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Heterotopic Ossification Has Some Nerve

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Abstract

Heterotopic ossification, defined as the formation of bone in abnormal anatomic locations, can be clinically insignificant or devastating and debilitating, depending on the site and duration of new bone formation. There are many causes of heterotopic ossification (HO), including soft tissue trauma, central nervous system injury, vasculopathies, arthropathies, and inheritance. One of the least understood components of HO is the interaction of the peripheral nervous system with the induction of this process. Recent work has shown that, upon traumatic injury, a cascade of events termed neurogenic inflammation is initiated, which involves the release of neuropeptides, such as substance P and calcitonin gene related peptide. Release of these peptides ultimately leads to the recruitment of activated platelets, mast cells, and neutrophils to the injury site. These cells appear to be involved with both remodeling of the nerve, as well as potentially recruiting additional cells from the bone marrow to the injury site. Further, sensory neurons stimulated at the injury site relay local information to the brain, which can then redirect neuroendocrine signaling in the hypothalamus towards repair of the injured site. While numerous studies have highlighted the important role of nerve-derived signals, both central and peripheral, in the regulation of normal bone remodeling of the skeleton,[1] this review focuses on the role of the local, peripheral nerves in the formation of heterotopic bone. We concentrate on the manner in which local changes in bone morphogenetic protein (BMP) expression contribute to a cascade of events within the peripheral nerves, both sensory and sympathetic, in the immediate area of HO formation.

Keywords

heterotopic ossification; peripheral nervous system; BMP2; neurogenic inflammation; sensory; sympathetic

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I. Heterotopic Ossification

I.A. Stages of Heterotopic Ossification

Heterotopic ossification (HO) appears to form *de novo* within tissues, presumably through the recruitment of stem cells and progenitors, which then undergo all stages of endochondral bone formation. Much speculation has suggested that injury to the tissue, through trauma, may lead to the recruitment of stem and progenitor cells to the injury site. Upon arrival, these progenitors are then exposed to osteoinductive factors that direct their differentiation towards the chondro-osseous fate. The newly formed ectopic bone is similar to skeletal bone, possesses a bone marrow cavity, and can often fuse the normal skeleton.

Studies from mouse models of HO,[2,3] where bone formation is induced through delivery of bone morphogenetic protein 2 (BMP2), show a series of changes within the soft tissues, including nerves, vessels, and muscle. One of the initial changes observed at the site of new bone formation is the appearance of brown adipocytes. These cells are capable of utilizing their uncoupled aerobic respiration to reduce localized oxygen tension and effectively pattern the newly forming cartilage condensations.[4] These unique cells are also able to express angiogenic factors, such as VEGF-A and -D, which can enhance rapid, new vessel formation.[5] This vascular ingrowth must occur for the transition of avascular cartilage to bone.[6] Therefore, it is not surprising that a mechanism exists for regulating both local oxygen tension and vessel growth as a component of HO. Interestingly, just prior to chondrogenesis, expression of markers of endothelial adhesion (E-selectin, SDF-1, CXCR4, VCAM) and vascular remodeling are elevated, simultaneous to the appearance of proliferating inflammatory-like cells (CD68+, SMA+, SMMHC+, Lysozyme M+) within the tissues (personal communication[7]). These inflammatory-like cells lose these more primitive markers upon expression of the chondrocyte/Schwann cell marker Sox9, forming a sharply demarcated perichondral region delineated by the Sox 9 expression.[7] Further, we find that these cells appear to be adjacent to the oxygen reducing brown adipose, and thus, form a three dimensional architecture where the brown adipose may be regulating chondrogenic differentiation through hypoxia.[4,7] As the cartilage and bone form, they appear to surround and engulf the muscle tissues, with significant muscle hypertrophy, and death. It is unclear what governs the inflammatory response after induction of bone morphogenetic protein (BMP) signaling, but recent studies in using these mouse models suggest a regulatory role for peripheral nerve signaling. In these models, BMP2 appears to lead to neuroinflammation, which involves recruitment of mast cells and neutrophils, activation of platelets, and significant expansion of myeloid progenitors (Salisbury et al., in preparation), suggesting that peripheral nerve stimulation by BMP2 may be involved in the induction of HO.

I.B. Clinical Scenarios of HO

Although many have speculated that HO is a heterogeneous disorder stemming from a number of different causes, study of the literature, across the different fields, reveals striking similarities to animal models. One commonality appears to be the enhanced expression or release of BMP2, at a time when stem cells and progenitors are identified within the tissues. Clearly, traumatic injury to skeletal bone and muscle leads to the increased expression of BMPs at the injury site, and recent reports suggest not only a role for this protein in bone

formation, but also a critical role in muscle regeneration and repair.[8] Clever et al. showed that many components of the BMP signaling pathway were activated within 24 hours of injury, and played a critical role in myoblast progenitor expansion.[8] It is unclear how BMPs can be involved in muscle stem cell expansion and repair, and still induce bone formation. One possibility is that the levels of BMP within the tissue may be a defining factor. Often, in cases of significant muscle injury, the adjacent bone is also disrupted, which could lead to the substantial release of BMPs within the local environment. Since BMP expression within the muscle must be turned of in order for the muscle progenitors to differentiate, perhaps lower levels of BMPs are required for limited times. When these higher levels of BMP are found, the results are shifted towards endochondral bone formation. Alternatively, there could be a secondary mechanism evoked, beyond the alteration in BMP expression. It is intriguing that in certain types of traumatic injury, such as myositis ossificans traumatica, which appears to result from muscle trauma, HO occurs without injury to the skeletal bone.[9] Beiner et al.[9] demonstrated that the inflammatory response evoked appears to rapidly destroy the muscle fibers, which are replaced with heterotopic bone. Clinically, it is unclear what the inductive components are that lead to HO, but the data suggests that BMP expression upon injury may play a key role.

One of the most common causes of heterotopic ossification in the general population is central nervous system injury. Heterotopic ossification is an especially challenging problem for spinal cord injury patients who have an elevated risk for HO. While such patients often lose all motor and sensory function below the level of the spinal cord injury, the distal nerves themselves remain viable and functional, although they no longer communicate with the brain. Indeed, neuronal activity in the lower extremity of spinal cord injured patients can be abnormally high, frequently causing spasticity.

Surprisingly, studies of HO in cardiovascular tissues have striking similarities to HO at other sites. Two primary sites within cardiac tissues appear to form bone: cardiac valves and within atherosclerotic plaques. Although the mechanisms that govern aortic valve (AV) degeneration are largely unknown, many of the pathways involved in embryonic formation of the valve appear to be disrupted in AV degeneration.[10] Sucosky et al. recently demonstrated that shear stress within the valve, from alterations in blood flow, appeared to rapidly enhance BMP2/4 signaling. The authors further suggested that the increase in BMP2/4 signaling led to localized inflammation and degeneration of the valve tissue.[11] Intriguingly, the valve contains peripheral nerves, which undergo neuroinflammatory remodeling during AV degeneration.[10]

Like the valves, shear stress and changes in hemodynamics have been suggested to be responsible for HO formation in atherosclerotic plaques.[12] Several BMPs have been detected in atherosclerotic plaques, including BMP2.[13] Elevation in BMP signaling through shear stress and a reduction in blood flow is thought to be responsible for early vascular inflammation.[14,15] Interestingly, Yao et al. demonstrated that increased BMP signaling led to the elevation of the endothelial adhesion molecules CD68, E-selectin, VCAM and ICAM-1.[14] They speculated that induction of BMP signaling in cardiac tissues induces monocyte infiltration through elevation in these endothelial adhesion molecules.[14] Further, the authors speculate that the elevated BMP signaling could also lead to osteochondrogenic

lineage reprogramming of smooth muscle cells.[16] Intriguingly, the mechanisms evoked in cardiovascular HO are very similar to what is observed in the BMP2 mouse model, again suggesting that this disorder may follow a common mechanism, regardless of the location of onset.

One of the best examples of the direct correlation between heterotopic bone formation and enhanced BMP signaling is the genetic disease fibrodysplasia ossificans progressiva (FOP).[17] Recently, Shore et al.[18] identified an activating mutation in the activin receptor type 1, a bone morphogenetic protein type 1 receptor, in patients with FOP, presumably leading to the formation of HO in skeletal muscle, tendons, and ligaments. This activating mutation leads to BMP signaling. However, the receptor activity can still be enhanced upon addition of BMP protein,[18] suggesting that there is a threshold level of BMP required for induction of bone formation. Interestingly, in patients that possess the mutation, even minor trauma to the muscle appears to rapidly induce HO, presumably by the rapid elevation in BMP expression in muscle after injury.[8] Perhaps this trauma releases BMPs within the muscle itself,[17] providing the small amount of additional stimulus to form the bone. Alternatively, Kitterman et al. showed the formation of HO along the needle track after childhood vaccinations in patients with FOP, suggesting that peripheral nerves, such as sensory neurons, may also contribute to induction of the bone formation.[19] BMPs have been shown to be expressed in normal peripheral nerves regulating neuronal function, and BMP signaling appears activated upon peripheral nerve damage, suggesting that BMPs play a role in the peripheral nerve's response to injury.[20]

We have highlighted the most common areas for heterotopic ossification to occur. However, the risk of HO within the general population is fairly low, approximately 5%, suggesting that it is still a very rare event. This most likely contributes to our lack of mechanistic knowledge of the subject. However, recent statistics from the military suggest that as many as 60% of all military casualties[21] are reported to have some form of HO. These numbers are staggering and have led researchers to question what is behind the significant increase in incidence. One possible reason is the type of injuries sustained in the military population. Approximately 60% to 70% of traumatic injuries are a direct result of blast or burn injuries associated with improvised explosive devices (IEDs), which can have dramatic effects on peripheral and central nervous system signaling, but can sometimes, paradoxically, leave the body's tissues with undetected or minimal damage.[21] One commonality among these types of injuries appears to be trauma to the peripheral nervous system. Here we examine the potential link between the peripheral nervous system and induction of heterotopic bone formation.

II. Heterotopic Ossification and the Sensory Nervous System

II.A. TRPV1 Sensory Neurons and Heterotopic Bone

Little is known about sensory nerves and bone. Studies in our own laboratory suggest a functional role for these nerves in HO. Recent studies in mice lacking TRPV1 (transient receptor potential cation channel V1) sensory neurons have shown these mice develop significantly less heterotopic bone after induction with BMP2, as compared to the normal counterpart (Salisbury et al., in preparation). Dissection of this sensory pathway after BMP2

induction showed a significant elevation in both substance P (SP) and calcitonin gene-related peptide (CGRP), which was absent in mice lacking TRPV1 sensory neuron function.

The small diameter, afferent sensory fibers of the peripheral nervous system (PNS) are of major importance in the release of SP and CGRP, and subsequent inflammatory effects. Within the tissues, these nociceptive primary afferent neurons respond to noxious mechanical, thermal, or chemical stimuli, providing feedback on pain and temperature.[22] Upon injury or inflammation, noxious stimuli activate these nociceptive, sensory fibers, which release neuropeptides both in the periphery, leading to neurogenic responses, and centrally to transmit the nociception to the central nervous system. The vanilloid (capsaicin) receptor TRPV1 is a nociceptive, ion channel located on sensory nerve endings that is activated by some of these noxious stimuli and involved in the mediation of pain sensation.[23,24] Capsaicin, the compound in hot chili peppers which gives them “heat,” is one chemical stimuli that can activate TRPV1, causing the ion channel to open, leading to an influx of calcium and sodium ions into the sensory neuron and triggering depolarization of the neuron. At normal levels, capsaicin binding transmits the sensation of pain. However, high doses of capsaicin lead to a massive influx of ions, resulting in cell death of sensory neurons expressing TRPV1.

II.B. Neurogenic Inflammation and Heterotopic Bone

While TRPV1 activation sends afferent signals to the central nervous system for the communication of pain, it also leads to neurogenic inflammation by the release of SP and CGRP within the tissue.[25] Indeed, TRPV1 is highly coexpressed with the substance P-positive and CGRP-positive neurons of the dorsal root ganglion.[26] This neurogenic inflammatory process is mediated by the release of neuropeptides from sensory nerves, which in turn act on target cells in the periphery, such as mast cells, to produce inflammation[22,27] (Fig. 1).

Intriguingly, BMP has been shown to upregulate CGRP, as well as SP, expression in sensory neurons cultured from dorsal root ganglia,[28] suggesting this molecule plays a role in producing these neuroinflammatory responses. Therefore, release of BMP2, such as during the induction of HO in soft tissue, initiates neurogenic inflammation within the local environment (Fig. 1). It is important to note that the small diameter, capsaicin-sensitive sensory neurons, which are critical in generation of neurogenic inflammation, are themselves activated upon injury and trauma, consequently augmenting the inflammatory response produced by the sensory nerves in scenarios of HO involving traumatic injury. The ability of BMP signaling to evoke this mechanism may, in part, explain why patients with an inherited form of HO, FOP, exhibit an increase in mast cell density within the lesional area of heterotopic bone, as compared to unaffected tissues.[29]

These pro-inflammatory neuropeptides bind to receptors expressed on mast cells, stimulating their activation and subsequent release of a variety of enzymes and inflammatory factors from intracellular granules within the mast cell, a process referred to as degranulation[30,31] (Fig. 1). Upon degranulation, mast cells release a variety of mediators, including serine proteases, such as chymase and tryptase, histamines, and cathepsins, which are associated with many types of tissue remodeling.[30] In addition, many sensory nerve

terminals are lined with receptors for the various mast cell mediators, which, upon activation, can lead to further release of SP and CGRP, creating a positive feedback loop for the perpetuation of neurogenic inflammation.[30] Studies in our BMP2-induced mouse model of HO support a role for mast cell degranulation in the progression of HO (Salisbury et al., in preparation). Mice treated with cromolyn, which is known to inhibit mast cell degranulation, prior to BMP2-induction, develop a significantly smaller heterotopic bone lesion than untreated animals (Salisbury et al., in preparation).

Mast cell proteases released upon degranulation are also linked to remodeling of the peripheral nerve.[32] Upon injury to the nerve, Schwann cells associated with the nerve start to repair the damaged nerve sheath.[33] This phenomenon holds greater significance when given the current findings that stem cells, which contribute to other tissues, are stored within the nerve sheath. Recently, Adameyko et al.[34] demonstrated the presence of a primitive stem cell within the nerve that contributed to melanocytes within the skin. Additionally, in patients with the complex disease neurofibromatosis, cells cannot migrate from the nerve; therefore, they remain within the nerve sheath and form the characteristic nerve-associated tumors of the disease.[35] These patients also display skeletal and skin abnormalities, including partial, early closure of the growth plate, bone loss, and café au lait spots within the skin. These phenotypes hint at a mechanism where stem cells for bone and melanocytes also reside in the nerve and become trapped in this disorder, leading to improper bone formation and skin pigmentation. Finally, studies in the developing sciatic nerve isolated from rats revealed three distinct stem cell populations within the nerve: one, a population of multipotent, self-renewing progenitors, presumably derived from the neural crest,[36] which contribute to the generation of peripheral nerves;[37] two, a population that appeared to generate Schwann (glial) cell precursors, which express glial fibrillary acidic protein (GFAP); three, a population of smooth-muscle like cells, which appeared to be absent from other nerve structures, but the authors speculate could contribute to more mesenchymal lineages. Interestingly, these cells were SMA+, SMMHC+ similar to the cells identified as tentative chondrocytes[13,38–40] and the prechondrocytes we observed in our model of HO. All of these studies point to a pool of stem cells within the nerve, with the potential to contribute to the structures of bone, including chondrocytes and osteoblasts.

In addition to nerve remodeling, the mediators released by mast cells can elicit a variety of pro-inflammatory effects within the tissues. In concert, SP and CGRP, along with activating mast cells, can induce other immune cells, including monocytes, macrophages, lymphocytes, and platelets.[22,41,42] Both neuropeptides are potent vasodilators.[43] We have observed an elevation of platelets in the blood early after injection of BMP2-producing cells, and at later times, we have observed an elevation of neutrophils (Salisbury et al., in preparation). Platelets play a critical role in wound healing and hemostasis, as well as in repairing bone fracture.[44] Induction of the sensory neuropeptides, whether by injury, BMP, or a combination of the two, modulates the local immune response, thus promoting the progression of HO.

II.C. Sensory Neuropeptides and Skeletal Bone

Intriguingly, capsaicin-sensitive sensory neurons and sensory neuropeptides have been implicated in the maintenance of the normal skeleton as well.[45] Capsaicin-induced denervation of the sensory neurons results in a loss of trabecular bone volume, decreased osteoblast activity, and impaired bone formation. Additionally, there is evidence that CGRP plays a fundamental role in osteoclast formation and function. Several studies showed that CGRP inhibits the formation of osteoclasts and that capsaicin-induced denervation leads to impaired recruitment of osteoclast precursors.[45] Both SP and CGRP have also been identified to promote osteogenesis *in vitro*.[46,47] Consequently, these neuropeptides appear to have the potential to interact with some of the principal cells, osteoblasts and osteoclasts, involved in bone formation and remodeling of the normal skeleton. This may suggest a similar potential in the formation of heterotopic bone formation, although these mechanisms have not currently been examined.

Additional evidence for the role of the peripheral nervous system, in particular the sensory nerves, in *de novo* bone formation comes from a number of clinical observations and basic science studies on the healing of fractured bone. Several animal studies have shown that transection or denervation of the complete peripheral nerve leads to an impaired healing of fractures.[48–50] While these studies examined the effects of combined motor, sensory, and autonomic denervation, a more recent study by Apel et al. further demonstrated that sensory denervation alone impairs fracture healing.[51] Using a model of capsaicin-denervated animals, which impairs the CGRP- and SP-positive nerve fibers of the PNS, the authors showed that sensory denervated animals displayed a fracture callus that is significantly larger and less ossified, with reduced mechanical strength, compared to fractures in animals with intact sensory nerves. In line with these results, clinical studies have revealed that the levels of the sensory peptides, such as CGRP and substance P, are significantly increased in patients within 24 h of bone fracture.[52] Following fracture of the rat tibia, studies have also shown a substantial increase in CGRP-expressing neurons that colocalize with new bone formation,[53] and a significant increase in the number of SP-positive nerve fibers.[54] In addition to fracture models, studies examining the repair of an experimental bone defect model in the rat tibia also demonstrated an increase in the number of nerve fibers expressing substance P and CGRP within the first few days following the defect, which returned to normal by 3 weeks.[55] All of these observations suggest that peripheral nerves, particularly the sensory component, are closely involved in fracture healing and bone repair following injury. Further, the data supports a global mechanism for bone formation involving the sensory neurons and neuroinflammation. Neuroinflammation mediated by the sensory nerves can lead to not only vasodilation, extravasation, and the recruitment of potential progenitors, but also potential nerve remodeling and the release of progenitors that contribute to bone formation.

III. Heterotopic Ossification and the Sympathetic Nervous System

BMPs have been demonstrated, *in vitro*, to induce development of sympathetic neurons from neural crest cell cultures. Additionally, *in vivo* studies revealed that delivery of the BMP antagonist, noggin, to the chick embryo during the time of sympathetic neuron

differentiation prevented expression of noradrenergic marker genes and generation of sympathetic nerves.[56] More recent studies, using conditional knockout embryos, have further defined the mechanisms by which BMP signaling regulates sympathetic nervous system (SNS) development, including a role for BMP signaling in survival of SNS precursors and SNS differentiation and proliferation.[57] Moreover, BMP2 has been shown to induce neurotransmitter and neuropeptide expression in rat neonatal sympathetic neurons.[58] Given the defined and important role of BMP during these key developmental events, it would not be surprising to observe BMP involvement in regulating SNS function during heterotopic bone formation within the adult organism.

III.A. Sympathetic Nerve Regulation of HO

As mentioned, one of the earliest steps in our mouse model of HO is the biogenesis of brown fat, approximately two days following injection of BMP2-producing cells.[4] These brown fat cells are critical for patterning of the local oxygen environment necessary for further cartilage and bone formation. While the exact mechanism by which BMP2 induces the rapid production and expansion of brown fat is currently under investigation, the induction of brown adipose tissue (BAT) has been shown to involve the SNS. Interestingly, heterotopic ossification in *Misty Grey Lean* mice, which lack functional brown adipose,[59] led to enhanced bone formation.[4] In these studies, the white adipose appeared to compensate for the loss of brown adipose, by utilization of its lipid to induce a hypoxic environment. Thus, the contribution of BAT in this model could be considered inhibitory, since we obtained a greater response in bone formation. However, the utilization of the white adipose, which is unable to uncouple, in this model, is at the expense of creating considerably reactive oxygen.[60] Recently, the mutation in *Misty Grey Lean* mice was identified to be in a protein known as *dock 7*,[61] which is known to be involved in axonal migration. This suggests a possible relationship between the potential nerve cell migration and expansion from the sensory neurons and the production of brown adipose through SNS stimulation.

Noradrenaline release from sympathetic neurons stimulates β_3-adrenergic receptors abundantly expressed on brown fat cells, ultimately directing a number of proteins involved in the upregulation of a brown fat phenotype.[62] In support of sympathetic regulation of BAT, administration of β_3-adrenergic receptor agonists increases BAT in mice, dogs, and primates[63]; adult humans with enhanced noradrenaline release, due to rare tumors of the adrenal glands, also develop more abundant brown fat deposits. Therefore, the SNS likely has a role in controlling the induction of BAT during HO (Fig. 2).

Interestingly, the production of BAT through sensory nerve stimulation during the initial stages of HO leads to further stimulation of sensory neurons within the local environment. Since sensory neurons, particularly the small diameter, afferent sensory fibers of the PNS, respond to thermal stimuli, heat produced by the brown adipose will continue to induce signaling and resultant neuroinflammation. Brown adipocytes, in addition to their ability to generate hypoxic stress within the tissue, are known for their function in heat generation, or thermogenesis.[62] Brown adipocytes exclusively express UCP1 (uncoupling protein 1), which is capable of uncoupling the electron transport chain from the generation of ATP to the generation of heat.[62] Therefore, an additional outcome of BAT activation is the release

of heat within the local environment. Thus, the initial pulse of BMP ultimately sets in motion a cascade of neuronal signaling events that propagate and reinforce each other to lead to heterotopic bone formation.

Finally, one of the other factors released by mast cell degranulation is serotonin in lipid vesicles,[64] although its function is unknown. It is conceivable that the serotonin released from mast cells leads to the stimulation of sympathetic neurons at the site of injury. Surprisingly, serotonin has been reported to have two opposing actions on bone remodeling. When released outside the hypothalamus, the hormone appears to inhibit bone formation, but when used as a neurotransmitter, it exerts positive effects on bone mass, by enhancing formation and limiting desorption.[65]

III.B. SNS Regulation of Osteoblasts

The SNS has also been linked to the regulation of orthotopic bone mass.[66] Inhibitors of sympathetic signaling, such as the β-blocker propranolol, have been shown to increase bone mass in wild-type mice, and reduce bone loss in ovariectomized mice and rats.[67] This sympathetic regulation of bone mass was further attributed to signaling mechanisms activated through β_2-adrenergic receptors expressed on osteoblasts. While in the normal skeleton this sympathetic signaling mechanism appears to inhibit the formation of bone, the potential efect on heterotopic bone is currently unknown. However, these studies provide further evidence for an additional cell type involved in bone formation and potentially under the control of sympathetic signaling (Fig. 2).

The SNS may be regulating osteoblasts directly, or regulating progenitors of osteoblasts. We[68] and others[69] have shown that the hematopoietic stem cell (HSC) is the precursor for the osteoblast. Intriguingly, the SNS has been implicated in the recruitment and mobilization of HSCs.[70,71] Sympathetic signaling has been demonstrated to regulate the release of stem cells from the bone marrow.[72] Activation of β_3-adrenergic receptors expressed on stromal cells within the bone marrow niche leads to the downregulation of Cxcl12, a chemokine critical for stem cell attraction within the marrow.[70] Consequently, decreased expression of Cxcl12 within the bone marrow microenvironment encourages stem cell mobilization from the marrow to the peripheral circulation. Upon mobilization, these stem cells could then recruit to the area of new bone formation for further differentiation. This may suggest another pool of potential progenitor cells, in addition to the primitive stem cells within the local, peripheral nerves. It is also possible that the nerve-associated stem cells are progenitors to the HSC. Indeed, the large numbers of neural markers on HSCs has been noted before.[73] Additionally, it has been previously reported that such neural stem cells can rescue lethally irradiated animals.[74,75] Future studies aimed at further understanding and identification of these various progenitor sources, under the control of neuronal signals, will provide a new area for potential treatment and prevention of HO.

IV. Conclusions

As we have outlined, a number of recent studies are beginning to shed light on the role of the peripheral nerves in the production of HO. Sensory stimulation, by injury and BMP release, can evoke local, neuroinflammatory processes, which ultimately enable the

recruitment of progenitors for chondroosseous differentiation. Neuroinflammation within the local environment may lead to the activation of the sympathetic nervous system, through the release of mast cell serotonin. Intriguingly, stimulation of the SNS then continues to trigger the sensory nervous system, through generation and thermogenesis of the brown adipose. Sensory neurons also transmit information regarding the local environment to the CNS and hypothalamus, potentially regulating both heterotopic bone formation and skeletal remodeling. This relationship is unclear, but, often, in clinical scenarios that favor HO, it appears to be at the expense of the adjacent skeletal bone, suggesting the production of HO is perhaps a response to replace the skeletal bone.

While heterotopic ossification is considered an aberrant process, its origins may stem from the critical need to maintain an intact skeleton for survival. In fact, it may be the peripheral nervous system that plays a key role in the surveillance required to preserve normal, functional bone. On one hand, the PNS may relay information to the CNS, to regulate the everyday remodeling of the normal skeleton, critical for maintaining homeostasis within the organism. This information may arise from mechanosensors on osteocytes, which provide additional signaling between the PNS and skeletal bone.[76] However, when the body sustains a traumatic injury, and the normal environment becomes altered through trauma and BMP release, the sensory nerves may be the first in line to detect any damage to the bone itself. Once these nerves “sense” these alterations within the local environment, they may initiate a program of regeneration of the bone and soft tissues, over the normal, remodeling mechanisms. The sensory nerves signal to the CNS to override the remodeling program, to set in motion the mechanisms to rebuild *de novo* bone. In certain instances, this mechanism may generate new bone in incorrect places, and result in HO. Thus, knowledge of peripheral nerve regulation of HO may be translatable to other repair mechanisms and may provide invaluable insight into the body's ability to detect and regenerate those tissues most valuable for survival, including the bone.

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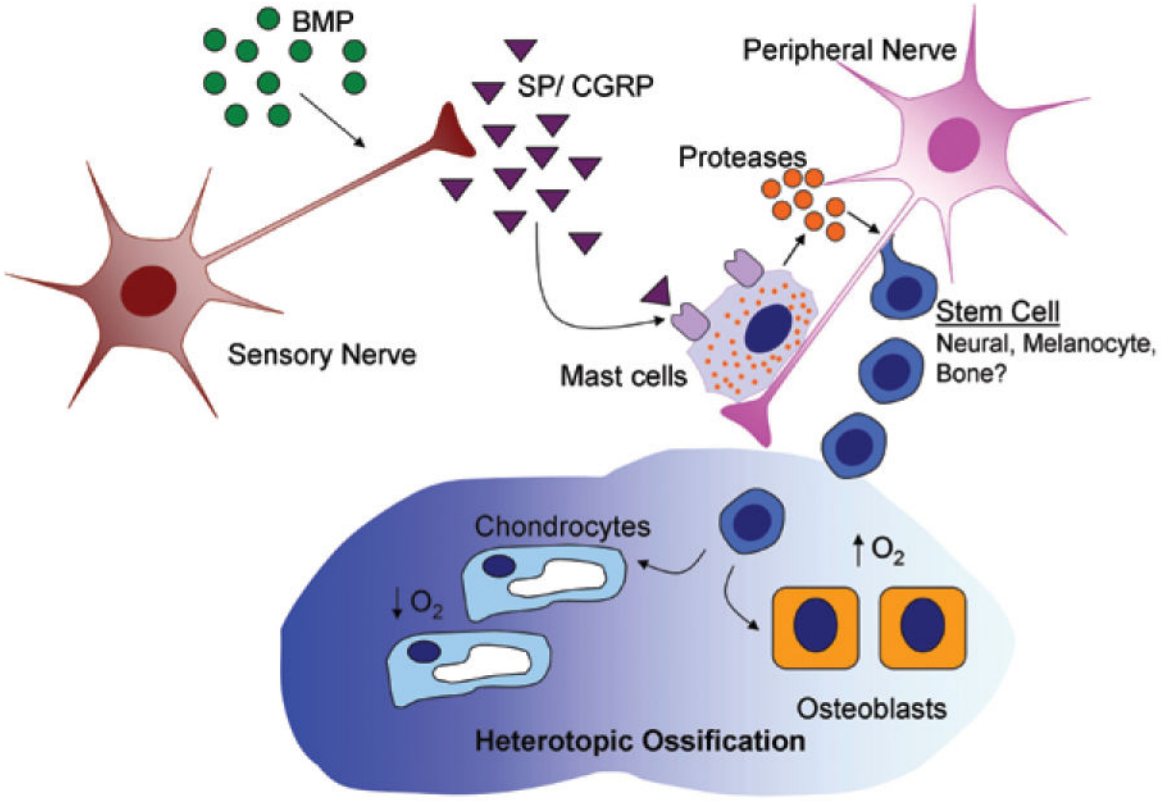

Figure 1.

Schematic representation of the tentative neuroinflammatory mechanism and its relationship to heterotopic ossification. BMP2 can induce recruitment of mast cells and nerve tissue remodeling, through activation of sensory neurons and release of Substance P (SP) and calcitonin gene-related peptide (CGRP).

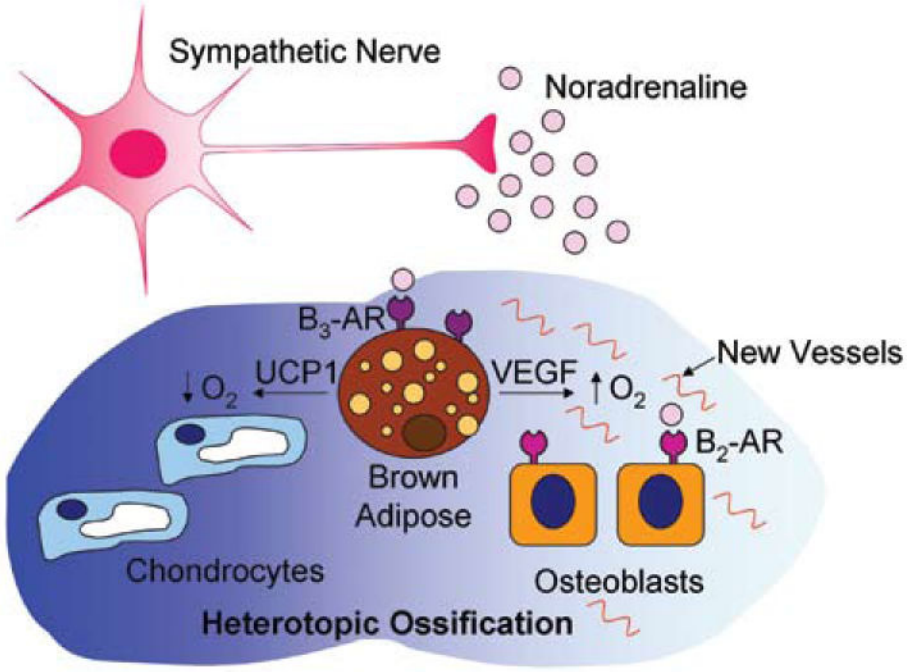

Figure 2.

Schematic representation of the tentative interaction of the sympathetic nervous system and heterotopic ossification. Activation of the sympathetic nervous system, through sensory stimulation, leads to regulation of adipose, particularly the rapid appearance of brown adipose within the area of HO. The brown adipose appears to be critical to bone patterning and formation.