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Review

A review of the differing roles of dead and live osteocytes

Toshihisa Komori *

Department of Cell Biology, Unit of Basic Medical Sciences, Nagasaki University, Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan

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ABSTRACT

Background: Osteocytes form a network through gap junction-coupled cell processes and canaliculi throughout bone; this network extends to the osteoblasts in the bone surface. The osteocyte network is considered to function in mechanosensing and mechanotransduction. However, the lack of suitable animal models makes it difficult to clarify the function of osteocytes.
Highlight: Any kind of osteocyte death results in necrosis, whereby the intracellular contents, including immunostimulatory molecules, which activate osteoclastogenesis, are released through the canaliculi to the bone surface. This leads to enhanced bone resorption in the damaged region of the bone. Overexpression of *Bcl2* in osteoblasts reduces the number of osteoblast processes, resulting in a reduction in the numbers of osteocyte processes and canaliculi. The osteocytes gradually die without enhancement of bone resorption because a severe reduction in the number of canaliculi interrupts the release of intracellular contents to the bone surface. *Bcl2* transgenic mice at 4 months of age, in which the osteocyte network is disrupted, are an appropriate mouse model for the evaluation of osteocyte function. These mice show that the osteocyte network enhances bone resorption and inhibits bone formation under physiological conditions, and that these osteocyte functions are augmented under unloaded conditions. Under such conditions, *Rankl* upregulation in osteoblasts and Sost upregulation in osteocytes are, at least in part, responsible for enhanced bone resorption and suppressed bone formation, respectively.
Conclusion: Dead osteocytes induce bone resorption, while live osteocytes enhance bone resorption and inhibit bone formation under physiological conditions, while their functions are augmented under unloaded conditions.

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1. Introduction

Osteocytes form a network in bone that is composed of two communication systems. One is an intracellular communication system that functions via gap junction-coupled cell processes, and the other is an extracellular communication system that functions

* Tel.: +81 95 819 7630; fax: +81 95 819 7633.
E-mail address: komorit@nagasaki-u.ac.jp

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through the canaliculi. The two communication systems extend to osteoblasts on the bone surface [1]. Both are required for the survival of osteocytes because osteocytes acquire nutrients, oxygen, and survival signals through them [2]. Furthermore, osteocytes release secreted factors through the extracellular communication system to the bone surface and vascular canals in the bone. The osteocyte network is thought to be an ideal mechanosensory system involved in mechanotransduction, by which mechanical energy is converted into electrical and/or biochemical signals [3–8]. However, the lack of suitable animal models makes proving this difficult.

2. Induction of bone resorption by osteocyte death

Osteocyte death occurs during aging, after menopause, under unloading, and in pathological conditions such as microcracks; the death of osteocytes is closely coupled to bone resorption [9–11]. Furthermore, bone resorption is severely enhanced by inducing osteocyte death [12]. Therefore, it is generally accepted that osteocytes inhibit bone resorption. However, it has to be considered that apoptotic osteocytes cannot be eliminated by macrophages because osteocytes are embedded in bone, and the canaliculi are too thin for macrophages to pass through. The membranes of apoptotic osteocytes are finally ruptured, resulting in secondary necrosis. Therefore, any type of osteocyte death ends in necrosis. After rupture of the cytoplasmic membrane, most of the intracellular contents are released through the canaliculi to the bone surface and the vascular canals in the bone. These contents include immunostimulatory molecules, including the so-called damage-associated molecular pattern (DAMP) molecules, such as the S100 family molecules, high-mobility group box 1 (HMGB1) protein, purine metabolites, heat-shock proteins, and uric acid [13,14]. The release of such immunostimulatory molecules actively recruits and activates macrophages, and promotes the production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1. These induce receptor activator of nuclear factor κ-B ligand (Rankl) expression in macrophages, leading to the acceleration of osteoclastogenesis [13,15]. Therefore, osteocyte death plays an important role in the replacement of damaged bone (Fig. 1A). However, this does not mean that live osteocytes inhibit bone resorption.

3. Animal models for the evaluation of live osteocyte function

The easiest way to analyze the functions of osteocytes in vivo is to ablate them. However, osteocyte death results in necrosis, which induces bone resorption. A typical example is osteocyte ablation by the diphtheria toxin. Transgenic mice expressing the diphtheria toxin receptor under the control of the *Dmp1* promoter, which directs the transgene expression to osteoblasts that are going to be embedded into the bone matrix and osteocytes, show enhanced bone resorption resulting in severe osteoporosis after injection of diphtheria toxin [12]. Therefore, in this mouse model the effects of osteocyte death but not the functions of live osteocytes are observed.

Gap junctions are composed of connexin 43, which is encoded by *Gja1*. In *Gja1* conditional knockout mice produced using *Dmp1* Cre transgenic mice, the intracellular communication system is disrupted but the extracellular communication system through the canaliculi remains intact. As both communication systems are necessary for osteocyte survival, osteocyte apoptosis occurs in these mice [16]. Indeed, this osteocyte apoptosis results in necrosis, and immunostimulatory molecules are released through the intact canaliculi to the bone surface. In fact, osteocyte apoptosis is increased, osteoclast number and surface area are increased at the endocortical surface, and the marrow cavity is enlarged in this mouse model [16]. Therefore, the effects of osteocyte death also mask the functions of live osteocytes in this mouse model.

Overexpression of *Bcl2* in osteoblasts reduces the number of osteoblast processes, and finally results in reduced numbers of osteocyte processes and canaliculi [2]. Bcl2 is able to form a complex with actin and gelsolin, which functions to decrease gelsolin-severing activity to increase actin polymerization, and to suppress cell adhesion, spreading, and motility [17]. Therefore, overexpression of *Bcl2* is likely to reduce the number of osteoblast processes by altering cytoskeletal organization. When osteoblasts with a reduced number of processes become osteocytes, the canaliculi are poorly formed. This is because the canaliculi are the pathways for osteocyte processes. As both intracellular and extracellular communication systems are necessary for osteocyte survival, the osteocytes gradually die by apoptosis due to the insufficient supply of nutrients, oxygen, and survival factors [2]. Indeed, the cytoplasmic membranes of apoptotic osteocytes are ruptured and secondary necrosis occurs. However, the severe reduction of canaliculi interrupts the release of immunostimulatory molecules to the bone surface and the vascular canals in the bone. Therefore, the effects of osteocyte death are negligible in this mouse model, although the effects of overexpression of *Bcl2* in osteoblasts have to be considered [2,18].

4. Phenotypes of *Bcl2* transgenic mice

In *Bcl2* transgenic mice under the control of the 2.3-kb *Col1a1* promoter, the levels of transgene expression are high during the growth period (2–6 weeks of age), reduced in adults (10 weeks of age), and low after 4 months of age [18]. The phenotypes differ depending on the expression levels of the transgene. The osteoblast number and bone volume are increased at 6 weeks of age. The osteoblast number remains high at 10 weeks of age but bone volume and bone formation rate are similar to those in wildtype mice. This indicates that the function of individual osteoblasts is impaired in *Bcl2* transgenic mice. This is also evident in the *Bcl2* transgenic mouse line with high transgene expression because the bone volume and bone formation rate are less than those in wildtype mice at 10 weeks of age [2]. The differentiation of primary osteoblasts from *Bcl2* transgenic mice is also decelerated compared with that in wildtype mice. The role of primary osteoblasts in supporting osteoclastogenesis in a co-culture with bone marrow cells are similar for *Bcl2* transgenic mice and wildtype mice, indicating that overexpression of *Bcl2* in osteoblasts has no effect on osteoclastogenesis [18]. Osteocyte apoptosis gradually increases, TUNEL-positive cells reach 75% at 4 months of age, and both intracellular and extracellular communication systems are completely disrupted. The number of osteocytes is increased at 10 weeks of age but reduced at 4 months of age in *Bcl2* transgenic mice compared with wildtype mice at each respective age. As the transgene expression levels are low after 4 months of age, they are insufficient to reduce the number of osteoblast processes. Therefore, bone remodeling occurs gradually and the normal osteocyte network is observed in nearly half of the cortical bone at 8 months of age [18].

5. The functions of osteocytes under physiological conditions

Bcl2 transgenic mice at 4 months of age are an appropriate mouse model for the evaluation of live osteocyte function [19]. Although 75% of lacunae in the cortical bone are TUNEL-positive in these mice, TRAP-positive osteoclasts are significantly reduced in