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Osteocytes: An Essential Knowledge

Rosini Sergio[1], Rosini Stefano[2], Saviola Giannantonio[3] and Molfetta Luigi[4]*

[1]*Biomaterial Reserach Center - Livorno, Italy*

[2]*Smile-Restyle. - Livorno, Italy*

[3]*Istituti Clinici Scientifici Maugeri, IRCCS Castel Goffredo, Castel Goffredo, Italy*

[4]*University of Genoa, DISC Department, School of Medical and Pharmaceutical Sciences, Research Center of Osteoporosis and Osteoarticular Pathologies, Italy*

***Corresponding Author:** Molfetta Luigi, University of Genoa, DISC Department, School of Medical and Pharmaceutical Sciences, Italy.

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Abstract

Osteocytes regulate homeostasis and bone turnover, exercise control over calcium and phosphate homeostasis, influence osteoblasts and osteoclasts activity, as well as acting as mechano-sensors in regulating bone responses. One of the substances expressed by Osteocytes is Sclerostin which acts by reducing bone formation through the inhibition of osteoblasts activity. Osteocytes play a role in the repair of bone microdamages by a remodeling of the damaged structure. This last effect is due to the ability to send signals that activate the expression of the nuclear factor RANKL and M-CSF which operate the recruitment and activation of OCs. Abnormalities in osteocytic morphology have been found in osteoporosis, osteoarthritic and osteomalacia bone. Alterations of the canalicular system affect the OCi signals and cause damage to the mechanical properties of the bone. The in-depth knowledge on the role of osteocytes has made it possible to tackle many skeletal pathologies with drugs aimed at influencing their activity and to envisage the possible development of osteometabolic new drugs.

Keywords: *Bone; Osteocytes; Metabolism; Sclerostin*

Introduction

Before the studies on osteocytes (OCi) made us aware of the basic importance of these cells in the maintenance of bone homeostasis, the researchers' attention had focused mainly on osteoblasts (OBs) and osteoclasts (OCs) [1]. The lack of attention to OCi, despite the fact that they represent over 95% of all cells present both on the surface and within the bone matrix, is probably due to the greater difficulty to investigate on them linked to the fact that these cells are embedded more or less deeply into the bone; their peculiar location and inaccessibility in mineralized matrix have hampered the cellular and molecular analysis of osteocytes, and there have been few osteocyte-specific markers. Only the knowledge and availability of advanced technologies made it possible to shed more light on the physiology of OCi and on the control exercised over OBs and OCs and consequently on bone remodeling, as well as the control exercised over calcium and phosphate homeostasis while acting as mechanosensory cells that coordinate the skeleton's response to loading or unloading, and as endocrine cells targeting other tissues such as kidney [2].

Osteocytes: Morpho-functional characteristics

Osteocytes represent the terminally differentiated state of the osteoblast lineage and are embedded within the mineralized bone matrix produced by the same OBs; OCi appear as stellate cells enclosed in bone gaps (lacunae) from which numerous canaliculi depart in the mineralized matrix inside which the dendritic extensions (50 - 60 per cell) of the osteocyte cells reside in order to form a network that connects the OCi to the periosteal and endocortical surfaces of the compact bone, the Haversian channels, to the blood vessels and to the surface of the spongy bone to form what is considered a syncytium [3,4]. The OCi are the longest-lived cells of our body with an average life of over 20 years, with their dendritic processes they are exposed to the fluid present in the lacunae and canaliculi whose flow depends on the extravascular pressure and on the mechanical load over the bone. The shear stress of the canalicular flow has numerous effects on OCi such as the release of intracellular calcium, the release of NO, ATP, PGE2, BMPs and other substances as well as regulating the prolongation of dendrites. In addition to influencing bone metabolism, modulating responses to structural stress, OCi also express numerous substances capable of acting on bone cells and influencing the turnover by coordinating the function of OCs and OBs. Although OCi are recognized as mechanosensory cells, the evidence of this function has only been indirect and demonstrated by the fact that mice suspended by the tail, and therefore in the absence of load, do not present any increase in bone resorption if the OCi have been ablated [5,6].

One of the substances expressed by the OCi, through the SOST gene is Sclerostine which acts by inhibiting the osteoblastic activity and bone formation. Another substance expressed by OCi is the inhibitory factor of the Wnt signal, or DKK1 that acts together with Sclerostine on OBs by inhibiting their differentiation and the activity.

To confirm this mechanism there are pharmacological experiences that show how the breakdown of the DKK1 and SOST genes leads to an increase in bone mass as occurs in Van Buchem's disease and in Sclerosteosis [7]. Similarly, the pharmacological inhibition of Sclerostine, by means of monoclonal antibodies, leads to a marked anabolic effect similar to that obtained following intermittent treatment with PTH which is able to negatively regulate the expression of Sclerostine in OCi. The activity of Sclerostine is also inhibited by Estrogen by a mechanism not yet well defined [8]. As sensors of bone stress and microfractures, OCi also play a role in the repair of bone microdamages by operating a process of remodeling of the damaged structure starting with the apoptosis of damaged OCi. Osteocytes can enlarge their lacunar volume by removing the bone from their perilacunar bone matrix in a process called osteocytic osteolysis or perilacunar remodeling. The mechanism involves the production by the OCi of the RANKL and M-CSF factors that operate the recruitment of OCs and their activation that seems to be due to a direct contact between the OCs and the RANKL bound to the membrane of the OCi dendrites. The same are also able to produce osteoprotegerin (OPG). Conversely, they can also promote perilacunar bone formation, thereby reducing the lacunar volume [9] (Figure 1).

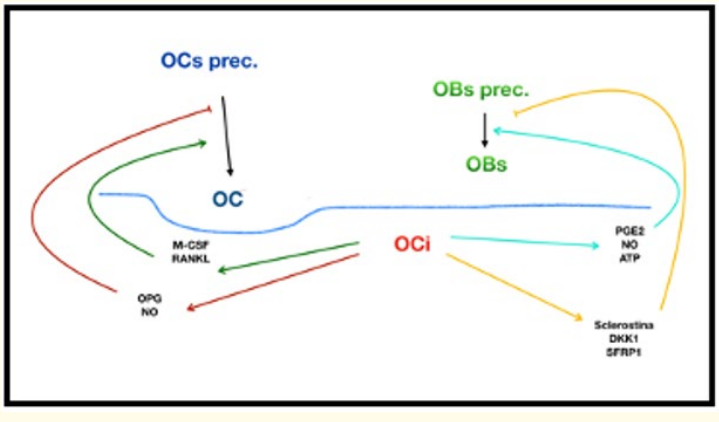

Figure 1: *Osteocytes can enlarge their lacunar volume by removing the bone from their perilacunar bone matrix in a process called osteocytic osteolysis or perilacunar remodeling; The OCi through their cellular processes inside the canaliculi also communicate with the OBs and with the lining cells at the surface of the bone.*

It should be remembered that the differentiation of OCs from hematopoietic progenitors also requires the coordinated action of other nuclear transcription factors such as c-Fos, nuclear factor of activated T cells NF-ATc1 and NF-kB [10]. In addition to regulating bone turnover, OCi also play a role in the mineralization process through the production of certain proteins that are expressed during the initial stages of bone matrix and dentin formation and mineralization. Osteocytes operate perilacunar resorption with the mechanisms used by the OCs themselves, that is, releasing TRAP and cathepsin K, carbonic anhydrase and other factors typical of osteoclastic resorption [11]. The OCi through their cellular processes inside the canaliculi also communicate with the OBs and with the lining cells at the surface of the bone [12] indirectly regulating, through these, also the differentiation of the OCs through the modulation of the expression of RANKL by the OBs themselves. Lining cells cover about 90% of the bone surface and are in contact with OCi and OBs [13].

Histopathology of osteocytes

An altered condition of the osteocyte network may therefore be responsible for increased expression of RANKL, M-CSF and bone resorption. Not well defined is the mechanism that regulates the transmission of signals between OCi and OBs [14]. The experimental data obtained in animals confirm how OCi can transduce different signals, i.e. pro-osteoclastogenic as a response to the absence of load or anti-osteoclastogenic in the presence of normal bone load. Several bone pathologies are linked to an increased death of OCi whose consequence is found in the increased degree of bone fractures and, in particular, in the pathogenesis of osteonecrosis, a disease characterized by the death of bone tissue with no regeneration and responsible for a collapse of the necrotic bone [15].

About 60 years have passed since the first reports of glucocorticoid-induced osteonecrosis [16] but only several years later it was possible to begin to understand the pathogenetic causes related to glucocorticoids (GCs). The skeleton damages related to GCs treatment are due to a direct effect on OBs, OCi and OCs through the increased apoptosis of OBs, and OCi and a lengthening of the OCs lifespan [17]. These alterations are associated with an increase in the degree of apoptosis of OCi and a decreased presence of VEGF and bone vascularization; the decrease in vascular and hydraulic support could be the prelude to a decreased strength of the bone and to an osteonecrosis. In fact, the primary histological lesion is the osteocyte apoptosis that tends to persist over time because, due to their localization, they are not subject to phagocytosis and the decreased degree of turnover, due to excess GCs, delays their replacement. It has been observed that GCs in low doses induce OCi autophagy while high doses lead to apoptosis. Abnormalities in osteocyte morphology have been found in several musculoskeletal diseases such as, for example, in osteoporosis where there is an altered orientation of the lacuno-canalicular system and decreased connectivity between neighboring OCi. Even in the osteoarthritic bone there is a decreased connectivity of the OCi without changing the orientation, differently in the osteomalacia bone there is normal connectivity but with dendritic disorientation. These alterations of the canalicular system affect the signals of the OCi and lead to damage to the mechanical properties of the bone. The resulting bone fragility is considered to be linked to the loss of the ability to register microdamages and to repair them.

Particular interest relates to some considerations reported by some authors [18,19] based on previous studies that report how the incidence of fractures in subjects treated with GCs was higher than those found in post-menopause despite the fact that in the former a higher BMD was maintained. Otherwise, it is surprising to observe how the antifracture activity of some drugs such as bisphosphonates (BPs) or estrogens, is more important than we could expect from the increase in BMD achieved. Among the various hypotheses to justify these evidences, one that has assumed a certain predictive value is linked to the apoptosis of the OCi due to GCs and to the inhibition of the same apoptosis exerted by the drugs mentioned above [20]. The studies of Plotkin., *et al*. have shown how Alendronate, and BPs in general, inhibit the apoptosis of OCI and OBs through various mechanisms including the activation of ERKs factors similar to that induced by PTH. Continuous treatment with PTH and similarly also daily intermittent treatment, induce a suppression of the SOST gene in OCi and the inhibition of Sclerostin expression [21]; a work by Yajima., *et al*. [22] reports that after parathyroidectomy in subjects with secondary hyperparathyroidism the death of OCI intensifies demonstrating the protective role of PTH on OCi. However, the similar protection found

for Calcitonin seems to be linked to a different mechanism mediated by an increase in cAMP production. Vitamin D also influences the number of OCi and the perilacunar space in both animals and humans; however, the mechanism of this action is not well defined [23]. In a recent work [24] the active Vit D metabolite (1,25(OH)2D) is considered able to increase the number of OCi in the first phase of maturation and to increase their turnover in addition to the production of FGF23 [25]. The production of FGF23 by osteocytes, in addition to the active metabolite of Vit D, is also positively regulated by the serum concentration of phosphate. The FGF23 has an important role in regulating the homeostasis of phosphates by inhibiting renal resorption in synergy with PTH.

A further influence on the physiology of OCi is also exerted by estrogens that maintain bone mass by decreasing the lifespan of OCs and increasing that of OBs and OCi [26]. Further light on the effects of estrogen on OCi is given by a further recent work [27] in which it is shown how estrogen induces in OCi the expression of Semaphorin 3A (Sema3A) which acts in an autocrine manner on its neuropilin-1 receptor (Nrp1), promoting survival and maintaining bone homeostasis. It is also shown that serum Sema3A levels tend to decrease with age by providing a mechanistic explanation of the action of estrogen and impaired bone production after menopause. It may be interesting to observe that TNF-α and IL-1 tend to generate estrogen deficiency and induce OCi apoptosis [28]. OCi have developed various mechanisms to defend themselves against potentially harmful events such as hypoxia, immobilization or other pathological conditions and can activate an autophagy process in addition to also undergoing necrosis and apoptosis. Autophagy is a process of controlled self-digestion and recycling of some non-essential cellular components generated as a result of harmful stresses in order to use cellular energies for survival until the stress immediately ceases; if the stress or suffered damage persists, apoptosis intervenes. Although OCi are very long-lived cells, they also do not escape the inevitable process of senescence that involves a reduction in the number and shape of OCi and a change in the same cell biology that tends to vary towards a secretory phenotype SASP (senescence-associated secretory phenotype) with the production of cytokine, chemokine, MMPs as well as various factors that induce senescence also in neighboring cells and with the consequence of an alteration of the remodeling and bone damages [29].

It has been estimated that about 2.5% of OCi die every year from which a life of about 1 to 50 years can be calculated [30]; what is less known is the reason that involves cellular apoptosis. It is established that aging accelerates the death of OCi as a result of increased oxidative stress or deficiency of sex hormones and the excess of GCs that seems to occur with aging. The lack of an adequate mechanical stimulus as well as an excess load, also increase the apoptosis of the OCi. In addition to undergoing apoptosis phenomena, OCi can also undergo necrosis or even present autophagy, all conditions that send particular signals to other cells, as well as leading the bone structure towards a condition of micropetrosis with filling of the lacunar space after the loss of the cell. Similar events can also be a consequence of microfractures, in this case the damaged osteocytes and the neighboring ones release factors that recruit OCs at the site to be remodeled in addition to directly starting a repair process. A study in rats showed a spatio-temporal relationship between the induction of a bone microdamage, OCi apoptosis and the release of pro-osteoclastogenic signals. The results show that apoptosis is essential for the initiation of local bone remodeling but that the signals, RANKL - MCSF - VEGF in addition to OPG, are processed by neighboring non-apoptotic cells and in staggered times [31]. The study provide also evidence that RANKL expression by OCi is low in adult cortical bone OCi but is inducible by injury. The finding that apoptotic cells are essential to the initiation of bone remodeling but do not themselves carry out RANKL-based pro-osteoclastogenic signaling indicates a division of labor among osteocytes in response to damage.

Conclusion

The most recent knowledge acquired on the biology of OCI, have allowed the knowledge of many mechanisms that regulate homeostasis and turnover of bone tissue, as well as the molecular events by which OCi influence the function of OBs and OCs; knowledge that made it possible to address many skeletal diseases with drugs aimed at influencing the activity of OCi and to envisage the possible development of other drugs for an increasingly targeted intervention on bone diseases.

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