abundance of RET receptors. GDNF synthesis in neurons, in tandem with RET receptors, activates the MAPK, Erk, and Akt pathways—all pivotal in bone formation [118–121].

NT-3 augments the expression of Sox9 and Runx2 by activating the endothelial-mesenchymal transition. TrkC-specific inhibitors can alleviate NT-3-induced HO formation [52, 122]. Activated macrophages regulate NT-3 secretion, which, in turn, hastens HO through the Erk signaling pathway [123]. NGF-β, GDNF, and NT-3 can drive the differentiation of BMSCs into neurons [124, 125].

In summary, neurotrophic factors can signal through both the Trks and the p75NTR [15]. In addition to promoting survival and differentiation, neurotrophic factors also function through interactions with other receptors and ion channels. Neurotrophic factors can selectively bind with specific Trks. Trks function by regulating the Erk and PI3K signaling

pathways. On the other hand, p75NTR can activate NF-κB and Jun N-terminal kinase (JNK).

Furthermore, BMP-2 has been revealed to enhance the interaction between peripheral nerves and bone, ensuring bone health in synergy with neurotrophin [14]. Engineered sensory nerves releasing NGF significantly stimulate the osteogenic differentiation of BMSCs via the NGF-TrkA pathway. Simultaneously, NGF may also foster bone healing through BMP-2 [126]. BDNF can boost BMP-2 gene synthesis, presenting therapeutic possibilities for treating periodontal defects [127, 128]. BDNF also promotes the expression of ALP, type I collagen as well as OCN, which play a significant role in bone formation [127]. NT-3 has been found to considerably raise BMP-2 and TGF-β levels, thereby hastening bone formation following tibial fractures in rats [128].

Glutamate, the primary neurotransmitter within the central nervous system, experiences an increase in release following neural injury [129]. The glutamate receptor N-methyl-D-aspartate receptor (NMDAR) which is associated with signal transduction in the central nervous system, is highly expressed in osteoblasts and osteoclasts [130]. Intense staining of NMDAR has been observed on osteoblasts and osteoclasts in human osteophyte tissues. Inhibition of NMDAR can suppress bone resorption and the activation of the NF-κB signaling pathway [131]. NMDAR inhibitors have been shown to suppress the expression of bone formation markers such as OCN, type I collagen, as well as ALP [132]. Furthermore, the activation of NMDAR can promote the production of bone remodeling markers mediated by parathyroid hormone [133]. Sympathetic Nervous System modulates bone remodeling through the signaling of beta-2 adrenergic receptor (B2AR) located on osteoblasts [134]. A specific inhibition and deficiency of B2AR in osteoblasts results in a reduction of bone resorption and an increase within the bone density [135, 136].

TGF-β and HO

TGF-β is integral for tissue homeostasis, directing various cellular activities, including proliferation, differentiation, apoptosis, and migration [137–139]. It orchestrates a vast range of biological processes, activating both non-Smad and Smad pathways, with the intricate involvement of upstream and downstream signaling molecules [140, 141]. Additionally, TGF-β is crucial in modulating HO and bone organogenesis [142].

Traumatized human tissue samples show pronounced increases in markers associated with bone growth. Notably, TGF-β expression, a marker for tissue fibrosis, escalates in injured tissues, subsequently leading to HO [143]. During bone remodeling, TGF-β regulates the recruitment of MSCs, ensuring bone homeostasis [144]. It also fosters the early differentiation of osteogenic progenitor cells and stimulates chondrocyte proliferation [145]. The resorption activity of osteoclasts can also activate TGF-β thereby inducing the progression of HO in patients with ankylosing spondylitis [146].

Neurologically, TGF-β expression intensifies in areas of nerve injury, helping to mitigate neuroinflammation [147, 148]. The TGF-β family has a seminal role throughout neurodevelopment, with profound clinical relevance to both injured and pathological nervous systems [149, 150]. Elevated expression of TGF-β1 and TGF-β has been noted in humans with posttraumatic spinal cord injuries [151–153]. Similarly, TGF-β is markedly expressed at injury sites after peripheral nerve damage [154, 155]. When introduced after chronic nerve injuries, TGF-β bolsters axonal regeneration [156]. Post spinal cord injuries in rats manifest as a significant upsurge in Ephrin type-B receptor 2 expression, accompanied by enhanced TGF-β1 secretion from activated astrocytes [157]. Mouse glial cells also exhibit increased TGF-β expression following trauma [158, 159]. Notably, the circular RNA Plek has been observed to amplify TGF-β1 after spinal cord injuries [160].

On a molecular level, TGF-β activates intracellular Smad signaling, a pathway influenced by a diverse set of factors and routes. Moreover, TGF-β collaborates with various pathways, such as MAPK, Wnt, Notch, and Akt/mTOR, all of which are pivotal for bone metabolism [161, 162]. WNT/β-catenin, acting as a mediator of the TGF-β/BMP signaling pathway, can regulate the differentiation of progenitor cells into osteoblasts and inhibit the apoptosis of osteoblast [161]. Smad serves as a platform for integrating MAPK/RTK signals with the TGF-β/BMP pathway. Ser203, Thr178 and Ser207 residues in the Smad3 linker region serve as phosphorylation sites for Erk1/2. TGF-β activating kinase and TAK binding protein activate MAPK through BMPR [163]. The MAPKs/TAK1 signaling pathway plays a role in the differentiation of MSCs and bone formation [161]. BMP2 can regulate MAPKs and activate the PI3K pathway [164]. The Notch pathway is active in the early stages of osteoblast differentiation, and disruption of Notch signaling genes leads to reduced osteogenesis and bone mass [165]. The subchondral bone microenvironment undergoes changes due to high levels of active TGF-β protein, leading to an accumulation of osteoprogenitor cells and an influx of new blood vessels [166, 167]. Additionally, mutations in the TGF-β1 gene locus in Camurati–Engelmann disease have been associated with long bone diaphysis hyperostosis and sclerosis, while TGF-β signaling anomalies can result in aneurysmal osteoarthritis syndrome [137].

Osteoclasts have been shown to resorb TGF-β in bone marrow, inducing HO [146]. Matrine can inhibit HO by obstructing the migration and osteogenic differentiation

of MSCs induced by TGF-β in mice [168]. Additionally, MSCs can secrete TGF-β, modulating synaptic transmission and neuronal excitability in dorsal root ganglia [169]. When activated, TGF-β stimulates the formation of cortical bone [170, 171] emphasizing its pivotal role in managing HO and aberrant formation of subchondral bone [172, 173].

Therapeutic strategies for HO may be based on the suppression of the TGF-β signaling pathway [171, 174] which has been implicated as a crucial stimulator of human cartilage production [175]. TGF-β exerts a divergent effect on MSCs, inhibiting their osteogenic differentiation [176] while mitigating neural damage when administered in mice [177].

NGF significantly enhances the production of TGF-β1 and the expression of P75NTR, known for its pronounced stimulatory effect on the amniotic membrane [178, 179]. Moreover, the mRNA level of NGF in chondrocytes is upregulated by TGF-β via the ALK5-Smad2/3 signaling pathway [180, 181]. The role of TGF-β is highlighted during osteoarthritis progression, recruiting MSCs to stimulate bone formation within the subchondral bone marrow [167]. It also fosters axonal regeneration after nerve injuries, increasing the mRNA concentration of TGF-β1 within the distal nerve stump and enhancing NGF mRNA levels in rats and mice [155, 182–185].

The TGF-β signaling pathway, modulated by sensory nerves, facilitates cranial suture closure upon NGF binding to TrkA and serves as an autocrine factor on cells via TGF-β receptor (TβR) activation, stimulating the release of NGF [186, 187]. The absence of NGF and the inhibition of its receptor TrkA both suppress chondrocyte differentiation and the progression of HO [10]. Cellular domains rich in NGF promote the development of TrkA + sensory nerve fibers and the interruption of TrkA signal transduction inhibits the fracture healing [188]. Blocking NGF helps to alleviate bone destruction in mice model of bone tumors [100]. Inhibition of TGF-β1-induced Smad2/3 pathway activation decreases NGF expression [189]. Marine compounds have been identified to prevent HO by inhibiting the migration and osteogenic differentiation of MSCs via the TGF-β/Smad2/3 pathway [168]. TGF-β also promotes the expression of NGF in chondrocytes via the Smad2/3 signaling pathway [180]. TGF-β activates two Smad signals leading to an increase in the levels of NGF within the pancreatic stellate cells [190]. TGF-β1 can enhance the survival of Dorsal Root Ganglia (DRGs) mediated by NGF [185].

TGF-β signaling protects damaged neurons during the early stages of TBI through Smad3 activation and is essential for cell motility, enhancing serum expression and secretion levels of TGF-β1 [191, 192]. This cytokine enhances the expression of GDNF via TβR, protecting spinal sympathetic neurons from apoptosis [193–196]. TGF-β exerts a potent trophic effect on midbrain dopaminergic neurons, and the signal transduction of classical neurotrophic factors may be influenced by TGF-β [196]. The combination of TGF-β with NT-3 and NT-4 promotes the survival of more neurons and the neurotrophic function of GDNF requires the involvement of TGF-β. It is ubiquitously distributed in mature mouse bone marrow and is expressed extensively within cartilage [195].

Research shows the prevalent presence of TGF-β signaling pathways within sensory nerves [197] and in astrocytes, it not only enhances protein expression but also modulates its own signaling pathway [198, 199]. Systemic injection of a TGF-β neutralizing antibody attenuates HO in BMP-induced spontaneous HO models in mice [24]. With intact BMP receptors, MSCs serve as BMP target cells in bone, with BMP being an effective inducer of osteoblast differentiation in vitro [22]. The TGF-β signaling pathway in astrocytes can inhibit inflammation and alleviate neuronal injury within the central nervous system [200] (Fig. 1).

Other Factors Inducing HO

Inflammation serves as a pivotal inducer of HO. Neuroinflammatory cascade is activated following the neural injury [201]. This process leads to the migration of chondro-osseous progenitor cells. TBI triggers a series of complex inflammatory responses activating the NF-KB, JNK and TAK1 signaling pathway, which is closely related to bone metabolism [202, 203]. Sensory neurons release neuroinflammatory molecules leading to the recruitment of hypertrophic cells [3]. The involvement of inflammatory responses, mediated by macrophages and mast cells, is observed in the development of HO subsequent to nerve injury [204, 205]. Macrophages are integral in promoting HO, facilitating the inflammatory response, and expressing cytokines such as TGF-β1, BMP, and Substance P (SP), which encourage the differentiation of MSCs [206]. There is a recognized association between HO, abnormal chondroprogenitor differentiation, and TGF-β1-producing monocytes or macrophages [207, 208]. However, TNF-α secreted by M1 macrophages has been implicated in the bone erosion associated with rheumatoid arthritis [209]. In the mouse model of HO induced by BMP or injury, the depletion of monocytes can promote the differentiation of endothelial cells into endochondral formation, ultimately leading to HO and an increase in bone density [207]. In addition, BMP-2 also plays a directly role in sensory neurons inducing neurogenic inflammation and resulting in the activation of osteoblasts [3]. TGF-β1 are closely associated with HO and abnormal differentiation of cartilage progenitor cells after musculoskeletal trauma [208].

Oncostatin M, produced by activated macrophages, stimulates osteogenic differentiation and mineralization of myocytes in individuals with spinal cord or brain damage, contributing to HO development [206, 210]. Following

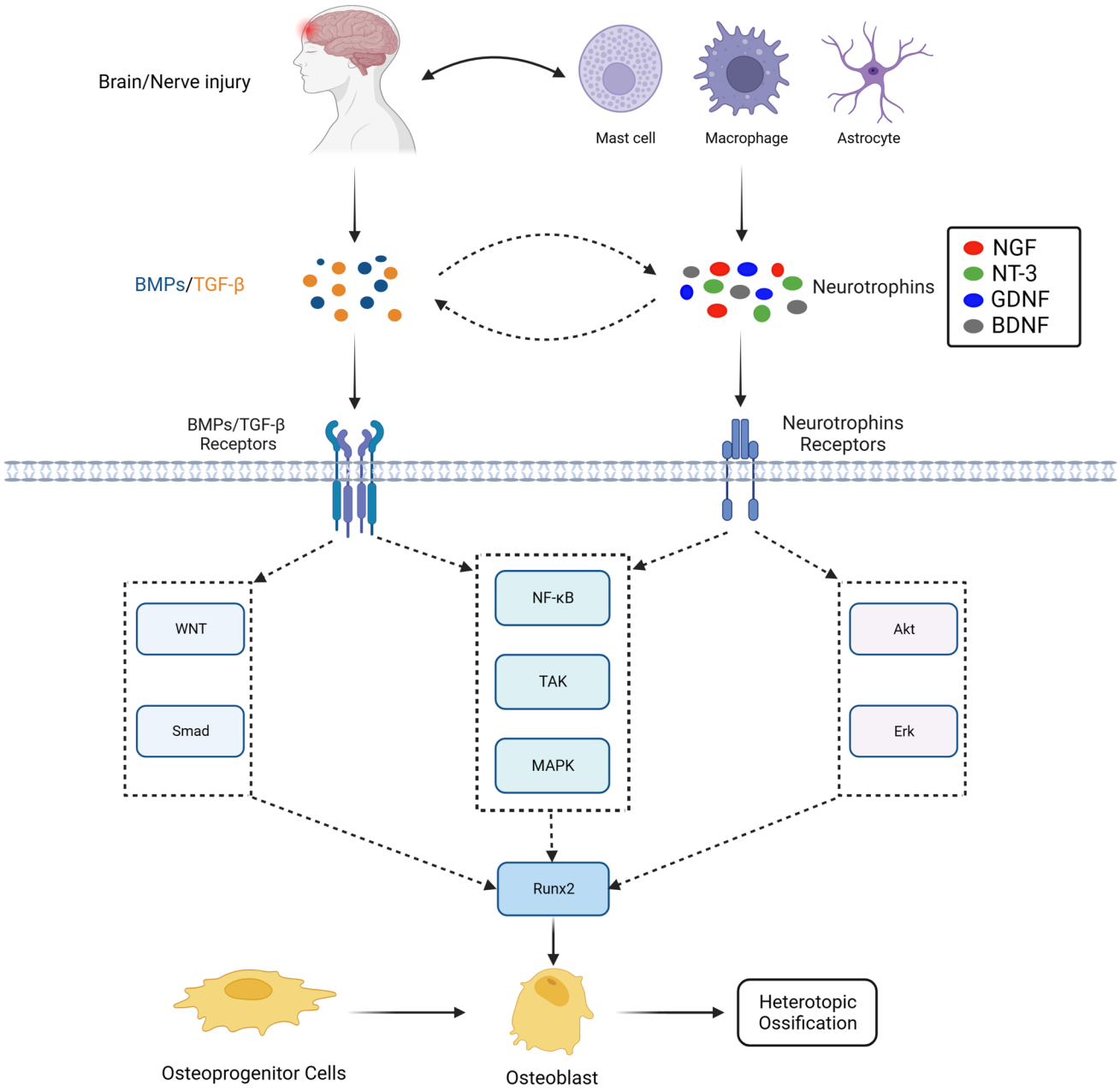

Fig. 1 Role of neuromodulation in HO after nerve injury. Blood levels of BMPs and TGF-β rise in response to damage to brain or nerve fibers, and these molecules directly influence intracellular signaling pathways to contribute to HO. Mast cells, macrophages and astrocytes are activated and accompanied by the release of neurotrophic factors following nerve injury. These neurotrophic factors in conjunction with BMPs and TGF-β combine with its corresponding receptor respectively and activate the signaling pathways to promote the progression of HO

nerve injury, an upregulation of SP is observed, which is released by diverse cell types in the nervous system, including macrophages, neurons, and dendritic cells [211]. This molecule interacts with neurokinin receptors, playing a role in the differentiation of chondrocytes and osteoblasts.

The established interplay between inflammation and oxidative stress under various pathological conditions is a critical aspect of disease progression. During the formation of HO, there is a notable dysregulation in the redox process. Macrophages exhibit antioxidant properties through nuclear factor E2–related factor 2 (Nrf2) aiding in the formation of chondrocytes [212]. Inhibition of Nrf2 can significantly alleviate HO. Nrf2 positive chondrocytes can prevent hypoxia, thereby facilitating the development of HO [213]. NGF/TrkA promotes the vitality of BMSCs under hypoxic conditions through the activation of the Nrf2 pathway [214].

A prolonged inflammatory response ensues characterized by the production of reactive oxygen species (ROS) and upregulation of Nrf2 expression following SCI [215]. This heightened inflammatory response induced by the injury can promote HO. CH6-MF NPs loaded with BMP2 siRNA can effectively scavenge ROS and actively deliver siRNA to MSCs and osteoblasts which effectively inhibits osteogenic differentiation under inflammatory conditions in vitro [216]. The Hedgehog signaling pathway regulates the antioxidant pathway affecting the generation of ROS in tendon-derived stem cells, thereby promoting trauma-induced tendinopathy [217]. Photo-crosslinked nanoparticles responds to the acidic and ROS in the inflammatory microenvironment to suppress HO [218].

Furthermore, the posttraumatic hypoxic microenvironment increases the availability of hypoxia-inducible factor-1 (HIF-1α). The subsequent upregulation of HIF-1α regulates the gene expression of BMPs and neuropilin-1, impacting mechanisms of HO, such as bone resorption and osteogenesis [219, 220]. Moreover, neuroinflammation following nerve injury induces the release of calcitonin gene-related protein, influencing bone metabolism by promoting chondrogenic differentiation of fibro/adipogenic progenitors [221].

In this detailed nexus of interactions, each molecule and cell type play a critical and interconnected role in the development of HO post-nerve injury, illustrating the complexity of the physiological responses that are involved.

Conclusion

HO is a complex pathological condition with numerous associated risk factors. While it is rooted in the pathological differentiation of pluripotent stem cells, it also shares similarities with typical physiological processes. However, our grasp of the cellular origin, etiology, and underlying mechanisms of HO is still not comprehensive. After nerve injury, neurotrophic factors have been shown to play a role in the development of HO, acting through various signaling pathways and in conjunction with local inflammation and immune responses. At present, there are no established treatments specifically for HO. Nonetheless, there is potential that future preventive and therapeutic strategies could utilize innate neuromodulatory mechanisms.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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