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Mechanisms underlying non-malignant skeletal pain

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Despite the high prevalence of non-malignant skeletal diseases that are associated with chronic pain as well as the impact of skeletal pain on society, the mechanisms driving non-malignant skeletal pain are not fully understood. In the past decade, improved biochemical, immunohistochemical and imaging techniques have helped to characterize the sensory and sympathetic innervation of different bone compartments in healthy tissue. New methods are being developed to determine how patterns of innervation change with aging or under pathological conditions. Importantly, several preclinical models of acute and chronic bone injury have been established that are providing a clearer understanding of the mechanisms underlying skeletal pain and are guiding development of mechanism-based therapies to more effectively treat skeletal pain.

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Burden of skeletal pain

Skeletal pain is caused by a diverse group of non-malignant disorders and conditions including trauma-related fractures, osteoporotic fractures, osteoarthritis, vertebral degeneration, sickle cell anemia, among others [1•,2]. These conditions not only result in chronic pain and physical disability, but also result in reduced mobility, cardiovascular dysfunction and impaired cognitive health. All of which diminish the functional status and quality of life of the patient [3]. The incidence in the world of some of these skeletal diseases is currently high and it is projected to dramatically increase in the next decade [4]. In 2010, it was estimated that osteoporosis affected more than 200 million people and 8.9 million osteoporotic-related fractures occur each year worldwide [5]. Low back pain affected over 100 million individuals in the world in 2014, and it is estimated that in 2018 about 251 million people suffered from knee osteoarthritis [5]. It should be noted that most of the skeletal diseases occur primarily in the elderly [4]. Given that life expectancy is increasing worldwide, the burden of skeletal pain on individuals and society is projected to increase [3].

In the present review, we summarize what is known regarding the mechanisms driving non-malignant skeletal pain due to bone damage by trauma, osteoporosis and osteoarthritis (OA) from preclinical models and human studies. We are not including discussion of joint pain due to inflammation or autoimmune arthritis as it is beyond the scope of this review, however these models have been discussed in excellent reviews by others [6,7].

Sensory innervation of normal bone

Sensory neurons are classified based on size, neurochemical phenotype, functional properties, and pattern of innervation. Thickly myelinated Aβ fibers predominantly detect non-noxious tactile or mechanical sensation. Thinly myelinated Aδ fibers detect predominantly noxious mechanical stimuli. Unmyelinated C fibers detect multiple types of noxious stimuli (thermal, mechanical, chemical) and are further subdivided into peptidergic and non-peptidergic subsets containing calcitonin gene related peptide (CGRP) or binding isolectin B_4 (IB_4), respectively. Cutaneous tissues contain virtually all sensory neuron subtypes. Skeletal tissue including mineralized bone, bone marrow, and periosteum are innervated by a more restrictive population of only thinly myelinated Aδ fibers and unmyelinated peptidergic C fibers a majority of which express transient receptor potential cation channel subfamily V member 1 (TRPV1) in mice [1•]. Previous studies have shown that mouse bone is minimally innervated by small unmyelinated non-peptidergic IB_4^+ fibers (See [1•] for references); however, it is possible that the distribution of subsets of sensory neurons in skeletal tissue is species-dependent as injection of retrograde tracers into the intramedullary space of the tibia in rats labels a small percentage of IB_4^+ non-peptidergic afferents (See [6] for references). The majority (>80%) of sensory neurons that innervate the bone contain tropomyosin receptor kinase A (TrkA), the endogenous receptor for nerve growth factor (NGF) (See [1•] for references).

In recent years, single cell RNA sequencing has been used for transcriptional profiling of sensory neuron subsets in

mice [8] and have revealed much greater heterogeneity within broad neurochemically defined subsets. Physiological and behavioral studies using transgenic mice (Cre driver and reporter) that label molecularly defined populations of sensory neurons often combined with retrograde tracing are beginning to describe distinct roles for neuronal subsets in unique aspects of nociceptive processing within cutaneous [9,10] and visceral tissue [11]. However, the contribution of subsets of nociceptors to skeletal pain has not been studied. Future studies utilizing transgenic mice in combination with optogenetic and chemogenetic approaches may better define the distribution and contribution of molecularly defined subsets of neurons in skeletal related pain behavior (ongoing versus movement evoked pain) and disease processes (See Table 1).

Possible mechanisms driving skeletal pain in different diseases/trauma

Direct activation of mechanosensitive nociceptors due to trauma or bone injury

Early studies suggested that a direct activation of nerve fibers innervating periosteum or bone marrow may result in skeletal pain [12,13]. In awake humans, a direct mechanical stimulation of the periosteum elicited an immediate sharp arresting pain at a lower threshold than stimulation of the ligaments, fibrous capsule of the joints, tendons, fascia and muscle [13]. In canine whole-nerve recordings in a branch of the tibial nerve, neural activity was increased after application of noxious mechanical or chemical stimuli to the bone marrow [12]. Elegant studies performed by Nencini and Ivanusic, using a novel *in vivo* bone-nerve preparation in rats, demonstrated that Aδ nociceptors that innervate the tibial bone marrow respond to high threshold noxious mechanical stimulation (increasing intra-osseous pressures) in an intensity dependent manner [14].

Acidosis

During different skeletal diseases such as osteoporosis, osteoarthritis and lower back pain there is a pathological bone resorption, which is mediated by osteoclasts. Osteoclasts reabsorb bone by maintaining an acidic extracellular microenvironment (pH 4.0–5.0) at the osteoclast-mineralized bone interface [15]. These protons

Table 1

Proposed classification and characterization of sensory neuron subsets that innervate bone/joints

Size/Myelination	Fiber class (CV)	Neurochemical marker	General role in somatosensation	Localization in bone/joint	Potential role in bone/joint pain
Small	C fibers (0.5 – m/s)	Peptide-rich (CGRP$^+$, TRKA$^+$,CHRNA3$^+$,PIEZO2$^+$)	"Silent" mechano-nociceptors	• ~50 % of knee joint in mice • Distribution in bone unknown	• Normally unresponsive to movement of bone/joint • Sensitized to mechanical stimuli/movement by inflammatory mediators (i.e. NGF)
		Peptide-rich (CGRP$^+$, SP$^+$, TRKA$^+$, TRPV1$^+$)	Polymodal nociceptors (Chemical thermal, mechanical)	• ~Dense innervation of joints and bone in mice/rats	• Sensitized and activated by low pH/acidosis • Contribute to dull aching ongoing bone pain
		Peptide-poor (IB4$^+$, P2X3$^+$, TRKA$^+$, MrgpD$^+$)	Mechanical nociceptors	• Sparse innervation of joints and bone in mice • ~20 % in rat tibia	• Role in nonmalignant skeletal pain unknown
Medium	Aδ fiber (5 – 30 m/s)	Peptide-rich (NF200$^+$, CGRP$^+$, TRKA$^+$) (Subsets: 5-HT3AR$^+$/NPYR2$^+$)	High threshold mechanoreceptors (AHTMR)	• Dense innervation of joints and bone in mice/rats • Distribution of subsets (5-HT3AR$^+$/NPYR2$^+$) unknown	• Detect noxious mechanical stimuli due to increased intraosseous pressure or distortion of periosteum/bone • Sensitized to mechanical stimuli/movement by inflammatory mediators • Contribute to sharp intense movement evoked bone pain
Large	Aβ fiber (30 – 70 m/s)	Neurofilament-rich (NF200+) (Subsets: TRKC$^+$/PV$^+$/PIEZO2$^+$)	Low threshold mechanoreceptors (LTMR) Proprioceptors	• Sparse innervation of joints and bone in mice • Some specialized encapsulated endings i.e. Pacinian corpuscles in periosteum of human/rats • Distribution of subsets (TKRC$^+$/PV$^+$) unknown	• Role in nonmalignant skeletal pain unknown

Abbreviations: CV: Conduction velocity, CGRP: Calcitonin gene related peptide, CHRNA3: Cholinergic receptor alpha-3 subunit, MrgpD: Mas-related G-protein coupled receptor member D, NGF: Nerve growth factor, NPYR2: Neuropeptide Y2 receptor, PV: Parvalbumin, 5-HT3AR: Serotonin receptor subtype 3A, TRKA: Tropomyosin receptor kinase A, TRKC: Tropomyosin receptor kinase C, TRPV1: Transient receptor potential cation channel subfamily V member 1.

then activate different channels such as the TRPV1 and/or the acid-sensing ion channel-3 (ASIC-3), which are expressed in the primary afferent neurons (nociceptors) innervating bone (See [1•] for references). In support of this, pre-clinical studies have shown that mice with ovariectomy-induced bone loss (a preclinical model of post-menopause osteoporosis) have increased thermal and mechanical hyperalgesia, which is blocked by treatment of TRPV1 and ASIC antagonists [16,17]. Furthermore, treatment with drugs that inhibit bone reabsorption, such as bisphosphonates, result in alleviation of pain and recovery of functional ability in patients with osteoporosis even in the absence of fractures [18,19]. All these results taken together suggest that acidosis due to increased and pathological bone resorption may partially contribute to the development of skeletal pain. It should be noted that enhanced bone resorption and pain may be dissociated as not all patients with osteoporosis or osteoarthritis experience pain [20,21]. Additionally, in mice with chronic osteoporosis due to ovariectomy, treatment with bisphosphonates only has minor analgesic effects despite significantly reversing bone loss [22].

Sensitization of mechanosensitive and 'silent' nociceptors by various growth factors and cytokines in the bone

NGF was originally discovered as a neurotrophic factor essential for the survival of sensory and sympathetic neurons during development [23]. In the adult, NGF has a major role in nociceptor sensitization following injury by acting mainly through its high affinity cognate receptor, TrkA regulating synthesis and expression of ion channels, receptors, and signaling molecules involved in the development of chronic pain (See [24] for references). A previous study has shown that NGF is upregulated in the sciatic nerve and skin four weeks after tibia fracture in rats [25]. Likewise, both levels of NGF and TrkA are increased in the synovial fluid and serum of patients with osteoarthritis who underwent total knee arthroplasty [26]. Furthermore, recent studies provide direct evidence that NGF activates and sensitizes nerve fibers innervating the rat tibial bone marrow [27,28]. Direct application of NGF into the medullary cavity increases the spontaneous action potential discharge, reduces thresholds of activation, and increases discharge frequency of bone nociceptors in response to mechanical stimulation (intra-osseous pressure) [14]. Consistent with physiology studies, peripherally restricted antagonism of NGF-trkA receptor signaling is effective for controlling bone pain in animal models of fracture and osteoarthritis as well as in people with osteoarthritis and low back pain [29–31]. Collectively, these studies suggest a pivotal role of NGF on peripheral sensitization of nociceptors in different skeletal diseases. Recent studies by Nencini *et al.* have shown that other growth factors including glial cell line-derived growth factor, neurturin and artemin activate and sensitize bone afferent neurons and may have an important role in the pathogenesis of inflammatory bone pain [32].

Several cytokines may have a role in driving skeletal pain including TNFα, IL-1α, IL-6 (See [24] for references). They can directly activate receptors expressed on nerve fibers innervating skeletal tissues or indirectly by inducing release of pain mediators such as prostaglandins, which in turn can activate or sensitize nociceptors [33]. In rats with tibia fracture followed by cast immobilization, there is upregulation of these cytokines in the sciatic nerve and skin four weeks after injury [34] and in synovial fluid of humans with osteoarthritis [26]. Furthermore, the levels of these cytokines are positively correlated with the magnitude of pain in people with osteoarthritis. Preclinical and clinical studies show that blocking TNF alpha, IL-1beta and IL-6 or their receptors results in a reduction of skeletal pain [27]. Classic electrophysiological studies by Schaible and Grubb assessed the mechanical responsiveness of afferents that innervate the cat knee joint before and after acute inflammation. Afferents with conduction velocities in the Aδ range exhibited reduced thresholds becoming activated by movement in the normal working range or in response to innocuous pressure after injection of inflammatory agents into the joint. Additionally, a significant percentage of unmyelinated C fibers insensitive to mechanical stimulation in the normal joint developed responsiveness to mechanical stimulation and ongoing activity following acute inflammation. It has been proposed that these 'silent' nociceptors are key contributors to bone and joint pain under pathological conditions [35]. A recent study in mice identified a population of peptidergic C-fibre mechanoinsensitive 'silent' afferents that preferentially innervate deep somatic tissue and express the cholinergic receptor alpha-3 subunit (CHRNA3). This population of fibers comprises 50% of peptidergic C fibers that innervate the knee joint, are $TrkA^+$ and become responsive to mechanical stimulation following exposure to NGF [36]. Future studies examining the distribution and functional role of, this subset of neurons in bone and skeletal pain models are warranted.

Plasticity of sensory and sympathetic nerve fibers innervating bone with age and in non-malignant skeletal diseases

Chronic skeletal pain caused by conditions such as osteoarthritis, fractures, and low back disorders becomes more prevalent with age [3,4]. Recent studies in rats and mice have shown that unlike bone mass and strength which decrease with age, the density of $CGRP^+$ and $NF200^+$ sensory nerve fibers in different bone compartments (periosteum, mineralized bone, and bone marrow), is not significantly different between young, middle-aged and old rats or mice [37,38]. Based on these observations and data demonstrating that the density of innervation of the bone is positively correlated with the severity of pain

following injury in animal models [39], it has been suggested that maintenance of the density of sensory nerve fibers that transduce noxious stimuli in bone tissue with aging may partially explain why the incidence and duration of skeletal pain increases with age [37].

Another potential mechanism of skeletal pain generation is pathological sprouting of sensory and sympathetic nerve fibers surrounding sites of injury or around regions of disease-induced bone remodeling. It has been reported that mice with non-healed femoral fractures [40] and rats with tibial injury (drilling through the tibia) [41], have a marked sprouting of sensory and sympathetic nerve fibers, and this nerve remodeling correlates with the magnitude of pain-like behaviors. Human studies have also reported that human chronic discogenic back pain, may in part be due to growth of $TrkA^+$ nerve fibers into normally aneural and avascular areas of the human intervertebral disc [42]. Likewise, sympathetic and sensory nerve fibers have been reported to be present within vascular channels in the tibiofemoral articular cartilage (a tissue that under healthy conditions is avascular and aneural) of humans with mild and severe osteoarthritis [43]. These reports suggest that following injury or disease of the skeleton, nerve fibers do not seem to act as static structures, instead they undergo a dramatic remodeling which may partially contribute to the development of chronic skeletal pain.

Spinal and supraspinal sensitization associated with nonmalignant bone pain models

While the majority the studies on skeletal pain have focused on peripheral sites, recent studies suggest an important role of spinal and supraspinal circuits in contributing to skeletal pain. Previous electrophysiological studies demonstrate that application of intraosseous pressure into the femoral bone marrow results in a barrage of firing of spinal cord neurons and causes referred pain and hyperalgesia in the corresponding skin areas [44] indicative of central sensitization. Likewise, increased intraosseous pressure in the tibial bone marrow increases the expression of c-Fos in the ipsilateral superficial dorsal horn [45]. Studies in mice with femur fracture demonstrate increased spinal c-Fos in superficial and deep dorsal horn for several days [29]. Treatment with anti-NGF before fracture reduced the persistent increase in c-Fos suggesting that spinal release of neurotransmitters from TrkA containing afferents contributes to central sensitization following traumatic injury to the bone. Additionally, biochemical studies have shown increased levels of several pro algesic factors IL-1β, TNFα, IL-8, IL-10, NGF in the spinal cord following a tibia fracture [34,46,47]. Thus, several factors may contribute to central sensitization following non-malignant bone pain.

The involvement of supraspinal circuits in non-malignant skeletal pain have minimally been examined in animal models. In the rat mono-iodoacetate (MIA) model of osteoarthritis, loss of descending inhibition [48] and increased facilitation from the RVM [49] have been reported to contribute to amplifying skeletal pain at later stages of MIA. Consistent with these findings, patients with severe knee OA pain had significantly less diffuse noxious inhibitory controls than healthy controls and functional imaging studies performed in patients with osteoarthritis showed increases in activation in facilitatory brain regions including the PAG [50] and RVM [51], facilitatory brain regions during cutaneous stimulation of referred pain areas. These studies suggest that centrally acting medications that augment descending inhibition including norepinephrine and serotonin reuptake inhibitors maybe effective for treating some patients with chronic osteoarthritis [52].

New therapies and conclusions

In the last decade, a significant amount has been learned from anatomical, physiological and behavioral studies in preclinical models of acute and chronic nonmalignant skeletal pain. All these advances are providing a better understanding of the mechanisms underlying skeletal disease as well as guiding the rational development of mechanism-based therapies to more effectively treat skeletal pain. Several novel therapeutic interventions to treat skeletal pain are in clinical trials including anti-NGF antibodies (Tanezumab), anti-IL-6 receptor antagonists (Tocilizumab), and anti-TNF antibodies (Adalimimab). Neurolytic strategies for silencing sensory neurons that innervate arthritic joints are also being explored clinically utilizing high dose capsaicin, cryoneurolysis, or radiofrequency ablation and have demonstrated pain relief in patients with osteoarthritis; however, the long term impact of these approaches are still being investigated [52]. Future research using clinically relevant animal models and improved methods to better define phenotype, physiology and plasticity of discrete populations of sensory neuron that innervate bone following trauma, degeneration or inflammation of skeletal tissue will help to identify new potential targets for treating skeletal pain.

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Conflict of interest statement

Nothing declared.

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