BONE PHYSIOLOGY—UPDATE AND IMPLICATIONS FOR NEW THERAPIES

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Bone remodeling is a continuous process that is essential for the biomechanical integrity, function and response to various stimuli of the skeleton. Bone remodeling occurs unsynchronized in many microscopic sites and is composed of a short phase (2–3 weeks) of bone resorption by specific cells, the osteoclasts, followed by a long phase (3–4 months) of bone formation by the osteoblast. Secondary mineralization that will finalize the cycle, may take another 12–18 months. Imbalance between bone formation and bone resorption will lead to changes in bone quantity and quality, that may be expressed as clinical signs and symptoms. A negative balance in bone remodeling is the pathophysiological mechanism that will lead to post-menopausal, senile and glucocorticoid-induced osteoporosis, to mention some examples. Major advances in the understanding of the origin, differentiation, function and the various control mechanisms of the osteoclast, the osteoblasts and its derivative cells, as the osteocyte, have been achieved during the last few years. A major common, though not exclusive pathway that controls the various stages in osteoclasts' development, function and life span, the receptor activator of Nf kappa B (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG), had been elucidated. The discovery of the Wnt, low density lipoprotein-like protein 5 (LRP5), beta-catenin pathway as a major regulator of osteoblasts' recruitment, differentiation and function as well as its negative and positive regulators and the cross-talk between osteoblasts, osteocytes and osteoclasts, are major breakthroughs in understanding bone physiology and pathophysiology.

There are new therapies already in use, in clinical trials and being developed that affect bone resorption and/or bone formation and are based on this newly-gained knowledge. These drugs may reduce the rate of bone fractures and its devastating clinical effect in osteoporosis, prevent disuse bone loss and enhance bone repair and fracture healing.

A short review of the above-mentioned mechanisms will be discussed.

HIF-1 ALPHA REGULATES ECM SECRETION IN THE HYPOXIC GROWTH PLATE

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Growth plate chondrocytes are professional secretory cells, engaged in the secretion of extracellular matrix (ECM) to form the cartilaginous template of developing bones during endochondral ossification. Unlike most other tissues, the growth plate is avascular and therefore hypoxic. The ability of chondrocytes to survive and secrete vast amounts of ECM under hypoxic conditions raises questions concerning the mechanism underlying this adaptation. Hypoxia inducible factor-1 (HIF-1), a key regulator of cellular hypoxic response, is necessary for chondrocyte survival under hypoxia, limiting the ability to study its effects by a loss-of-function approach. In order to bypass this limitation and investigate the possible role of HIF-1 in ECM secretion by growth plate chondrocytes, we utilized mice with temporally activated cKO of Hif-1**α**, the oxygen sensitive subunit of HIF-1, in these cells (Hif-1**α**f/fCol2ERCre).

We show here for the first time that Hif-1**α** inactivation in the growth plate results in the intracellular accumulation of major ECM components in the hypoxic central region of the growth plate. This was accompanied by ER stress and activation of the unfolded protein response. In addition, the content of the ECM surrounding the cells was reduced and resulted in cell-matrix detachment. Taken together, these results indicate that Hif-1**α** inactivation in the growth plate inhibits proper folding and secretion of cartilage ECM under hypoxia. To further understand the molecular mechanisms by which HIF-1**α** regulates cartilage ECM folding, we examined the involvement of HIF-1**α** in posttranslational modifications of cartilage ECM. We show that upon Hif-1**α** inactivation, there was a reduced expression of collagen prolyl-4 hydroxylase subunits, which are required for collagen hydroxylation. Moreover, the growth plate was significantly more hypoxic and protein glycosylation was impaired.
In this work, we establish a new role for HIF-1**α** in the regulation of cartilage ECM folding and secretion under hypoxia. In addition, we provide evidence for the importance of HIF-1**α** in post-translational modifications and biosynthesis of cartilage ECM.

A SERM-LIKE ACTIVITY OF DIETARY COMPOUNDS IN BONE AND CANCER CELLS

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Various phyto-nutrients, carotenoids, polyphenols and isothiocyanates were found by us to inhibit estrogen signaling, in breast and endometrial cancer cells. In addition, we and others have shown that these phyto-nutrients induce the antioxidant response element (ARE) and the Nrf2 transcription factor. Using overexpression of Nrf2 and siRNA for this gene, we demonstrated that Nrf2 is involved in the phytonutrient-induced inhibition of the estrogenic activity. Although the effect of estrogens in breast and endometrial cancer is harmful, it is beneficial for bone formation. Thus, we investigated the effect of the phyto-nutrients on estrogenic activity in osteoblasts. We found that the dietary compounds, which inhibit estrogenic activity in cancer cells, did not inhibit and even stimulated the expression of estrogen-induced genes in osteoblast-like cells. The effect of glucocorticoids in bone is opposite to that of estrogens and glucocorticoid treatment leads to bone resorption and osteoporosis. Thus, we determined whether phytonutrients inhibits glucocorticoid activity in bone. We found that the expression of glucocorticoid-dependent bone-destroying gene (RANKL) and the glucocorticoid inhibition of bone-supporting genes (osteocalcin, osteoprotegerin) were both reversed by the phytonutrients. The phytonutrients increased estrogen receptor-α level in bone cells nuclei but reduced its level in nuclei of breast cancer cells and did not affect the level of glucocorticoid receptors. As discussed above, Nrf2 was found to be involved in the inhibition of estrogenic activity in breast cancer cells. In contrast, in bone cells, over-expression of Nrf2 enhanced estrogen-induced transcription but reduced glucocorticoid-induced transcription, similar to the effect of the phyto-nutrient. In addition, reduction of Nrf2 level, by siRNA, leads to a decrease in phytonutrient supported activity of estradiol in bone cells. In addition to their positive effect on osteoblasts which can lead to increased bone formation, the dietary compounds were found to interfere with RANK-Ligand dependent osteoclastic differentiation, which can lead to reduction in bone resorption. In conclusions, dietary phyto-nutrients, which inhibit estrogenic activity in cancer cells, do not inhibit and even stimulate estrogen signaling in osteoblastic bone cells but inhibit the deleterious effects of glucocorticoids in these cells. The results suggest that Nrf2 is partially involved in these activities.

BONE LOSS IS FAT GAIN – THE ROLE OF SIRTUIN 1 IN BONE

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Bone loss is an inevitable consequence of aging. While for most organs, aging pathologic processes accumulate with advancing age and different diseases occur in different individuals, bone loss occurs virtually in everyone if they live long enough, resulting in most in osteoporosis. Age-related bone loss is characterized by reduction in the osteoprogenitor pool in the marrow accompanied by increased marrow adipogenesis leading to decreased bone formation.

Sirtuin 1 (*Sirt1*), the mammalian homologue of yeast Sir2, is a member of the sirtuin family of highly conserved NAD^+-dependent deacetylases that was found to regulate life span in lower organisms and affect metabolic processes in mammals. First identified for its role in chromatin remodeling associated with gene silencing, *Sirt1* was then discovered to be a mediator of the life-extending effect of calorie restriction in lower organisms. *Sirt1* deacetylases histones and a host of key regulatory proteins affecting transcription and function. Although there is no definite evidence that *Sirt1* regulates lifespan in mammals, over-expression of *Sirt1* in mice confers protection against obesity, impaired glucose tolerance, and Alzheimer's disease. Its role in bone and in osteoporosis has not been studied yet.

We have recently uncovered that *Sirt1* is a major regulator of bone mass by influencing osteoblast differentiation from its mesenchymal marrow stem cell (BM-MSC) progenitor. Using mice with a germ line mutation in *Sirt1*, we show that *Sirt1* haplo-insufficient mice have a dramatic reduction in bone mass, accompanied by increased marrow adipogenesis. Importantly, we identified a novel bone-specific target of *Sirt1*, a critical inhibitor of bone formation, which is negatively regulated by *Sirt1*. These findings have potential CLINICAL implications suggesting that *Sirt1* is a target for promoting bone formation as an anabolic approach for treatment of osteoporosis.