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Review Article

Netrin-1: A Complex New Player in Many Pathologies

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Abstract

Osteoarthritis (OA) is a disease characterized by progressive destruction of cartilage although currently, more correctly, considered a disease of whole joint. Increased subchondral bone turnover is a key moment underlying osteoarthritic process determined by an altered relationship between the activity of osteoblasts, osteoclasts, and osteocytes. While the systemic hormonal regulation of the remodeling process has to occur via factors arriving at individual remodeling sites via bloodstream, the way by which local regulatory factors exert their action on individual cell populations has been subject of constant and progressive knowledge. Initial subchondral bone and cartilage damage starts with release of some factors responsible for joint pain. For a long time, the release of NGF from damaged cells in osteochondral junction has been considered as main event and trigger of pain carried, via various nociceptive fibers, to the spinal cord.

Recently other factors have entered this complex mechanism; one of the emerging player is a member of laminin-like protein family, Netrin-1, which was initially identified as a molecule involved in axonal guidance but also in non-neural activities like angiogenesis, inflammation, tissue remodeling and cancer. Netrin-1, as a part of the classic axon guidance family (netrins, semaphorins, ephrins and slits), was shown to coordinate cell migration and axonal growth as a chemoattractant or chemorepellent, depending on the receptors expressed by target cells.

Recent research has revealed a link between Netrin-1 and the development of multiple inflammatory diseases such as ischemic reperfusion injury, atherosclerosis, diabetes. Nonetheless the effects of Netrin-1 are not exactly the same in all disease or inflammatory condition. It has been also found that Netrin-1 orchestrate organogenesis, angiogenesis, tumorigenesis. In inflammation, as in neuronal development, Netrin-1 plays a role directing migration of leukocytes, especially monocytes in inflamed tissues with impact on acute and chronic inflammation.

Keywords: *Netrin-1; Inflammation; Osteoarthritis; Rheumatoid Arthritis*

Introduction

It is part of the natural order of things to have to continually revise and expand scientific knowledge in most diverse areas of sciences, an event that also occurs very often regarding the mechanisms underlying human pathologies. Such consideration is also confirmed with respect to the knowledge gained so far about the mechanisms that regulate degradation of osteoarticular structures and pain that often accompanies them.

While the hallmark of Osteoarthritis (OA) is the progressive destruction of cartilage, OA is now considered a disease of whole joint [1].

The subchondral bone may show sclerosis, combined with osteophytes [2]. often associated with inflammatory changes in the joint such as synovitis [3]. Furthermore, OA joints may show bone marrow lesions [4,5].

It is an established concept that increased subchondral bone turnover is a key moment underlying the osteoarthritic process and that this process is determined by an altered relationship between the activity of bone cells, such as osteoblasts, osteoclasts, and osteocytes.

Bone remodeling

At the maturity bone is subject to a remodeling process which is tightly regulated to secure repair of microdamage and replacement of old bone with new bone through sequential osteoclastic resorption and osteoblastic bone formation under the regulation of a wide variety of calcitropic hormones. During normal bone remodeling, the amount of resorbed bone is completely replaced in location and amount by new bone. While the systemic hormonal regulation of remodeling process has to occur via factors arriving at individual remodeling sites via bloodstream, the way by which local regulatory factors exert their action on individual cell populations has been subject of constant and progressive knowledge and have included several growth factors and cytokines like interleukins (IL), tumor necrosis factors (TNF), transforming growth factors (TGF), colony stimulating factors (CSF), Insulin like growth factors (IGF), fibroblast growth factors (FGF), platelet derived growth factors (PDGF) and bone morphogenetic proteins (BMP) formed by both monocyte cells in the marrow space or circulation, as well as by bone cells in the bone multicellular unit (BMU).

To accomplish normal physiological bone remodeling, the proper coupling of bone formation and bone resorption requires direct communication among different bone cells. Cells of the osteoblast lineage (osteoblasts, osteocytes, and bone-lining cells) and bone-resorbing cells (osteoclasts), together with their precursor cells, are organized in specialized units called bone multicellular units (BMU). Bone remodeling follows coordination of distinct and sequential phases. In the resorption phase, osteoblasts respond to signals generated by osteocytes or PTH, recruiting osteoclast precursors to the remodeling site and releasing activating factors like M-CSF and RANKL (Receptor activator of nuclear factor kB ligand) while osteoprotegerin (OPG) is also released to counteract the RANKL. Mononuclear cells of the monocyte/macrophage lineage in the bone marrow or in the circulation are generally considered to be osteoclast precursors, which are attracted to resorption sites and then differentiate into osteoclasts in response to M-CSF and RANKL [6]. A large number of other factors are involved in the process which are described in several other specific reviews as that is not the purpose of this review [7,8].

Osteoarthritis and rheumatoid arthritis

It is established that increased subchondral bone turnover is a key moment underlying the osteoarthritic process and that this process is determined by an altered relationship between the activity of bone cells, such as osteoblasts, osteoclasts, and osteocytes. Subsequently a local increase in bone formation lead to a subchondral bone sclerosis.

OA represents one of the most frequently occurring painful conditions. Pain is the major OA symptom, involving both peripheral and central neurological mechanisms. OA pain is initiated from free axonal endings located in synovium, periosteum and tendons. The nociceptive message involves not only neuromediators and regulating factors such as neuronal growth factor (NGF) but also central

modifications of pain pathways. OA pain is a mixed phenomenon where nociceptive and neuropathic mechanisms are involved in both local and central levels.

Osteo-articular pain can be caused by numerous conditions such as trauma, inflammation, an auto-immune reaction, but also by genetic alterations, pathologies associated with aging, or cancer.

The resulting pain remains complex, with different manifestations as are the multiple etiologies and, consequently, also the different therapeutic strategies aimed at affecting the multiple mechanisms. Joint tissues have the ability to send signals, thermal, chemical, mechanical originating locally, through numerous and diverse nerve fibers (nociceptors) that go to the spinal cord where they release, in the synaptic space, small molecules (Glutamate, ATP) and peptidergic neurotransmitters (substance P, CGRP (Calcitonin Gene Related Peptide)).

These substances act on receptors present on interneurons within the spinal cord and on neurons along special tracts to various regions of the brain. The interneurons that modulate the pain signal are both inhibitory and release GABA and glycine, and excitatory glutamate and ATP.

It should be recognized, however, that there is not yet complete knowledge about the processing of information derived from joint tissues.

In this review, we wanted to focus on the event that follows the initial subchondral bone and cartilage damage with the release of some factors responsible for joint pain. For a long time, the release of NGF from damaged cells in the osteochondral junction has been considered as main event and trigger of pain stimulus carried via the various nociceptive fibers to the spinal cord in addition to stimulating TrkA (tropomyosin-related kinase) receptors on A-delta and C fibers and initiating the expression of CGRP and substance P responsible for increased angiogenesis, fluid leakage and edema formation.

In more recent times other factors have entered this complex mechanism; one of the emerging player is a member of the laminin-like protein family, Netrin-1, which was initially identified as a molecule involved in axonal guidance but also in non-neural activities like the angiogenesis, inflammation, tissue remodeling and cancer [9]. Netrin-1, as a part of the classic axon guidance family (netrins, semaphorins, ephrins and slits), was shown to coordinate cell migration and axonal growth as a chemoattractant or chemorepellent, depending on the receptors expressed by the target cells [10,11].

Recent research has revealed the link between Netrin-1 and the development of multiple inflammatory diseases such as ischemic reperfusion injury, atherosclerosis, diabetes.

A study of Ranganatan., *et al*. (2013) shows that Netrin-1 suppresses COX-2 expression and PGE2 production in neutrophils *in vivo* and *in vitro*. The same study demonstrate that Netrin-1 blocks PGE2 production but not its activity. Moreover, Netrin-1 regulates COX-2 expression through inhibition of IκBα degradation [12]. Netrin-1 plays also a role directing the migration of leukocytes, especially monocytes in inflamed tissues with an impact on acute and chronic inflammation [13].

Ly., *et al*. (2005) have reported that, outside the central nervous system, Netrin-1 can modulate the migration of granulocytes, monocytes, and leukocytes in and outside the inflamed tissue and promotes macrophage differentiation to an alternative activated phenotype [14,15].

Collectively, most studies show that the regulation of Netrin-1 is critically involved in acute and chronic inflammation. The expression of Netrin-1 and its various receptors are differentially regulated depending on the tissue, the stimulus, the source, and the target cell.

In 2015 Mediero., *et al*. showed that Netrin 1 is a key factor in osteoclast differentiation and stimulates a marked increase in the number of mature osteoclasts similar to classical osteoclast differentiation factors such as M-CSF and RANKL [16].

Recent studies report that in synovial fluid of OA patients, Netrin 1 concentration correlate negatively with that of CTXI (type 1 collagen cross-linked C-terminal telopeptide) [17]. This observation indicates that Netrin 1 may also be a negative regulator of bone destruction in non-inflammatory conditions. Furthermore, Netrin 1 treatment potently suppressed the bone erosion associated with experimental arthritis in mice, suggesting a prophylactic potential of Netrin 1 in Th17-associated bone disease in humans. It was also found that the osteoclast inhibitory function of Netrin 1 is the strongest among the axon guidance cues released from osteoblasts. The study highlights that osteoblasts and synovial fibroblasts rapidly release humoral factors that protect against excessive bone destruction during acute inflammation.

A work of Shouan Zhu., *et al*. has investigated the role of OCs in the subchondral bone remodeling as well as the role in sensory innervation. The study shows that osteoclast-initiated subchondral bone remodeling mediates OA pain, and nociceptors are generated during aberrant subchondral bone remodeling in early phase of OA [18].

In addition to its role in axon guidance, Netrin-1 has been suggested to be a potent vascular mitogen [19,20]. These findings suggest that inhibition of aberrant subchondral bone turnover can reduce the presence of Netrin 1 and sensory innervation and attenuate articular cartilage degeneration.

Other player

Coordination between resorption phase and neoformation, that regulates bone remodeling, is determined not only by molecules or factors generated by the cells present in the resorption site or released from organic and mineral matrix dissolved by the OCs. This concept is certainly not wrong or at least it is not entirely correct because many neurological signals influence this coordination and play a double role, both as guides for the formation and direction of nerve fibers and as signals that activate the function of bone cells.

Besides substances such as Netrin 1, other molecules like Semaphorins (Sema) and Slit are expressed in the bone microenvironment where they mediate relationships between bone cells. Often the same substance can exert different effects on cells where specific receptors are present and in a paracrine or autocrine manner and with different effects.

The brain is the most complex organ in the body with trillions of specific synapses whose formation depends on the correct direction of axons and dendrites during development of the nervous system and it is for this function that neurons themselves express guidance substances and specific receptors.

At the bone level there is contact between the membranes of axons and osteoblastic cells and communication via released substances such as e.g. Glutamate and Substance P but also other substances as Sema, Netrin and Slit have a role as substances that in addition to being guides for axons movement, are also members of a neuroskeletal network. They play an essential role in neural development and are expressed in bone microenvironment to mediate interaction between osteoblasts and osteoclasts [21,22].

Sema3A can inhibit the differentiation of osteoclasts by suppressing RhoA (Ras homolog family member A) and enhance differentiation of osteoblasts mediated by β-catenin. Sema4D, expressed by osteoclasts, has been shown to inhibit osteoblast-mediated bone formation after interaction with the OB receptor Plexin B1 [23].

The Slit family controls axonal rejection and axonal guidance by binding to Robo (Roundabout) receptors, which are essential in nerve development. Slit family is also involved in regulation of cancer development, cell migration, cell proliferation, angiogenesis and plays an essential role in bone metabolism [21].

There is much evidence that Slit, as a new bone coupling factor, can regulate bone formation and resorption. For example, Slit3 can promote bone formation and inhibit bone resorption through Robo receptors. Slit2/3 acts on osteoclasts through the Robo1/3 receptor to inhibit the migration and fusion of osteoclasts, resulting in the inhibition of osteoclast formation. Slit3 can also bind to Robo1/2 receptor on osteoblasts and promote the migration and proliferation of osteoblast lines, thus promoting bone formation.

Discussion

The pain caused by excessive bone remodeling can also be due to the increased acidity that occurs under OCs following the secretion of hydrogen ions and local stimulation of receptors sensitive to pH variation (ASIC, TRPV-1), consequently, the decreased osteoclastic remodeling leads to a dual pain-inhibiting effect: a reduced release of Netrin 1 and a lower local acidity.

The demonstration that the increased remodeling has a direct role in pain generation is reported il the work of Zhu., *et al*. 2019 which show that reduction of osteoclast formation by knockout of receptor activator of nuclear factor kappa-B ligand (Rankl) in osteocytes inhibits the growth of sensory nerves into subchondral bone, dorsal root ganglion neuron hyperexcitability and pain hypersensitivity in OA mice. Moreover, the Authors demonstrate a possible role for Netrin-1 secreted by osteoclasts in inducing sensory innervation and OA pain through its receptor DCC (deleted in colorectal cancer). Same work reports that inhibition of osteoclast activity by alendronate modifies aberrant subchondral bone remodeling and reduces innervation and pain behavior at early stage of OA. However, Netrin 1 is not the only factor released during hyperactivity of OCs but NGF is also expressed in similar situations by activated bone cells and also by cartilage cells to stimulate the formation and development of sensitive nerve fibers. In addition, NGF also induces angiogenesis similarly to Netrin 1.

A work of Marsick BM., *et al*. (2010) suggests that NGF and Netrin 1 work together for this purpose [24].

RA and OA, although they recognize different etiologies, they both present alterations in the bone structure and pain.

High levels of Netrin 1 have been detected in synovial fluid of rheumatoid arthritis patients and is potently expressed in osteoblasts and synovial fibroblasts while IL-17 robustly enhances Netrin 1 expression in these cells.

RA is a bone-destructive disease caused by an autoreactive immune system generally characterized by proliferating synovial fibroblasts, severe joint inflammation, and bone erosion, accompanied by hyperactivated bone-degrading osteoclasts [25].

The work of Moriyama., *et al*. reports how Netrin-1 is a protein deriving from osteoblasts and synovial fibroblasts that limits multinucleation of osteoclasts protecting bone from excessive degradation during acute inflammatory processes and non-inflammatory condition, moreover when osteoblasts were stimulated with various cytokines, IL-17 was the most potent inducer of Netrin 1 expression among the cytokines typically elevated in RA. Furthermore, IL-17-induced Netrin 1 expression in osteoblasts was NF-κB-dependent. In RA synovial fluid, there was correlation between Netrin 1 and IL-17 concentration.

The reported data allow us to understand the importance of Netrin 1 in modulating basic events in the genesis of OA and RA pathologies. In fact, Netrin 1 is expressed in OB and fibroblasts during RA following stimulation of numerous cytokines with the task of limiting local bone remodeling activated by the inflammatory process and, in general, by activation of immune system. At same time, Netrin 1 is also expressed by activated local OCs inducing growth of nerve fibers in subchondral bone and cartilage, thus leading to excitation of dorsal root ganglion and hypersensitization [26].

Conclusion

Further studies will still need to be done to explain how two different cell types, OBs of mesenchymal origin and OCs deriving from myeloid cells, are able to express same factors with apparently different effects. A hypothetical explanation could consider the two induced effects, neuronal one and inhibition of multinucleation of OCs, as aiming at the same end. On the one hand, to reduce the presence of active OCs and therefore reduce bone erosion but in case of an erosive pathology already present, to stimulate the pain symptom necessary to trigger signals that can lead to an attenuation of serious symptoms of the ongoing pathology.

No less important will it be to know the relationships, that certainly exist, between the factors reported above as well as the relationships with the numerous substances that intervene in maintenance of tissue homeostasis as well as during various pathological events.

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