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Invited Mini Review

Interrelationship of Runx2 and estrogen pathway in skeletal tissues

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Two key molecules in skeletal tissues are bone formation master transcription factor Runx2 and the steroid hormone estrogen. It is well known that these two molecules play pivotal roles in bone homeostasis; however, the functional interaction between Runx2 and estrogen synthesis in skeletal tissues is largely unknown. Recent studies have indicated that there is a positive relationship between Runx2 and the estrogen biosynthesis pathway. In this review, a possible functional link between Runx2 and estrogen synthesis pathway in skeletal tissues will be discusses as well as the biological significance of this interaction. [BMB reports 2011; 44(10): 613-618]

INTRODUCTION

Bone is a dynamic tissue which is continuously resorbed by osteoclasts and formed by osteoblasts in a process called bone remodeling throughout the lifetime of the organism. Bone remodeling is the replacement of old bone tissue with new bone tissue in local bone areas, which is called the bone remodeling compartment (BRC), and this process is responsible for a gain in bone mass and change in skeletal form (1). BRC comprises the basic multicellular unit (consisting of osteoclasts, osteoblasts and osteocytes), the canopy of bone-lining cells and the associated capillaries. The remodeling process within BRC involves the coupling of bone formation and bone resorption. Runx2 and estrogen have been shown to play important roles in this process. However, the relationship between Runx2 and estrogen biosynthesis in bone is still not clearly understood.

Function of Runx2 and estrogen in bone tissues

The transcription factor Runx2, also known as Pebp2A, Cbfa1, Osf2 and AML3, plays essential roles in osteoblast differentiation and function (2). Ablation of Runx2 function in mice results in a complete lack of both intramembranous and endochondral ossification and shows an absence of osteoblast differentiation, as well as retardation of chondrocyte differentiation (3-5). Furthermore, many *in vitro* studies have shown that Runx2 is a positive regulator for the expression of bone matrix genes, including type I collagen, osteopontin, bone sialoprotein, osteocalcin, and matrix metalloproteinase 9 (6-10). The heterozygous mutation of Runx2 results in cleidocranial dysplasia, a dominantly inherited developmental disorder of bone, in both mice and humans (3, 4, 11-13). Moreover, Runx2 accelerates chondrocyte differentiation in response to the upregulation of Runx2 target genes, which include the indian hedgehog (*Ihh*) and type X collagen (*Col10A1*) (14, 15). Runx2 also plays an important role in estrogen deficiency-mediated bone resorption in adult mice (16). Together, these studies demonstrate that Runx2 acts as a master regulator of bone formation by modulating the activity of a cohort of target genes not only in early growth but also in postnatal bone development and maintenance stages.

Estrogen is essential in skeletal growth and development and the maintenance of bone health in both men and women (17). Estrogen synthesis starts from cholesterol and converts androgens like testosterone and androstenedione into estrogens by aromatase at the final step (Fig. 1). Deficiency of estrogen by mutation of aromatase (18-20), or mutation of estrogen receptor alpha (ERα) could lead to unfused epiphyses, osteopenia and even osteoporosis (21). Estrogen deficiency after menopause has been associated with increased bone turnover, which results in a reduced bone mass and an increased fracture risk. One of the key roles of estrogen is to modulate osteoclast generation and life span, which is revealed by a loss of expected increase of the number of osteoclasts in bone than expected and further decrease of cancellous bone after loss of estrogens in monocyte/macrophage cell lineage- or osteoclast lineage-specific ERα conditional knockout mice (22, 23). Although the stage specific roles of Fas ligand (FasL) during osteoclastogenesis (24) and osteoclast apoptosis (23) remain controversial, estrogen mediated upregulation of FasL through ERα in osteoclasts or osteoblasts has been shown to induce osteoclast apopotosis (22, 25). In addition, estrogen inhibits osteoclast formation through the upregulation of osteoprotegerin production (26). Therefore, mutation or ablation of genes in the pathway related to estrogen synthesis disrupts bone homeostasis (27).

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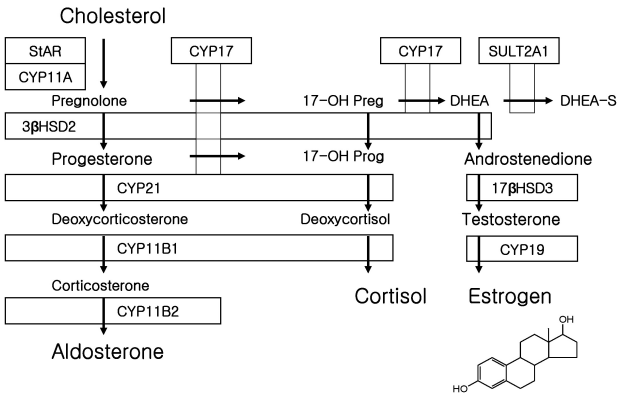

Fig. 1. Estrogen synthesis pathway. The biosynthesis of estrogens from cholesterol involves many enzymes and the final step is catalyzed by the enzyme aromatase (CYP19). CYP11A, cholesterol side-chain cleavage enzyme; CYP17, 17α-hydroxylase and 17,20-lyase; CYP21, 21-hydroxylase; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP19, aromatase; StAR, steroidogenic acute regulatory protein; SULT2A1, dehydroepiandrosterone-sulfotransferase; 17-OHPreg, 17α-hydroxypregnenolone; 17-OHProg, 17α-hydroxyprogesterone; 17βHSD3, 17β-hydroxysteroid dehydrogenase type 3; 3βHSD2, 3β-hydroxysteroid dehydrogenase type 2. Cartoon was modified from Pezzi, *et al*. (35).

Alternative usage of multiple aromatase promoters in various tissues

Aromatase catalyzes the conversion of androgen to estrogen and is encoded by a single gene (CYP19) in human. The human aromatase gene is comprised of a 30-kb coding region containing nine exons and a 93-kb regulatory region containing 10 untranslated exons I and is located in the chromosome 15q21.2 region (28-30). Aromatase is expressed in many tissues including the ovaries (31), muscle, skin, adipose tissue (32-34), placenta (35), and bone (36-40). The unusually large regulatory region contains 10 tissue-specific promoters that are alternatively used in various cell types (28, 29). Each promoter is regulated by a distinct set of regulatory sequences in the DNA and transcription factors, which bind to these specific sequences. The promoters specific for the ovary tissues (PII), bone tissues (I.4 and I.6), brain (I.f), endothelial cells (I.7), fetal tissues (I.5), and placenta (2a, I.1) are localized in tandem ~0, 73, 1, 33, 36, 43, 78, and 93 kb upstream of the ATG translational start site in exon II. The majority of aromatase transcripts in bone cells and tissues contain exon 1.4 and exon 1.6 in the 5'-untranslated region (5′-UTR) (Fig. 2).

Expression of aromatase in bone tissues

Multiple usage in the aromatase promoter has been well established and among them aromatase exon I.4 and I.6 show high activity in bone and bone cells. Indeed, osteoblast-specific aromatase gene expression in transgenic mice increases bone mass (41). Several groups have shown that bone tissue, as well as osteoblast-like cells in primary culture, expresses aromatase and that this expression is up-regulated in some physiological or pathological conditions such as bone fracture or osteoporosis, probably under conditions in which osteogenesis is stimulated (39, 42, 43).

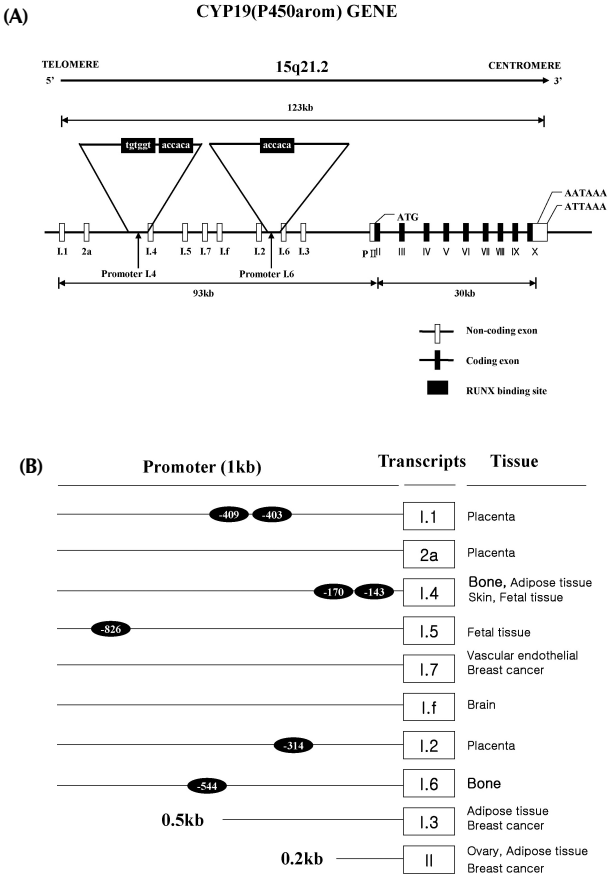

Fig. 2. Genome structure of aromatase gene and Runx binding sites of aromatase promoter found in various human tissues. (A) Structure of the human aromatase gene. Promoter (P) I.4 and I.6 are indicated as arrows. (B) Using the Transfac database and the TF search, the Runx binding site was identified in tissue-specific promoters that are alternatively used in various tissue types. Ellipses indicate the position of the Runx binding site.

With regard to the expression of aromatase in osteoblasts, many factors have been identified as a positive regulator for aromatase gene expression. For example, TGF-β1 enhances the expression of aromatase through promoter I.4 (43). Dexamethasone stimulates aromatase activity as well as the expression of aromatase mRNA in isolated human osteoblast cells (36). Oncostatin M and Forskolin strongly stimulates aromatase expression together with dexamethasone in human osteoblasts (44, 45). IL-1β is one of the most potent stimulators of aromatase expression in osteoblast-like cells obtained from the human fetus and the presence of dexamethasone is necessary for the induction of aromatase by this cytokine (39). IL-1β also directly enhances the mRNA expression and activity of aromatase in the human osteoblast cell line, HOS (46). Several potential cyto-

kines such as IL-1β, TNFα and TGFβ regulate the aromatase activity of osteoblast-like cells in the presence of dexamethasone (43, 47). Moreover, 1,25-Dihydroxyvitamin D_3 enhances the enzyme activity and gene expression of aromatase in the presence of dexamethasone (48). Recently, Runx2 was shown to stimulate aromatase gene expression in skeletal tissues (49). In bone and osteoblast cells, positive regulation of aromatase activity by glucocorticoid, Vitamin D_3, and Runx2 may contribute to the local production of estrogens, thus leading to protective effects against osteoporosis especially after menopause.

The importance of the aromatase in bone has been well characterized by two groups using aromatase deficient mice and both have reported osteoporotic phenotypes in these mice although the sexual dimorphic response reported by these two groups was somewhat inconsistent (50-52).

Interrelationship between Runx2 and estrogen synthesis pathway

Runx2 is a master regulator of osteoblast differentiation. Consistent with its role in the physiological control of osteoblastogenesis, Runx2 anabolically modulates bone formation and controls transcriptional signaling pathways that are linked to PTH (16), glucocorticoids (53) and vitamin D_3 (54). Runx2 is also a strong positive regulator of aromatase gene expression in osteoblastic cells (49). Runx2 was directly interact with the aromatase gene promoter and stimulated aromatase gene expression in a transient transfection assay. Estrogen production was elevated by forced expression of Runx2 in several osteoblastic cells. In contrast, estrogen production was decreased in bone marrow stromal cells derived from Runx2 heterozygous mice. Aromatase expression in the perichondrial and periosteal area was drastically decreased in Runx2 null mice (49). Therefore, aromatase gene expression was well correlated with the level of Runx2 expression in osteoblastic cells. Runx2 mediated increase in cellular estrogen production through upregulation of bone-specific aromatase gene expression may contribute to the maintenance of bone mass in conjunction with endocrine effects. Indeed, both cortical bone thickness and trabecular bone mineral density were lower in Runx2 heterozygous mice compared to wild type mice. Together, these results indicate that aromatase is a downstream target the Runx2 gene, and that Runx2 functions as a positive regulator for aromatase gene expression and estrogen production.

Positive and negative feedback loop between Runx2 and estrogen synthesis pathway

Recently, ERα was shown to directly interact with Runx2 and modulate its transcriptional function in the presence of estrogen (55). Interestingly, ERα inhibited Runx2 transactivation function and this inhibition was further enhanced in the presence of 17β-estradiol (49). These results suggest that local production of estrogen by Runx2 through upregulation of aromatase in bone can be regulated by both a positive and negative feedback loop, which can further contribute to the maintenance of bone homeostasis (Fig. 3). In addition to the interrelationship between Runx2 and ERα, Runx2 also controls the expression of genes involved in sterol/steroid metabolism and GPR30/GPER, a cell surface G-protein-coupled receptor for estrogen, which are important for osteoblast proliferation (56, 57). Runx2 is expressed in not only skeletal tissues but also in nonskeletal tissues such as ovary, testis and brain (58). Because the expression of Runx2 is not confined to skeletal tissues, the functional significance of Runx2 may also include modulation of aromatase gene expression in these new organs.

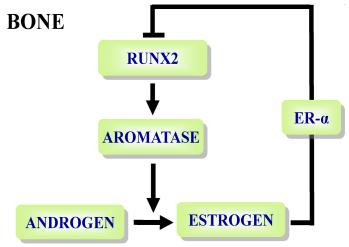

Fig. 3. Interrelationship between Runx2 and the estrogen pathway. Runx2 increases aromatase gene expression and results in estrogen production. Upregulated estrogen can inhibit Runx2 transactivation function through ERα.

CONCLUSION

Aromatase, a key enzyme for estrogen synthesis from androgen, is a Runx2 downstream target gene and local production of estrogen in bone may in part be due to the regulation of Runx2 mediated aromatase gene expression. Collectively, these results indicate a possible functional link between aromatase and Runx2 and a component of a physiological regulatory network between Runx2 and the estrogen pathway that can contribute to skeletal development and bone homeostasis.

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