The Biology of Osteocytes

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Introduction

Osteocytes are terminally differentiated, nonproliferative cells of the osteoblast lineage. They reside in the mineralized bone matrix and in newly formed osteoid. They are essentially locked inside small lacunar spaces in the hard substance of bone and, for obvious reasons, are considered nonmigratory. In all but a few of the more primitive vertebrates, these cells form an extensive, connecting syncytial network via small cytoplasmic processes in canaliculi. It was considered for some time that the bones of certain fish species contained no osteocytes. More recently, however, small osteocyte-like cells, devoid of cytoplasmic processes or obvious canicular connections, have been noted in the teleost fish [1]. In considering the “purpose” of osteocytes, the identification across species of any healthy bone naturally lacking them is important. Further studies are required in order to establish whether truly acellular bone exists. In any case, whatever their purpose, they appear to have been a “solution” for some time: dinosaur bones have been shown to contain osteocyte lacunae and connecting canaliculi [2].

What Do Osteocytes Look Like?

As with other cell types, the size of osteocytes varies between species. The relative size of the lacunae and canaliculi in which the cells reside is important when we consider the possibility that fluid and various molecules or solid structures may migrate through the system extracellularly. In the human, osteocytes are approximately 9 μm by 20 μm [3]. The murine osteocyte lacuna measures about 5 μm by 20 μm [4], with a gap present between the cell and the wall of the lacuna. Likewise, the cytoplasmic processes are approximately half the diameter of the canaliculi, which are 50 to 100 nm in diameter [4], leaving a substantial gap sufficient for two processes to lie side by side at points of gap junctional communication. However, this might not be a simple empty space; it has been noted that a mesh of extracellular material, primarily proteoglycans, is present in at least some volumes of canaliculi. It has been hypothesized that this extracellular meshwork assists in the amplification of mechanotransduction signals derived from fluid flowing in the system [5].

In lamellar bone, the distribution of osteocytes and their cytoplasmic processes within the network is not entirely random and is set at the time of new bone formation. The cells appear to display a polarity in terms of the distribution of their cell processes, such that the cell membrane facing the bone surface has the highest number (ie, the vascular dendrites) [6]. It has been suggested that the decision to incorporate an osteoblast into bone matrix may be dictated by the maximum functional length of these cell processes [6].

It is often stated that osteocytes are by far the most common cell type in bone (31,900 mm⁻³ in bovine bone to 93,200 mm⁻³ in rat bone) [3]. Why we need so many of this cell type is an interesting question. Are they simply acting to open up bone to fluid for mineral exchange, a “keeper of the canals”? Do they act as a high-number, low-activity component of an information transfer system, similar to nerve cells in the brain? Do they need to have such an extensive distribution in bone because they are important sensors with a small sensing range, necessary to identify nanoscale features such as fatigue cracks?

Historically, studies concerning numbers of cellular organelles have concluded that the osteocyte is relatively inert metabolically. However, osteocytes are capable of

Osteocytes, the most abundant cell type in bone, remain the least characterized. Several theories have been proposed regarding their function, including osteolysis, sensing the strains produced in response to mechanical loading of bones, and producing signals that affect the function of osteoblasts and osteoclasts and hence, bone turnover. This review also discusses the role of osteocyte apoptosis in targeted bone remodeling and proposes that the occurrence of osteocyte apoptosis is consistent with the description of apoptosis as an essential homeostatic mechanism for the healthy maintenance of tissues.
physiologically significant molecular synthesis and modification. The use of a communication system comprising large numbers of low-activity cells in a syncytial network is reminiscent of the nervous system. This system has been claimed to represent the most efficient design for the transmission of metabolically expensive signals over long distances [7].

In examining bones across the vertebrate species, a study by Mullender et al. [8] found that in trabecular bone the areal density of these cells broadly scales inversely with animal size. Other studies have found it less obvious that such a relationship exists. Indeed, a similar study that also included amphibians did not find such a relationship [9]. Nevertheless, even within this broad, negative allometric relationship, there exist a number of specialized bone types (often relatively temporary) that show distinct departures from the relationship. For example, woven bone and plexiform bone both tend to contain a higher density of osteocytes and to be manufactured and remodeled more rapidly than lamellar bone. Higher densities of osteocytes have been associated with some disease states in bone, such as osteoporosis [3], osteogenesis imperfecta [10], and 1,25(OH)2D3, loss [11].

Osteocytes are formed during the terminal differentiation of bone-forming osteoblasts. It is believed that during the process of bone formation an osteoblast will be left behind the upwardly advancing, newly formed osteoid material, to be entombed in matrix as an “osteoid osteocyte.” During the process of “burial,” the future osteocyte maintains contact with the advancing osteoblasts at the surface via an extending cellular process. The surrounding osteoid matrix then becomes mineralized under the control of enzymes, including osteocyte-derived casein kinase II [12]. It has been suggested that 10% to 20% of osteoblasts differentiate into osteocytes [13]. The cellular and molecular mechanisms that regulate this process are not fully understood. Recent work has described a role for matrix metalloproteinase-2 (MMP-2) in the regulation of osteocyte production and the generation of an appropriate canalicular system [14•]. The supply of oxygen and nutrients to osteocytes is thought to occur primarily via the extracellular fluid present in the lacunocanalicular system [15]. Of potential importance is the possibility that mechanical stimulation is required in order to distribute extracellular fluid sufficiently to maintain metabolic activity in the osteocyte. Restriction of mechanical stimulation (exercise) in rodents has been shown to increase levels of the hypoxia-induced transcription factor-1α (HIF-1α) protein in osteocytes [16] and to reduce the supply of marker molecules throughout the lacunocanalicular system [17]. Similar studies in humans are difficult to undertake, but the application of mechanical stimulation to human bone biopsy specimens in a specialized bioreactor has been shown to improve osteocyte viability through reduced apoptosis [18••].

Characteristic Molecular Markers and Receptors

Because of their location and the relative lack of phenotype-specific markers, the characteristics of primary osteocytes in culture have been described in very few studies. Avian postmitotic osteocytes have been isolated and purified based on the use of an antibody termed mAb OB7.3 [19], which is thought to be specific for the chicken PHEX endoprotease, a phosphate-regulating gene with homology to endopeptidases on the X chromosome. Studies have localized the osteoblast/osteocyte factor 45 (OF45)/matrix extracellular phosphoglycoprotein (MEPE) mRNA associated with mature osteoblasts and osteocytes throughout ossification in the skeleton [20]. In more recent studies, MEPE has been shown to be mechanically regulated in osteocytes [21]. MEPE belongs to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins and is thought to control mineralization within the canalicular system. Another possible marker of the osteocytic phenotype is the E11 antigen, which is expressed primarily during bone modeling and fracture healing at regions of the cell membrane in contact with the matrix; it has been proposed to function in the formation and maintenance of cellular processes and the adhesion of cells to the bone matrix [22]. Expression of integrins β1, [23], αβ3, and β3 [24] (Fig. 1) has also been suggested to play an important role in the adhesion of osteocytes to the bone matrix and their formation following differentiation of osteoblastic cells. A molecule that is also highly expressed in osteocytes is dentin matrix protein-1 (DMP-1). Its deletion in knockout models results in hypomineralization and defective osteocyte maturation [25•].

Most osteocytic characteristics in vitro have been based on the use of the murine osteocyte-like cell line MLO-Y4 (murine long-bone osteocyte Y4) [26]. MLO-Y4 osteocyte-like cells were isolated from the long bones of transgenic mice in which expression of the SV40 large T-antigen oncogene was targeted to osteocytes under the control of the osteocalcin promoter [26]. MLO-Y4 cells are characterized by cellular proliferation, long dendritic processes, osteopontin and connexin 43 expression, low collagen type I, and low alkaline phosphatase expression [26].

Osteocyte Responses to Hormonal and Neural Factors

Until recently, few studies have described osteocyte responses to changes in the level of hormones, cytokines, or growth factors. If the receptors known to be expressed by osteocytes are listed, it is apparent that the osteocyte expresses receptor protein for most of the hormones and cytokines known to be important in bone function, including estrogen receptor (ER) α and β [27], parathyroid hormone (PTH) [28], vitamin D3 [29], corticosteroids [30], and transforming growth factor (TGF)-β [31] (Fig. 1). Notable exceptions include the insulin-like growth factor receptors, insulin receptors, and growth hormone recep-