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Original Article

Hylonome

The osteocyte as a wiring transmission system

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Abstract

The mechanism of transduction of mechanical strains into biological signals remains one of the more baffling problems of skeletal homeostasis. The updated literature ascribes to osteocytes the function of sensing the strains induced into the bone matrix by mechanical stresses. Whether the osteocytes perform such function by themselves or they are helped by other cells is also unknown. Indeed TEM investigations carried out in our laboratory pointed out the existence of a functional syncytium among all the cells of the osteogenic lineage (COL: stromal cells, osteoblasts or bone lining cells, osteocytes). On the basis of this finding, we suggested that COL may reciprocally modulate their function not only by *volume transmission* (paracrine and autocrine stimulation) but also by *wiring transmission*, namely in a neuronal like manner. Thanks to their location, osteocytes should theoretically be the first cells of COL functional syncytium to sense mechanical strains, whereas stromal cells should be the first to be activated by hormonal molecules diffusing across the endothelial lining. Since PTH and Estrogen receptors have also been localized on osteocytes, and considering that such hormones have been suggested to modulate the sensitivity to strain of the bone mechanosensor, we suggested that the osteocyte syncytium may constitute the microscopic bone structure that sense both mechanical strain and biochemical factors and, at any moment, after having combined the two types of stimuli, issues the appropriate signals to the other bone cells by *volume* and/or *wiring-transmission*. Stromal cells, on the other hand, besides transmitting signals from vascular endothelium to bone cells, may control the differentiation and then direct the course of the osteoblasts around the vascular framework.

Keywords: Osteocytes, Bone Lining Cells, Stromal Cells

Introduction

It is a well established fact that, under the control of mechanical agents (body weight, force of gravity, muscular strength) and non-mechanical agents (hormones, vitamins, cytokines, growth factors), bone cells regulate bone homeostasis and take part in the maintenance of mineral homeostasis, by means of three processes: bone growth, bone modeling and bone remodeling. Bone growth and bone modeling are only devoted to the regulation of bone homeostasis, whereas bone remodeling takes part in the regulation of bone homeostasis as well as of mineral homeostasis, by respectively improving bone structure in response to mechanical demands and setting free calcium and phosphate ions during the reabsorbing phase.

In recent years Frost's mechanostat theory[1] and Utah paradigm[2] have greatly rationalized bone modeling and remodeling processes and what they involve at a bone macroscopic level. However what happens at the cellular level still remains to be defined. We do not know, for instance: a) how mechanical agents and non-mechanical agents interact at the cellular level; b) which is the mechanism of transduction of mechanical strains into biological signals; updated literature ascribes to osteocytes the function of sensing the strains induced into the bone matrix by mechanical stresses but, as we will discuss below, all cells of the osteogenic system are likely to be affected by mechanical strains; c) how osteocytes transmit mechanical stimuli to, and interact with, the other bone cells.

In the attempt to answer these questions we will first summarize the results of the morphofunctional investigations we carried out on the cell of the osteogenic lineage during the last three decades. Then we will discuss some functional implications.

The cells of the osteogenic lineage: morphological aspects

In the early 1970s, we showed that the exponential decrement of the appositional growth rate, which has been shown to occur during osteon formation by means of triple fluorochrome technique[3,4] depends on the diminution in size

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of the osteoblasts and their progressive flattening. At the beginning of osteon formation, when the appositional rate is high, the osteoblasts are big and prismatic, whereas towards the end of osteon formation, when the rate is low, they are smaller and flat[5].

Since these facts were also observed in trabecular bone, our conclusion was that the rate at which the bone tissue is laid down depends on the ratio between the volume of the osteoblasts and their secretory territory: the greater the osteoblast volume and the smaller its secretory territory, the higher the rate of bone apposition[5].

Additionally, we showed that during the edification of osteons, also the osteocytes decrease in size, in parallel to the decrement of osteoblast dimension and the appositional growth rate. This finding implies that the size of the osteocytes strictly depends on the size of the osteoblasts from which they differentiate: the bigger the osteoblasts the larger the size of the osteocytes[5]. The functional meaning of this fact is yet to be established. However, we recently found in human osteons, that the decrement in size of osteocytes from the cement line towards the Haversian canal is paralleled by a thinning of osteocytic-loose (collagen poor) lamellae and, consequently, by a diminution of the distance between non-osteocytic-dense (collagen rich) lamellae, whose thickness does not significantly change throughout the osteonic wall. Mechanically speaking, this fact involves an increase in collagen fibers, namely in bone strength, along the bone surfaces where stresses and strains reach the highest values[6].

In more recent years, we showed by transmission and scanning electron microscopy that the arborization of osteocytes is asymmetrical regarding both number and length of cytoplasmic processes. Vascular dendrites (those radiating toward the bone vascular surface) are more numerous[7] and incomparably longer than mineral dendrites (those radiating towards the opposite surface)[8-10]. Therefore osteocytes appear to be polarized cells, towards the bone surface where they come into contact with osteoblasts or bone lining cells.

Additionally we found that the number of osteocyte vascular dendrites coming into contact with each osteoblast is inversely proportional to the osteoblast size, namely to its bone forming activity. This fact suggests a possible inhibitory effect of osteocytes on osteoblasts[11].

In subsequent series of transmission electron microscope investigations we found that also bone associated stromal cells are dendritic elements. They form a continuous cytoplasmic network which extends from endothelial cells to bone lining cells or osteoblasts[12]. Since gap junctions were observed throughout all cells of the osteogenic system, including stromal cells, it seems likely that not only osteocytes but all cells of the osteogenic lineage are functionally connected in a syncytium.

On the basis of these findings, we postulated that the transmission of signals throughout the cells of the osteogenic system may occur by means of two mechanisms: volume transmission (VT) and wiring transmission (WT). VT corresponds to the well-known routes followed by hormones, cytokines and growth factors to reach the bone cells. The novelty of our hypothesis lies in the suggestion that the cells of the osteogenic lineage may communicate reciprocally and modulate their activity by WT, namely in a neuron-like manner[13-15]. Indeed some similarities do exist between osteocytes and neurons. Mineral cytoplasmic processes of osteocytes resemble neuronal dendrites in that they are shorter, thicker and may contain cell organelles, whereas osteocyte vascular cytoplasmic processes are longer, slender and do not contain organelles, thus resembling neuronal axons. Transmission of signals through osteocytes seems to occur by gap junctions instead of synapses, though it has been recently shown that osteocytes produce typical neurotransmitters like nitric oxide[16] and amino acid glutamate[17]. Additionally, we recently provided evidence that WT occurs along osteocytes in amphibian cortical bone[18].

Discussion and functional implications

It resulted from our morphological investigations that the osteogenic cellular system (stromal cells, osteoblasts or bone lining cells, osteocytes) constitutes a functional syncytium whose variously shaped cells play different roles and have different relationships with the surrounding environment. The cytoplasmic network of stellate stromal cells is bathed in the interstitial fluid, and extends from vascular endothelium to the cells carpeting the bone surface, i.e. osteoblasts or bone lining cells. Osteocytes display an asymmetrical dendrite arborization polarized towards osteoblasts or bone lining cells, and are enclosed inside bone microcavities filled with the bone fluid compartment, having a different composition from the perivascular interstitial fluid where stromal cells are located. Osteoblasts and bone lining cells form cellular laminae in between two networks of dendrites: on their vascular side they are in contact with stromal cell processes, whereas on their bony side they are in contact with osteocyte vascular dendrites. Moreover osteoblasts and bone lining cells separate the bone fluid compartment from the perivascular interstitial fluid.

In our opinion, one of the biggest mistakes made by the majority of researchers, particularly molecular biologists, was to consider the bones only in the active phases of formation or resorption, and thus only osteoblasts and osteoclasts were deeply studied. We should, however, bear in mind that osteoblasts and osteoclasts are transient cells; they constitute the “arms of a worker”. If we wish to detect where the “operations center” is, in order to understand how the processes of bone formation and bone resorption are first triggered and then modulated, we must focus our investigations on the events occurring in the bone cellular system starting from the resting, steady state.

According to our morphological studies, the resting phase is characterized by osteocytes, bone lining cells, and stromal cells, all connected in a functional syncytium, which extends from the bone to the endothelial lining (Fig. 1). We named this syncytium the Bone Basic Cellular System (BBCS),

because it represents the cellular background capable of triggering and driving both processes of bone formation and bone resorption, under the control of mechanical and non-mechanical agents.

It is likely that mechanical agents are first sensed by osteocytes and, in second instance, probably also by the other cells of the osteogenic lineage, whereas non-mechanical agents first affect stromal cells and then diffuse into the bone fluid volume to reach the bone lining cells and finally the osteocytes via their canalicular system. In our view BBCS represents the "bone operations center". This view is supported by the following facts: a) bone overloading and unloading respectively induce modeling-dependent bone gain and remodeling-dependent bone loss also in the adult skeleton, in which no or few osteoblasts and osteoclasts are present whereas BBCS is surely present, thus suggesting it intervenes in activating both bone formation and bone resorption; b) bone resorption was found to occur in regions less subjected to mechanical loading in biochemical osteoporoses[19,20], whereas in disuse osteoporosis it takes place uniformly throughout the skeletal segments[20,21], thus indicating that osteoclast activity is activated and driven by local signals which can but be issued by BBCS.

As regards osteoclasts, they are free cells that never become part of the osteogenic cell network; on the contrary, it seems likely that they should destroy stromal cells and bone lining cells, before reabsorbing the bone matrix and osteocytes. Therefore, strictly speaking, osteoclasts do not pertain to bone cells. They instead appear to be "workers" specialized in bone destruction and, when their activity is needed, BBCS calls them, probably by secreting osteoclast activating cytokines, and tells them where, when and how long they have to work. Osteoclasts are also under the control of blood derived systemic factors, whereas they should not be capable of sensing mechanical strains being free cells.

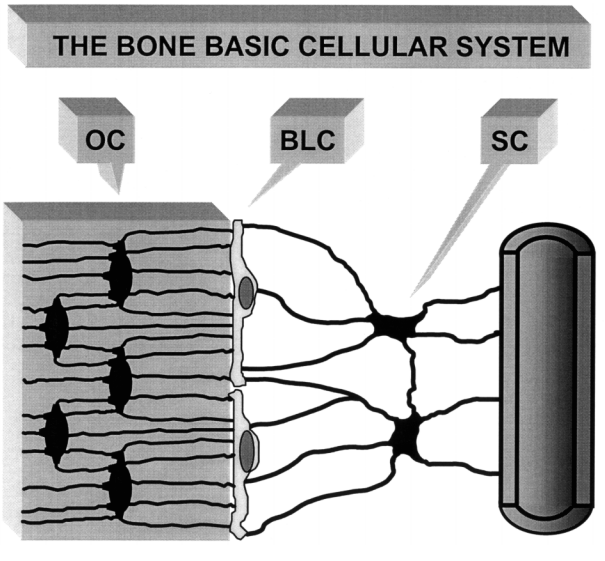

Figure 1. Schematic drawing of the basic cellular system of bone. From left to right: osteocytes (OC), bone lining cells (BLC), stromal cells (SC) and a vascular capillary. See text for explanation.

In conclusion, according to our working hypothesis all processes of bone formation and bone resorption, occurring in response to mechanical agents and non-mechanical agents, are triggered, modulated, and stopped by the BBCS functional syncytium. This appears to be the real "bone operations center" capable of sensing both mechanical strains and biochemical factors and, at any moment, after having combined the two types of stimuli it issues by wiring transmission and/or volume transmission the signals that activate the processes of bone formation or bone resorption. Future investigations are needed to define the mechanisms by which BBCS drives such processes and operates in modeling-dependent bone gain, remodeling-dependent bone loss, and remodeling-dependent bone structure.

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