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An overview of the endocrine functions of osteocalcin

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Osteocalcin is the most abundant non-collagenous protein produced in bone. It has traditionally been regarded as a marker of bone turnover and is thought to act in the bone matrix to regulate mineralization. However, emerging knowledge regarding osteocalcin has expanded to include functions in energy metabolism, fertilization, and regulation of cognition. Fully carboxylated osteocalcin binds to hydroxyapatite, thereby modulating bone turnover, whereas undercarboxylated osteocalcin in the circulation binds to osteocalcin-sensing receptors and acts as a hormone that affects multiple physiological aspects. In this review, we summarize the current knowledge regarding the hormonal actions of osteocalcin in various organs and potential cellular downstream signaling pathway that may be involved.

Keywords: Osteocalcin, Undercarboxylated osteocalcin, Bone derived hormone, Osteoblasts

Introduction

Classically, bone has been considered as an inert organ, only providing the supporting framework for the body, protection for soft tissues and functions as a regulator for mineral homeostasis, a storage system for minerals, namely, calcium and phosphorus. Over the last decade, mounting evidence has emerged that bone can act as an endocrine organ [1]. The bone matrix protein osteocalcin has been extensively investigated since studies first elaborated its endocrine function in regulation of energy metabolism [2–4]. Osteocalcin has traditionally been regarded as a bone turnover marker and thought to act in the bone matrix to regulate mineralization. However, emerging knowledge on the functionality of this protein expanded to include properties such as energy metabolism, fertilization, and regulation of cognitive functions. In this review, we summarize the current knowledge of hormonal actions of osteocalcin in various organs and potential cellular downstream signaling pathway involved.

Undercarboxylated Osteocalcin

Osteocalcin, also referred to as bone γ-carboxyglutamic acid (Gla) containing protein, is the most abundant non-collagenous protein produced in bone [5]. It is a small protein with a size of 5.6 kDa (49 amino acids long in humans) and is produced primarily by osteoblasts, although smaller amounts are made by odontoblasts or hypertrophic chondrocytes [6]. In osteoblasts, osteocalcin undergoes vitamin K-dependent post-translational modifications, which cause carboxylation at glutamic acid (Glu) residues in positions 17, 21, and 24. Vitamin K is first converted to an epoxide, and then reduced by vitamin K epoxide reductase to complete the carboxylation process [7].

These three Gla residues allow binding of osteocalcin to hydroxyapatite crystal in the bone matrix, leading to a disulfide bond between cysteine residues which stabilize the three-dimensional structure of osteocalcin [8].

Osteocalcin still containing one or more empty, that is, not carboxylated Glu residue, are denoted as undercarboxylated

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osteocalcin (ucOC) [9]. Of the total osteocalcin released into the bloodstream, 40–60% exists as either a partially or completely uncarboxylated form; these undercarboxylated forms have been known to perform hormone-like functions, including energy metabolism, fertilization, and regulation of cognitive functions [9–17].

Endocrine Functions of Osteocalcin

Previous reports have shown that ucOC upregulates β-cell proliferation and insulin production in the pancreas, while also increasing insulin sensitivity in adipose tissue and skeletal muscle [18–20]. The ESP (Ptprv) gene, first explored by Lee et al. [3], is expressed only in bone and encodes for a tyrosine phosphatase that suppresses carboxylation of osteocalcin, consequently affecting insulin production in the pancreas. Global and osteoblast-specific deletions of ESP produced hypoglycemic and anti-obese phenotypes.

Since ucOC regulates glucose metabolism, which provides energy to muscles, it may function as part of the endocrine axis between bone and muscle that favors adaptation to exercise. Mera et al. [21] demonstrated the role of osteocalcin signaling in myofibers in promoting uptake and utilization of glucose and fatty acids, which contributes to muscle adaptation during exercise. Acutely or chronically administered osteocalcin enhanced the exercise capacity of young mice and restored the exercise capacity of old mice to that of young mice (15-month-old mice vs. 3-month-old mice).

Concerning endocrine function in reproduction, Oury et al. [22] reported the regulatory role of osteocalcin in male fertility. Using leydig cell specifically cAMP response element binding protein (CREB) deficient mice, they showed that osteocalcin upregulates testosterone synthesis in a CREB-dependent manner, promoting enzymes expressions required for testosterone synthesis and germ cell survival.

Osteocalcin signaling in the regulation of cognition and anxiety in the brain were also demonstrated.

Oury et al. [23] observed a substantial increase in anxiety-like behavior, along with major deficits in spatial learning and memory, in *Osteocalcin*$^{-/-}$ adult mice and showed that brain development and the acquisition of cognitive function of offspring is influenced by maternal osteocalcin. The same research group demonstrated that the hippocampal region is smaller [23] and the corpus callosum region [24] is often missing in the *Osteocalcin*$^{-/-}$ mice compared to those of wild-type (WT) littermates. Serotonin, dopamine, and norepinephrine neurotransmitters were 20–50% reduced, and the accumulation of γ-aminobutyric acid increased by 15–30%, in both the midbrain and brainstem of *Osteocalcin*$^{-/-}$ mice [25].

Osteocalcin Sensing Receptor

G-protein-coupled receptor family C group 6 member A (*GPRC6A*) was recently identified as an undercarboxylated osteocalcin-sensing receptor [26]. Osteocalcin signaling has been demonstrated to be mediated by *GPRC6A* in various tissues, including pancreas, testes, adipose, and skeletal muscle [27–29]. Pi et al. [30] carried out computational modeling to probe the structural basis of osteocalcin binding to *GPRC6A* and predicted that the C-terminal hexapeptide docks to the extracellular side of the transmembrane domain of *GPRC6A*. Wei et al. [20] demonstrated *GPRC6A*-mediated osteocalcin signaling occurred in β-cell proliferation during development and adulthood in mice [20]. Our research team has recently reported that ucOC downregulates pancreatic lipase via *GPRC6A* in pancreatic acinar cells [31]. *GPRC6A*-mediated osteocalcin signaling has also been proposed in fat, skeletal muscle and hepatic tissues. *GPRC6A*$^{-/-}$ mice developed an increase in triglycerides and a decrease in both glycogen storage and cholesterol levels, along with hepatic steatosis. Glucose intolerance, insulin resistance and white fat accumulation were observed in *GPRC6A*$^{-/-}$ mice but not in WT mice [29, 32–36]. Pi et al. [37] showed that ucOC affects testosterone secretion and De Toni et al. [38] reported vitamin D production in testicular Leydig cells through a *GPRC6A*-dependent pathway.

Beyond *GPRC6A*-mediated osteocalcin signaling, Khrimian et al. [39] recently identified an orphan class C G protein-coupled receptor (GPCR), Gpr158, as an another osteocalcin-sensing receptor, which is expressed in neurons of the hippocampal CA3 region and mediates ucOC's regulation of cognitive function and memory.

Even though class C GPCRs, also called "nutrient receptors", are activated by numerous ligands, some studies do not show consistency in osteocalcin activation of class C GPCRs, including *GPRC6A*. Jacobsen et al. [40] reported that osteocalcin did not activate *GPRC6A* when expressed in Chinese Hamster Ovary cells. They reported that the *GPRC6A* receptor is internalized and constitutively recycled; consequently, these events do not seem to directly regulate the agonist-mediated receptor response. They further demonstrated that the expression level of *GPRC6A* was unaffected by a reduction in basal receptor signaling. Oury et al. [26] demonstrated that the effects of

osteocalcin in Leydig cells were not specifically mediated by *GPRC6A*. Importantly, even previous animal models are not consistent with regards to the influence of *GPRC6A* on glucose homeostasis in *GPRC6A*-deficient mice [29,41]. Further studies are required to identify additional osteocalcin-sensing receptors recognized by various cell types.

Osteocalcin Signaling and Transcriptional Regulation

Mounting studies have demonstrated the osteocalcin signaling pathway and its transcriptional effector in various cell types. The Karsenty group has demonstrated that osteocalcin induces a cAMP accumulation, indicating Gs coupling, but no Gq or extracellular signal-regulated kinase (ERK) pathway activation, in TM3 Leydig cells. They also reported that the CREB pathway is one mediator of osteocalcin signaling in myofibers by showing that CREB phosphorylation in myotubes is weaker after exercise using muscle-specific *GPRC6A* knockout mice [21]. The Quarles group also demonstrated that *GPRC6A* agonists can induce Gs coupling by showing that cAMP accumulation occurred in *GPRC6A*-transfected HEK 293 cells in response to four *GPRC6A* agonists (osteocalcin, testosterone, L-arginine, and divalent cations) [27,42]. Pi et al. [43] reported that osteocalcin leads to the downstream activation of serum-response element (SRE) and/or ERK (Gq pathway) in *GPRC6A*-transfected HEK 293 cells.

Park et al. [31] recently reported that ucOC downregulates pancreatic lipase expression in a cAMP/protein kinase A/ATF4-dependent pathway, indicating Gs coupling in pancreatic acinar cells. The NFκB-p65-dependent osteocalcin signaling pathway in vascular tissue was implicated in a study by Zhou et al. [44], demonstrating that osteocalcin reverses obesity-induced autophagic dysfunction and endoplasmic reticulum stress.

In contrast to the aforementioned studies, Jacobsen et al. [40] used a *GPRC6A*-transfected Chinese Hamster Ovary cell line to demonstrate that osteocalcin does not induce ERK signaling pathway or any of the other G-protein signaling pathways that were tested. Thus, further studies are justified to explore which ligand classes and signaling pathways are recruited by the *GPRC6A* receptor.

Conclusions

The studies presented above provide evidence that osteocalcin functions as an endocrine hormone, leading to a paradigm shift in our understanding of traditional bone physiology. Osteocalcin in circulation can now be considered to be a hormone-like factor that regulates energy metabolism, reproduction, and even brain function, such as cognition/mood, through endocrine links between bone and pancreas, muscle, adipose, testes, and brain. Considering the mounting evidence showing the strong regulatory effects of osteocalcin on various organs, it is highly likely that osteocalcin signaling also influences additional organs that have not been studied. Determining the extent to which osteocalcin's endocrine roles observed in animal models also manifest in humans will be a critical issue in osteocalcin research.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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