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Review

Regulation of energy metabolism by the skeleton: Osteocalcin and beyond

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ABSTRACT

The skeleton has recently emerged as an endocrine organ implicated in the regulation of glucose and energy metabolism. This function of bone is mediated, at least in part, by osteocalcin, an osteoblast-derived protein acting as a hormone stimulating insulin sensitivity, insulin secretion and energy expenditure. Osteocalcin secretion and bioactivity is in turn regulated by several hormonal cues including insulin, leptin, the sympathetic nervous system and glucocorticoids. Recent findings support the notion that osteocalcin functions and regulations are conserved between mice and humans. Moreover, studies in mice suggest that osteocalcin could represent a viable therapeutic approach for the treatment of obesity and insulin resistance. In this review, we summarize the current knowledge on osteocalcin functions, its various modes of action and the mechanisms implicated in the control of this hormone.

Introduction

Traditionally, bone has been viewed as a relatively static tissue, as a mere collection of calcified tubes only serving as a scaffold for other organs. In the past decade however a more complex picture of bone physiology has emerged. It is now clear that bone integrity and normal function depends upon, but also affects, other organs. For instance it is now known that bone plays a role in the maintenance of the hematopoietic stem cell niche, in the control of serum calcium and in the control of phosphate absorption by the kidney [1–3].

Presumably bone remodeling, the normal biological process by which bone tissue is constantly destroyed by osteoclasts and renewed by osteoblasts, is energetically costly for the rest of the body. Therefore, one can assume that bone remodeling is highly dependent on the energetic status of the organism. This view is supported by specific clinical observations. For example, anorexia nervosa and insulin-dependent diabetes mellitus are associated with osteoporosis, while a higher body mass index (BMI)[1] is generally associated with increased bone mass [4–8]. Together, these observations suggested the existence of a coordinated endocrine regulation of bone and energy metabolism. In other words, that one can expect to identify hormones or circulating factors controlling both bone and energy metabolism [9]. Over the past ten years, several such hormones have been implicated in the control of bone remodeling. Those include leptin and adiponectin, two adipocyte-derived

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[1] *Abbreviations used:* 11bHSD2, 11-β-dehydrosteroid dehydrogenase 2; ADRB2, β2-adrenergic receptor; ATF4, activating transcription factor 4; BGLAP, bone GLA protein; BMI, body mass index; Ccnd1, cyclin D1; Ccnd2, cyclin D2; Cdk4, cyclin-dependent kinase 4; CREB, cAMP response element-binding protein; DTA, diphtheria toxin fragment A; ECM, extracellular matrix; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; Esp, embryonic stem cell phosphatase; FoxA2, forkhead box protein A2; FoxO1, forkhead box protein O1; Fra-2, fos-related antigen-2; Ggcx, γ-glutamyl carboxylase; GLU, glutamic acid; GLA, γ-carboxyglutamic acid; GLP-1, glucagon-like peptide-1; GLU, glutamic acid; GPRC6A, G protein-coupled receptor family C group 6 member A; GSK-3β, glycogen synthase kinase-3 beta; GTG, gold thioglucose; Ins1, insulin I; Ins2, insulin II; InsR, insulin receptor; Nrf1, nuclear respiratory factor 1; Mcad, medium-chain acyl-coenzyme A dehydrogenase deficiency; OCN, osteocalcin; OPG, osteoprotegerin; OST-PTP, osteotesticular protein tyrosine phosphatase; Pepck, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PTPRV, protein tyrosine phosphatase receptor type V; RANKL, receptor activator of NF-kappa-B ligand; SNS, sympathetic nervous system; Tgl, triglyceride-lipase; Tnfα, tumor necrosis factor alpha; UCP1, uncoupling protein 1; Vkorc1, vitamin K epoxyde reductase complex subunit 1; WT, wild-type.

hormones implicated in the regulation of food intake and energy metabolism that also regulate bone mass [10,11]. Additionally, gut-derived hormones such as glucagon-like peptides 1 and 2 and serotonin have been shown to regulate both bone remodeling and energy homeostasis [12–15].

The demonstration of an endocrine control of bone mass by hormones otherwise implicated in the regulation of energy balance, combined with the general principle of endocrine feedback loops, raised an even more intriguing hypothesis, which is that bone cells themselves might secrete hormone(s) implicated in the regulation of glucose metabolism. This hypothesis led to the demonstration a few years ago that osteocalcin, an osteoblast-derived protein, was a hormone regulating glucose and energy homeostasis [16]. Since this initial discovery, several studies have expended and broaden our understanding of osteocalcin biology and more generally of bone as an endocrine organ. We will review here the most recent findings in mice and humans on the influence of bone on energy metabolism and the physiological regulation of bone endocrine function by intrinsic mechanisms and by hormonal signals. We will also try to integrate this knowledge into a unified model of osteocalcin regulation and action (Figs. 2 and 3).

Osteocalcin: a bone-derived hormone regulating energy metabolism

Osteocalcin is a small protein (46 a.a. in mouse and 49 a.a. in human) produced by bone and posttranslationally modified on specific glutamic acid (GLU) residues that are carboxylated to form γ-carboxyglutamic acid (GLA) residues (Fig. 1). Although this modification is usually associated with increased affinity for mineral ions, both loss- and gain-of-function in vivo experiments have failed to demonstrate a critical function for osteocalcin in extracellular matrix mineralization, at least in mice [17,18]. While osteocalcin is detected at high concentration in the bone extracellular matrix (ECM), it also possesses several characteristic of a hormone. For instance, osteocalcin is a cell specific molecule secreted by osteoblasts, it is first produced as a prepropeptide and its mature form is present in fair concentration in the circulation (between 100 and 1000 ng/ml in mice) (see Fig. 1A).

Analysis of the osteocalcin knockout mice ($Ocn^{-/-}$) revealed that they are abnormally fat suggesting that somehow osteocalcin might impact glucose metabolism (Fig. 2). A comprehensive analysis revealed that insulin secretion, glucose tolerance and insulin sensitivity are all decreased in $Ocn^{-/-}$ mice under normal chow diet [16]. In addition, islets number, β-cell area, β-cell mass and insulin content are reduced in the pancreas of $Ocn^{-/-}$ mice. Importantly, osteocalcin appears to transcriptionally regulate insulin biosynthesis, since *Ins1* and *Ins2* gene expression is stimulated in pancreatic islets by recombinant osteocalcin or by WT osteoblast supernatant while it is impaired in pancreatic islets co-cultured with supernatant from $Ocn^{-/-}$ osteoblasts [16,19]. It was shown through islets perifusion assays, that osteocalcin is also a potent insulin secretagogue due to its ability to increase cytosolic Ca^{2+} levels [20]. These studies thus suggested that osteoblast-derived osteocalcin control glucose metabolism by directly affecting pancreatic islets biology as well as insulin synthesis and secretion.

These observations then raised the question of how osteocalcin signals to β-cell to trigger its effect. Initially described as a cations and amino acid sensing receptor, the G protein-coupled receptor GPRC6A is expressed in many tissues including liver, skeletal muscle, brain, testis, bone and pancreatic β-cells [21–25]. Interestingly, $Gprc6a^{-/-}$ mice have more white fat compared to WT animals, are glucose intolerant, insulin resistant, exhibit histological features of hepatic steatosis and reduced testosterone levels [26]. Since $Gprc6a^{-/-}$ mice phenocopied $Ocn^{-/-}$ mice with regards to their

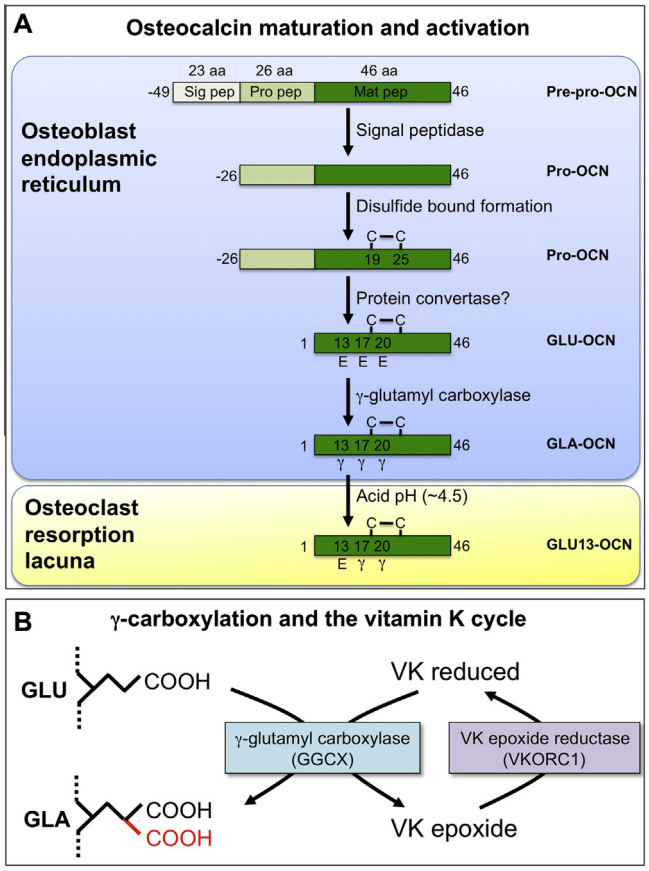

Fig. 1. Osteocalcin maturation and activation steps. (A) Osteocalcin is synthesized as a prepropeptide (Pre-pro-OCN). While transiting through the endoplasmic reticulum, pro-osteocalcin (Pro-OCN) is processed into mature osteocalcin by a mechanism that is still not well understood. Osteocalcin is post-translationnally modified on three glutamic acid residues (E13, E17 and E21) by the enzyme γ-glutamyl carboxylase (GGCX) in the endoplasmic reticulum. It is still unclear which of γ-carboxylation or osteocalcin processing occurs first. Since γ-carboxylation increases the affinity of osteocalcin for the mineral component of the bone, once secreted by osteoblasts, osteocalcin is stocked in this organ. The acidic pH generated during bone resorption is sufficient to decarboxylate osteocalcin, which will decrease its affinity for bone extracellular matrix and promotes its release into the bloodstream. (B) γ-Carboxylation is the addition of a carboxyl group to the glutamic acid (GLU) residue of a protein to generate a γ-carboxyglutamic acid (GLA) residue. This reaction relies on GGCX and requires vitamin K. The oxidized vitamin K epoxide generated by this reaction can be reduced by VKORC1 to allow another γ-carboxylation cycle to take place.

metabolic abnormalities, it suggested that GPRC6A might mediate osteocalcin function, at least in pancreatic islets. Indeed, it was shown that ERK phosphorylation induced by osteocalcin either in GPRC6A-overexpressing cells or in mice pancreas was prevented in absence of GPRC6A [27]. In addition, specific inactivation of *Gprc6a* in pancreatic tissue caused glucose intolerance in mice resulting from an impaired capacity to secrete insulin in response to glucose [25]. This latter study also demonstrated that osteocalcin action on pancreatic islets depends on GPRC6A since the capacity of osteocalcin to promote insulin secretion was abrogated in $Gprc6a^{-/-}$ islets. $Gprc6a^{-/-}$ pancreas are characterized by a decreased in β-cell area and β-cell mass similarly to $Ocn^{-/-}$ pancreas, but by a normal number of islets although their proliferative status is impaired due to a decreased in *Ccnd1* expression. Importantly, this work suggested that the osteocalcin/GPRC6A signaling cascade might be an important regulator of the massive β-cell proliferation that occurs shortly after birth in mice. It was also demonstrated that GPRC6A is the receptor mediating the function of osteocalcin in Leydig cells, where osteocalcin signals through GPRC6A to promote testosterone production and male fertility [28].