Molecular and Cellular Endocrinology 382 (2014) 521–526

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

Regulation of male fertility by the bone-derived hormone osteocalcin

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ARTICLE INFO

Article history:
Available online 19 October 2013

Keywords:
Osteocalcin
G-coupled receptor
Primary testicular failure
Testosterone

ABSTRACT

Traditionally, bone has been viewed as a relatively static tissue only fulfilling mechanical and scaffolding function. In the past decade however, this classical view of the bone has considerably evolved towards a more complex picture. It is now clear that the skeleton is not only a recipient for hormonal input but it is also an endocrine organ itself. Through the secretion of an osteoblast-derived molecule, osteocalcin, the skeleton regulates glucose homeostasis and male reproductive functions. When undercarboxylated, osteocalcin acts following its binding to a G-coupled receptor, *Gprc6a*, on pancreatic β cells to increase insulin secretion, on muscle and white adipose tissue to promote glucose homeostasis and on Leydig cells of the testis to favor testosterone biosynthesis. More recently, it was also shown that osteocalcin acts via a pancreas-bone-testis axis that regulates, independently of and in parallel to the hypothalamus–pituitary–testis axis, male reproductive functions by promoting testosterone biosynthesis. Lastly, in trying to expand the biological relevance of osteocalcin from mouse to human, it was shown that *Gprc6a* is a potential new susceptibility locus for primary testicular failure in humans. Altogether, these results shed new light on the importance of the endocrine role of the skeleton and also provide credence to the search for additional endocrine functions of this organ.

Contents

1. The classical view of the bone physiology

The skeleton is essential for locomotion and is defined primarily by its mechanical and scaffolding properties. This is critical for vertebrates to maintain a constant bone mass with high bone quality and excellent biomechanical properties. This is achieved by the ability of the bone to constantly renew itself through a mechanism called bone remodeling (Karsenty, 2006; Rodan and Martin, 2000). Bone remodeling is a biphasic process including the destruction of the preexisting bone (bone resorption mediated by the osteoclasts), followed by a second phase of *de novo* formation of the bone, (bone formation mediated by the osteoblasts) (Rodan and Martin, 2000; Harada and Rodan, 2003; Teitelbaum, 2000). Importantly, these two phases not only occur sequentially but also in a balanced manner to keep a constant bone mass throughout life. A mis-regulation of this balance leads to diseases, the most frequent being osteoporosis, which is caused by an increase of bone resorption in comparison to bone formation (Karsenty, 2006; Rodan and Martin, 2000; Teitelbaum, 2000; Khosla and Riggs, 2005; Nakamura et al., 2007; Oury, 2012). The regulation of bone (re)modeling is complex and involves mechanical stimuli, locally produced factors and many hormones. For instance, sex steroid hormones play a crucial role during the bone growth spurts of puberty, and for maintenance of bone mass (Harada and Rodan, 2003; Oury, 2012; Khosla et al., 2001; Riggs et al., 2002; Vanderschueren et al., 2004; Venken et al., 2006).

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http://dx.doi.org/10.1016/j.mce.2013.10.008

2. The novel dimension of bone physiology

Bone remodeling occurs throughout life in dozens of location in the skeleton, which is also one of the organs covering the largest surface in our body. Both the cellular events it entails and the surface covered by the skeleton suggest that this physiological process is costly energy-wise. Clinical observations support, fully, this view of bone (re)modeling. Specifically, the absence of food intake, as in anorectic children causes a near-total arrest of growth and low bone mass in adulthood (Legroux-Gerot et al., 2005; Misra and Klibanski, 2011a,b). Moreover, and unrelated to food intake, it has been known for a long time that the growth and integrity of both the female and the male skeleton are influenced by sex steroid hormones. The biological importance of this regulation is best exemplified by the fact that gonadal failure triggers bone loss in both genders and leads to osteoporosis in post-menopausal women (Riggs et al., 1998; Riggs and Melton, 1986). Taken together, this view of bone (re)modeling and these clinical observations suggest that there may be a coordinated regulation of bone mass or growth, energy metabolism and reproduction (Karsenty, 2006; Karsenty and Ferron, 2012).

Many genetic-based studies have shown that this hypothesis is true in both rodents and humans. The skeleton secretes at least two hormones. First, fibroblast growth factor 23 (FGF 23) regulating mineral metabolism through its control of the phosphate homeostasis that is intimately linked to bone health (Hori et al., 2011; Shimizu et al., 2012). Second, an osteoblast-specific secreted protein, osteocalcin, when undercarboxylated, acts as a multifunctional hormone. Osteocalcin acts on pancreatic β cells to increase their proliferation and insulin secretion (Karsenty and Ferron, 2012; Karsenty and Oury, 2012; Lee et al., 2007). It also promotes glucose homeostasis by acting in various tissues, such as muscle, liver and fat (Karsenty and Ferron, 2012; Karsenty and Oury, 2012; Lee et al., 2007) (Fig. 1).

Subsequently, two groups working independently showed that mice lacking the insulin receptor in osteoblasts (*Ins*Rosb−/−mice) were glucose intolerant and insulin insensitive when fed on normal chow; that is, they were a phenocopy of the osteocalcin-gene-deficient mice (Ferron et al., 2012; Ferron et al., 2010; Fulzele et al., 2010). Because mice lacking the insulin receptor in skeletal muscle or white adipose tissue do not display glucose intolerance when fed a normal diet (Bluher et al., 2002; Bruning et al., 1998), insulin must act in additional tissues to achieve glucose homeostasis. The fact that bone is such a tissue legitimizes the notion that this tissue is necessary for glucose homeostasis. In addition, *Ins*Rosb−/−mice had significantly less biologically active (undercarboxylated) osteocalcin in their sera, revealing that insulin signaling in osteoblasts is a determinant of osteocalcin bioactivity (Bluher et al., 2002; Bruning et al., 1998). In a manner that is both elegant and economical, insulin uses the interplay between osteoblasts and osteoclasts for that purpose. Specifically, insulin inhibits the expression in osteoblasts of the gene encoding osteoprotegerin (*Opg*) Ferron et al., 2010, which hampers osteoclast differentiation. In other words, insulin signaling in osteoblasts favors bone resorption, a process that occurs at pH 4.5 (Silver et al., 1988). Acidic pH is the only mechanism known to achieve decarboxylation of proteins (Poser and Price, 1979), therefore, bone resorption decarboxylates and activates osteocalcin (Ferron et al., 2010) (Fig. 2). Thus, in a feed forward loop, insulin signaling in osteoblasts promotes its own secretion by activating osteocalcin. Furthermore, mice and humans in which bone resorption is genetically impaired show a decrease in the undercarboxylated form of osteocalcin, resulting in glucose intolerance (Ferron et al., 2010) (Fig. 2).

The discovery of the hormonal functions of osteocalcin raised multiple questions with great biological and medical importance. Chief among them was to elucidate the signaling events triggered by this hormone in target cells. A prerequisite to address this question was the identification of a specific receptor for osteocalcin. Because most hormones have several functions, the next question was whether this was the case for osteocalcin.

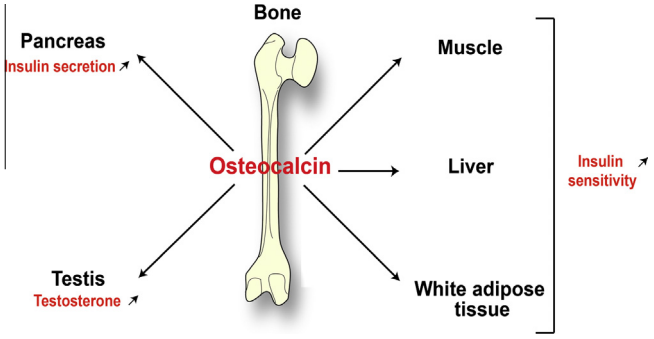

Fig. 1. The skeleton is an endocrine organ. The endocrine regulation of energy metabolism and male reproduction by the bone is mediated by osteocalcin, an osteoblast-specific secreted molecule. Osteocalcin regulates energy metabolism by increasing insulin secretion, favoring pancreatic-β-cell proliferation, and increasing insulin sensitivity in various tissues. In addition, it promotes male reproductive function by stimulating testosterone synthesis in Leydig cells.

3. Osteocalcin favors testosterone production by the Leydig cells of the testis

The well-known regulation of bone remodeling by gonads (Khosla and Riggs, 2005; Nakamura et al., 2007; Riggs et al., 2002; Vanderschueren et al., 2004; Kousteni et al., 2002) suggested

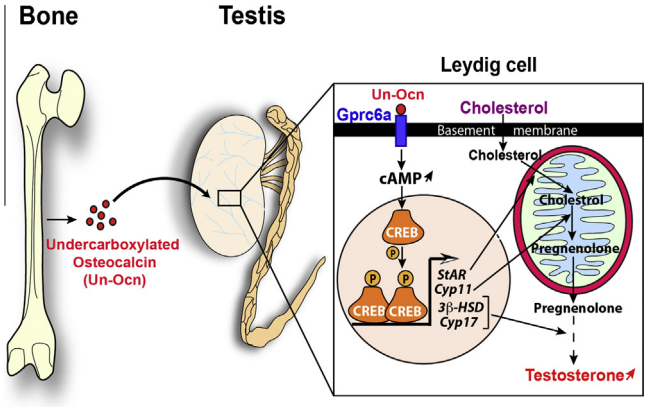

Fig. 2. Molecular mode of action of osteocalcin in regulating testosterone production. Osteocalcin has an unusual mode of activation that relies on the interplay between two specific bone cells: Osteoblasts and Osteoclasts. The osteoblasts produce and secrete an inactive form of this molecule (carboxylated) that is stored in the extracellular bone matrix (ECM). The activity of the osteoclasts create resorption lacunae in the ECM inducing a low PH (4.5) which is necessary and sufficient to bio-activate osteocalcin by promoting its undercarboxylation. The mechanism by which osteocalcin is activated is regulated in osteoblasts by insulin signaling, which inhibits the expression in osteoblasts of the gene encoding osteoprotegerin (*Opg*), which hampers osteoclast differentiation. Following its binding to *Gprc6a* expressed in Leydig cells, osteocalcin favors cAMP production that leads to the activation of the transcription factor CREB (cAMP response element binding). CREB activates the expression of several genes encoding the enzymes that are necessary for testosterone biosynthesis, such as *StAR*, *Cyp11a*, *3β-HSD* and *Cyp17*. Steroidogenic acute regulatory protein (StAR) is crucial for transport of cholesterol to mitochondria where biosynthesis of steroids is initiated. *Cyp11a* encodes the cholesterol side-chain cleavage enzyme (P450scc) that catalyzes the first and rate-limiting step, which converts cholesterol to pregnenolone. *3β-HSD* and *Cyp17* encode two enzymes required during the conversion of pregnenolone to testosterone. Testosterone is a sex steroid hormone require for many aspects of testicular functions, such as germ cell survival and spermatogenesis.