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Review

New insights into the biology of osteocalcin☆

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ABSTRACT

Osteocalcin is among the most abundant proteins in bone and is produced exclusively by osteoblasts. Initially believed to be an inhibitor of bone mineralization, recent studies suggest a broader role for osteocalcin that extends to the regulation of whole body metabolism, reproduction, and cognition. Circulating undercarboxylated osteocalcin, which is regulated by insulin, acts in a feed-forward loop to increase β-cell proliferation as well as insulin production and secretion, while skeletal muscle and adipose tissue respond to osteocalcin by increasing their sensitivity to insulin. Osteocalcin also acts in the brain to increase neurotransmitter production and in the testes to stimulate testosterone production. At least one putative receptor for osteocalcin, Gprc6a, is expressed by adipose, skeletal muscle, and the Leydig cells of the testes and appears to mediate osteocalcin's effects in these tissues. In this review, we summarize these new discoveries, which suggest that the ability of osteocalcin to function both locally in bone and as a hormone depends on a novel post-translational mechanism that alters osteocalcin's affinity for the bone matrix and bioavailability. This article is part of a Special Issue entitled Bone and diabetes.

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1. Introduction

Osteoblasts are specialized mesenchymal cells that are primarily responsible for the synthesis and deposition of the mineralized, collagen-rich matrix that composes bone tissue. Over the last decade, studies have elaborated an expanded biological function for the osteoblast that is focused on the actions of bone-derived osteocalcin [1,2]. Osteocalcin has routinely been used as a serum marker of osteoblastic bone formation and believed to act in the bone matrix to regulate mineralization, but new genetic and pharmacologic evidence now points to a hormonal role for the protein. These newly discovered actions link the energy demands of bone to global homeostasis [1,3] and close a number of open endocrine loops associated with the impact of nutrient availability [4,5], leptin [6,7], adiponectin [8] and insulin [9] on skeletal metabolism. In this review, we summarize the current knowledge of osteocalcin function beginning with the initial suggestion that the protein

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inhibits mineralization and ending with evidence of the hormonal actions of osteocalcin in humans.

2. Structure and post-translational modifications of osteocalcin

Osteocalcin, also referred to as bone γ-carboxyglutamic acid (Gla) protein or BGP, is a 46–50 amino acid, 5.6 kDa secreted protein that is produced primarily by osteoblasts [10]. Smaller amounts are also produced by odontoblasts of the teeth and hypertrophic chondrocytes. The protein was first isolated by Price et al. [11,12] from bovine and human bone and shown to represent the major fraction of Gla containing protein in bone. A second Gla-protein, isolated later by the same group, was termed matrix Gla protein or MGP [13,14]. Together, these two proteins belong to a distinct subgroup of the larger vitamin K-dependent protein family, the constituents of which are primarily involved in coagulation.

The human osteocalcin gene, *BGLAP*, is located on chromosome 1 at 1q25–q31 [15] and encodes an 11 kD, 98 amino acid pre-pro-protein (Fig. 1). The mature peptide is generated by sequential cleavage events that remove an endoplasmic reticulum signal sequence and the pro-sequence followed by γ-carboxylation of three glutamic acid residues at positions 17, 21, and 24. All three of the glutamate carboxylation events occur during a single binding of the immature peptide to γ-glutamyl carboxylase [16]. This enzyme utilizes CO_2, O_2, and vitamin K, supplied by the vitamin K cycle and circulation, as cofactors. With each γ-carboxylation cycle, vitamin K is converted to an epoxide, which is then reduced by vitamin K epoxide reductase to allow another round of carboxylation [17]. The mature, carboxylated osteocalcin protein is packaged into intracellular vesicles for secretion into the bone matrix [10,18].

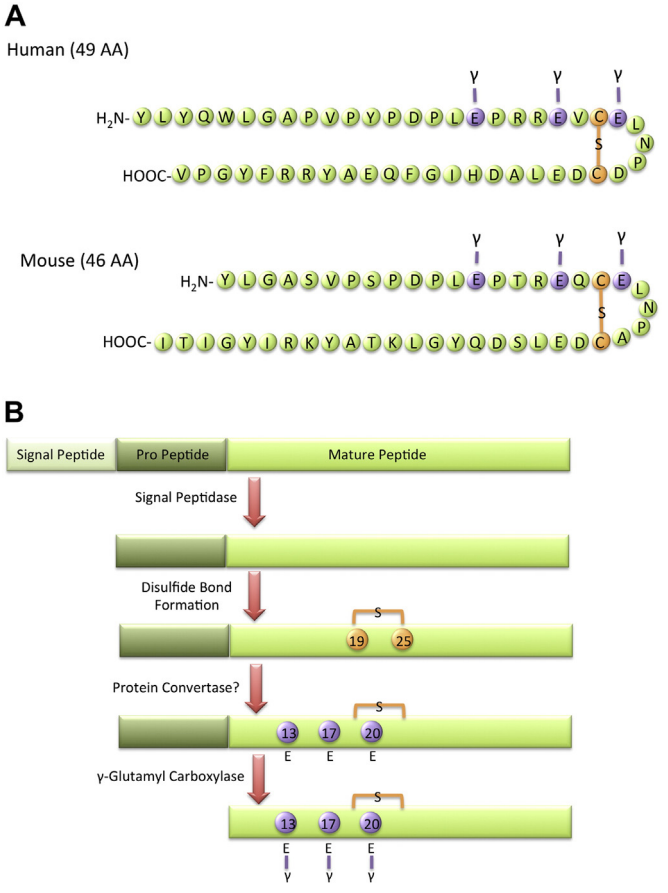

Fig. 1. Osteocalcin structure, translation, and processing. (A) The structure of osteocalcin is marked by three γ-carboxyglutamic acid residues and a disulfide bond. The human protein contains 49 amino acids with γ-carboxyglutamic acid residues at positions 17, 21, and 24 and a disulfide bond between cysteine residues at positions 23 and 29. The mouse protein is 3 amino acids shorter with γ-carboxyglutamic acid residues at positions 13, 17, and 20. (B) Osteocalcin is translated as a pre-pro-peptide. Following signal sequence cleavage the peptide translocates into the endoplasmic reticulum, where a disulfide bond forms. After processing in a potential protein convertase step, osteocalcin is γ-carboxylated on three glutamic acid residues.

All vitamin K-dependent Gla proteins are thought to have evolved from a common ancestor because they share both the ability to bind calcium and the same γ-carboxylase recognition sites [19,20]. The hepatic Gla proteins that function in blood coagulation bind to calcium-linked phospholipids whereas osteocalcin binds to calcium ions in hydroxyapatite [21].

The osteocalcin and MGP genes appear to have diverged from an ancestral gene that first emerged 500 million years ago before the evolution of jawless fish [22]. MGP appeared first with the development of cartilaginous structures, while a later genome duplication event coinciding with the emergence of bony structures led to the evolution of the osteocalcin gene. In most organisms, including humans, osteocalcin is encoded by a single gene that is highly conserved across species. However, mice contain a cluster of three osteocalcin genes, indicative of an additional duplication late in rodent evolution [23]. Two of these genes, osteocalcin gene 1 (OG1) and osteocalcin gene 2 (OG2), are expressed predominantly in the bone. The third gene, osteocalcin-related gene (ORG), is expressed primarily in the kidney. Although the exact function of ORG remains unknown [24], it has been suggested that ORG encodes nephrocalcin, a calcium-binding Gla protein important in calcium homeostasis [23].

3. Role of osteocalcin in mineralization

Mature osteocalcin is secreted into the bone micro-environment and then undergoes a conformational change that aligns its calcium-binding Gla residues with the calcium ions in hydroxyapatite. This property was initially proposed as a mechanism that enables osteocalcin to initiate the formation of hydroxyapatite crystals [12]. However, subsequent work was more compatible with the notion that osteocalcin functions as an inhibitor of bone mineralization. In support of this idea, osteocalcin inhibits the precipitation of calcium salts from saturated solutions [25], and chronic treatment of rodents with warfarin, an inhibitor of vitamin K-dependent γ-carboxylation, results in over-mineralization and the premature closure of the growth plate [26].

To more fully study the role of osteocalcin in bone formation, Ducy et al. [27] created an osteocalcin null mouse lacking both osteocalcin genes expressed in the bone. These mice exhibited an age-dependent increase in bone formation rate and bone mass compared to controls, without an impact on bone resorption. Subsequent analysis of the bone matrix using Fourier transform infrared microspectroscopy indicated that the mineral to matrix ratio was increased in older knockout animals. In addition, the hydroxyapatite crystal size was larger, suggesting that osteocalcin might regulate the rate of mineral maturation [28]. However, the deposition of dentin by odontoblasts was normal in osteocalcin null mice [29], and analysis of mice overexpressing osteocalcin in bone revealed a relatively normal state of mineralization [30]. Therefore, the precise role of osteocalcin within the bone matrix remains unclear, and osteocalcin's inhibitory effect on bone mineralization is likely to be considerably lower than that of MGP since the removal of this gene results in the calcification of the aorta and the progressive mineralization of the growth plate [30,31].

Other studies have led to the hypothesis that osteocalcin exerts a mechanical function within the bone matrix. As a result of its ability to tightly bind hydroxyapatite and form a complex with collagen through the matrix protein osteopontin [32–34], osteocalcin was proposed as means to bridge the matrix and mineral fractions of bone tissue. Such an arrangement is compatible with the formation of dilatational bands