

COMMENTARIES

Osteocyte Apoptosis Induces Bone Resorption and Impairs the Skeletal Response to Weightlessness

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Commentary on: Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab.* 2007 Jun;5(6):464-75.

It has been long proposed that the osteocyte network continually compares present mechanical strains to usual levels of strain, and triggers signals to osteoclasts or osteoblasts resulting in bone loss or gain, as needed. Whereas physiological levels of mechanical stimulation maintain bone mass, levels of strain that are too high or too low induce bone resorption. One mechanism by which osteocytes may trigger bone resorption is by undergoing apoptosis. Previous evidence demonstrated that either low or high levels of mechanical loading lead to increased prevalence of osteocyte apoptosis, which temporally precedes and is spatially associated with osteoclast recruitment and the subsequent increase in bone resorption (1;2). However, a direct demonstration of a cause and effect relationship between osteocyte death and bone resorption was lacking. Using a transgenic mouse model of inducible osteocyte ablation, Tatsumi *et al.* (3) show that osteocyte apoptosis is sufficient to trigger osteoclast recruitment and bone resorption. Moreover, the normal osteoclastogenic response to unloading is missing in bones from osteocyte-depleted mice, confirming that osteocytes are indispensable for the skeletal adaptation to weightlessness. Because osteocyte apoptosis is inhibited not only by mechanical stimulation but also by estrogens and bisphosphonates, the findings of Tatsumi *et al.* raise the intriguing possibility that preservation of osteocyte viability contributes to the anti-remodeling properties of these agents.

Regulation of the Executive Cells of Bone Remodeling by Osteocytes – the Sclerostin Paradigm

Osteocytes are ideally positioned to be the means by which bone adapts in response to mechanical stimuli. Compared to osteoblasts and osteoclasts, which are present on bone only transiently, in low number, and in variable locations, osteocytes constitute more than 90 percent of cells in bone and are strategically distributed throughout the entire bone volume. In addition, osteocytes form a syncytium among themselves and with cells on the bone surface via cytoplasmic processes that radiate from their bodies and travel along canaliculi excavated in the mineralized matrix. This network is perfectly suited to sense and respond to both mechanical and systemic stimuli by generating signals that affect osteoblasts, osteoclasts, and their progenitors in the bone marrow.

Despite significant progress in our knowledge about osteocytes in recent years, the mechanisms by which these cells control the function of osteoblasts and osteoclasts are just starting to emerge. Sclerostin is the first, undisputable mediator of the communication between osteocytes and the executive cells of bone remodeling. Osteocytes, but not other cells in bone, express sclerostin – the product of the *SOST* gene that antagonizes the action of Wnts and BMPs (4;5). Evidence from human diseases and experimental animals indicates that sclerostin acts in a paracrine fashion to inhibit bone formation (4;6;7).

Recently, it was shown that sclerostin expression is potently inhibited by two recognized stimuli that increase osteoblast number: parathyroid hormone and mechanical loading (8-10), thereby representing a novel mechanism of regulation of bone formation mediated by osteocytes.

Osteocyte Apoptosis: Regulation and Consequences

That osteocytes perceive changes in the level of both physical stimuli as well as circulating factors is evidenced by studies on the regulation of their lifespan. Osteocytes are long-lived cells. However, like osteoblasts and osteoclasts, they die by apoptosis, and decreased osteocyte viability accompanies the bone fragility syndrome that characterizes glucocorticoid excess and estrogen withdrawal (11-13). Conversely, preservation of osteocyte viability might explain at least part of the anti-fracture effects of bisphosphonates, which cannot be completely accounted for by changes in bone mineral density (14).

Osteocyte apoptosis is also regulated by mechanical forces. Thus, mechanical stimulation of osteocytic cells or authentic osteocytes protects them from the pro-apoptotic action of glucocorticoids, etoposide and other death inducers (15;16). Mechanistic studies indicate that the transduction of mechanical forces into intracellular signals is accomplished by a signalsome assembled at caveolin-rich domains of the plasma membrane and composed of integrins, cytoskeletal proteins and kinases, including the focal adhesion kinase FAK and Src, resulting in activation of the ERK pathway and osteocyte survival (15). *In vivo* mechanical stimulation also regulates osteocyte lifespan. Thus, an increased prevalence of apoptotic osteocytes is found in unloaded bones (1) or in bones exposed to high levels of mechanical strain (2). In both cases, increased apoptosis of osteocytes was observed before any evidence of increased osteoclast resorption. Moreover, apoptotic osteocytes in unloaded bones accumulated in areas that were subsequently removed by osteoclasts (1). Taken together

with the *in vitro* evidence, these findings had suggested that diminished mechanical forces eliminate signals that maintain viability, thereby leading to osteocyte apoptosis, and that dying osteocytes in turn become the beacons for osteoclast recruitment to the vicinity and the resulting increase in bone resorption (Fig. 1).

The report of Tatsumi *et al.* now provides direct evidence that the death of osteocytes is sufficient to recruit osteoclasts and to increase resorption. The authors generated transgenic (TG) mice expressing the diphtheria toxin receptor (DTR) under the control of the dentin matrix protein 1 (DMP1) promoter, which is only active in osteocytes (17). DTR is normally not expressed in murine cells; therefore, osteocytes are the only cells sensitive to the toxin in these TG animals. A single injection of DT resulted in rapid induction of apoptosis of 70-80% of osteocytes, and this was followed by increased osteoclasts and loss of bone. These findings demonstrate that osteocyte apoptosis is sufficient to trigger osteoclast recruitment and bone resorption. Taken together with the evidence that osteocyte apoptosis is inhibited by estrogens and bisphosphonates (12;14), the findings of Tatsumi *et al.* also raise the intriguing possibility that preservation of osteocyte viability contributes to the anti-remodeling properties of these agents. Future research is required to directly test this stimulating hypothesis.

Osteocytes: Primary Culprits for the Bone Loss Induced by Physical Inactivity

Mechanical loading is critical for the maintenance of bone mass, and skeletal unloading, as with reduced physical activity in old age, immobilization by bed rest, or total or partial motor paralysis, causes bone loss leading to disuse osteoporosis (18). Furthermore, the bone loss that ensues under microgravity conditions represents the most significant hindrance for long-term space flight (19). The rapid decrease in osteocyte viability with unloading had suggested that osteocytes are the first responders to the change in mechanical forces (1). Now, Tatsumi *et al.* demonstrate

that mice depleted of osteocytes are protected from the bone loss induced by tail

suspension, indicating that in the absence of osteocytes, bones are unable to elicit the

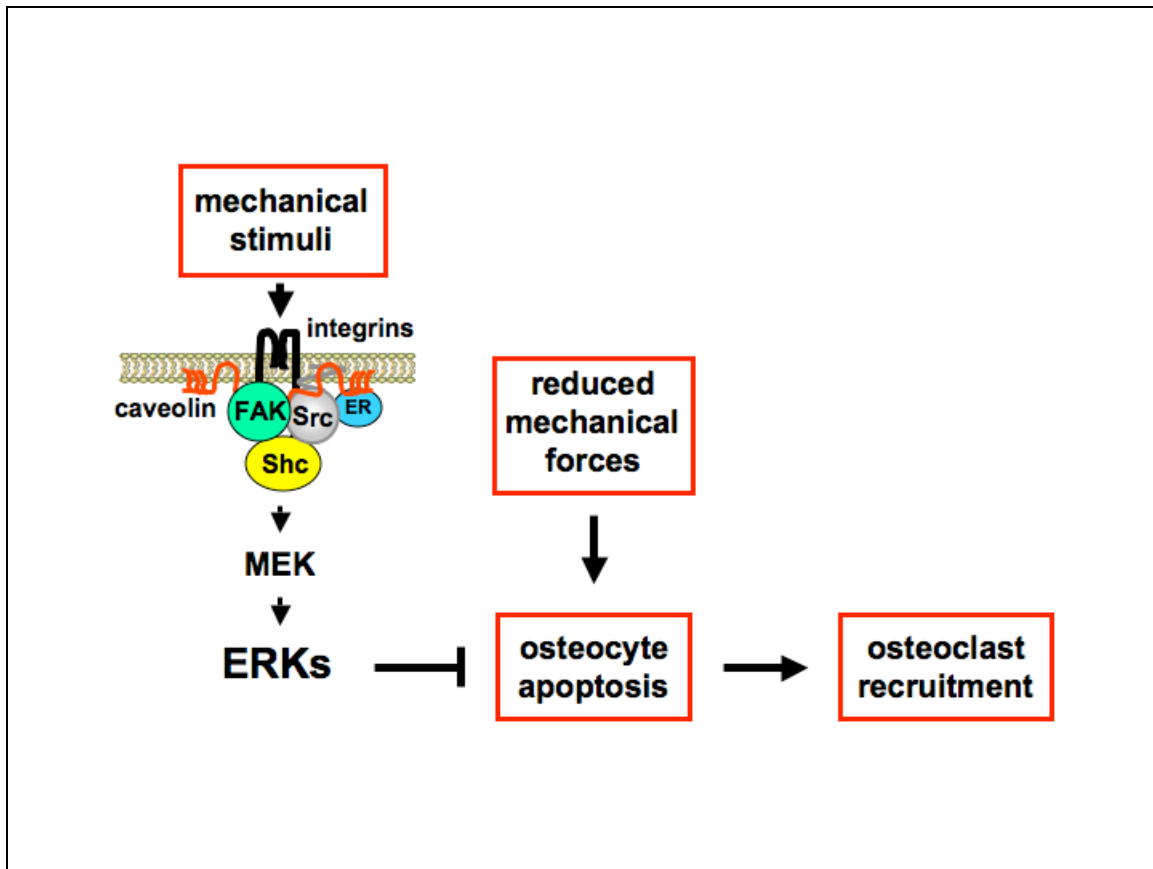


Fig 1. A signalsome comprised of integrins, cytoskeletal proteins and kinases, including the focal adhesion kinase FAK and Src, transduces physiological levels of mechanical stimulation into activation of the ERK pathway, leading to osteocyte survival (15). Reduced mechanical stimulation eliminates kinase-mediated survival signaling, thereby leading to osteocyte apoptosis (1). Apoptotic osteocytes, in turn, become the beacons for osteoclast recruitment and the resulting increase in bone resorption and bone loss (3).

normal osteoclastogenic response. These findings confirm that osteocytes are the primary culprit of the negative bone balance that ensues with weightlessness.

Osteocytes: Not Required for the Anabolic Response of Bone to Mechanical Stimuli?

Surprisingly, osteocyte-depleted mice were as responsive to loading as normal mice in the study by Tatsumi *et al.* Thus, the bone lost by tail suspension was recovered upon re-loading in TG mice that had undergone osteocyte ablation as effectively as in wild type mice. These findings raise the possibility

that the bone anabolic effect of loading is mediated by a mechanism that does not involve osteocytes. However, this provocative hypothesis requires confirmation by additional studies. It seems counterintuitive that osteocytes would be essential for the osteoclastogenic response of bone to unloading but dispensable for the osteoblastogenic response to loading. One possibility is that the osteocyte-depleted TG animals elicited an anabolic response through the remaining osteocytes. Increased osteoblast generation and bone formation induced by loading is defective in animals lacking the Wnt co-receptor LRP5 (20) and,

consistent with this finding, loading dramatically reduces the expression of the Wnt antagonist sclerostin in osteocytes (10). Therefore, one way to assure that the 20-30% of osteocytes that remained upon osteocyte ablation did not mediate the recovery of bone would be to determine whether, indeed, sclerostin expression did not decrease, and Wnt signaling was not triggered, upon re-loading in these mice.

Concluding Remarks and Remaining Questions

In conclusion, the osteocyte ablation model had revealed that osteocyte apoptosis is sufficient to initiate an osteoclastogenic response and that osteocytes are required for the skeletal adaptation to reduced mechanical forces. Whether living osteocytes continually produce molecules that restrain osteoclast recruitment, or whether in the process of undergoing apoptosis osteocytes produce pro-osteoclastogenic signals, remains to be determined. It is expected that intense investigations will take place in the near future attempting to identify the molecular mediators involved in the communication between osteocytes and osteoclasts.

Conflict of Interest: The author reports that no conflict of interest exists.

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