Camacho-Hübner C, Nilsson O, Sävendahl L (eds): Cartilage and Bone Development and Its Disorders.
Endocr Dev. Basel, Karger, 2011, vol 21, pp 42–51

Estrogen Signaling in Growth Plate Cartilage

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Abstract

It is well known that sex steroids, in particular estrogens, play an important role in longitudinal bone growth during puberty. High doses of estrogen therapy can reduce the final height of an individual, but such treatment is also associated with severe side effects. At the same time, attenuation of estrogen production by aromatase inhibitors increases adult final height, inhibiting bone turnover, which influences bone architecture and may increase the risk for vertebrae fractures. SERMs, which display either estrogenic and/or antiestrogenic effects, bind to ERs with different affinities and subsequently recruit comodulators of transcription in a tissue-specific manner.

The physiological effects of estrogens are mediated primarily by two known nuclear receptors, ERα [1, 2] and ERβ [3], each containing 6 domains (A–F) as detailed in figure 1. ERα and ERβ act as classical ligand-activated transcription factors, residing in the cytosol until binding their ligand and then being translocated into the nucleus, where the estrogen-ER complex interacts with EREs in the promoter regions of target genes. In addition, estrogens bind to subpopulations of ERα and ERβ associated with the plasma membrane, thereby rapidly activating a variety of intracellular signaling cascades.

Recently, a membrane-bound G protein-coupled estrogen receptor (GPR30) that rapidly mediates estrogen signaling was identified [4]. This receptor is expressed at high levels in the hypertrophic zone, and the expression level decreases during pubertal progression, suggesting that GPR30 is involved in modulating longitudinal bone growth [5]. The observation that estrogen treatment of mice lacking this receptor *(GPR30–/–),* does not influence the growth plate height or femur length

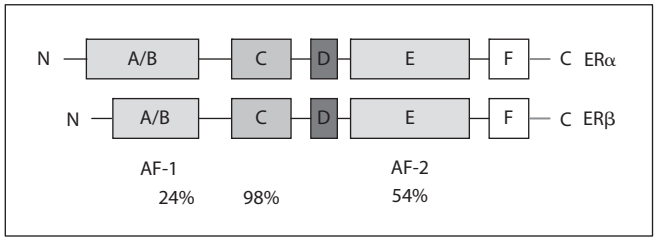

Fig. 1. Structure of the estrogen receptors. These receptors contain 6 domains (A–F). From the N to the C terminus: the A/B domain mediates ligand-independent activation (AF-1); the C domain contains the DNA-binding domain that binds to EREs in target genes; the D domain is a hinge that provides flexibility between the C and N termini. The E and F domains contain the site that binds estrogen and estrogenic compounds. The AF-1 region at the N terminus and the AF-2 region within the ligand-binding domain are involved in ligand-independent and ligand-dependent transcriptional activation, respectively. The percentages of homology between the A/B, C and E domains in these two isoforms are indicated below these domains.

[6] indicates that GPR30 is required for normal estrogenic responses in the growth plate.

Classical Mechanism of Ligand-Dependent ER Action

Ligand binding to the ER induces a conformational change that promotes ligand-receptor dimerization, and this dimeric complex is then translocated into the nucleus, where it binds to a specific ERE in the promoter regions of responsive genes and activates their transcription.

ERE-Independent Activation of Transcription

Studies have demonstrated that ERs can regulate transcription without binding directly to DNA through protein-to-protein interactions with a complex of transcription factors that have direct contact with DNA. In this way, ERs regulate the expression of a large number of genes that do not contain EREs. This mechanism, which is common among members of the nuclear receptor superfamily, is often referred to as transcriptional cross talk [7].

Ligand-Independent Effects on Transcription

Certain growth factors activate protein kinase cascades that lead to the phosphorylation and activation of nuclear ERs bound to EREs.

Nongenomic Action

Membrane E2-ER complexes activate protein kinase cascades, thereby altering the functions of various cytoplasmic proteins and/or regulating gene expression through phosphorylation and activation of transcription factors.

Endogenous Estrogen Production by Chondrocytes

Chondrocytes appear to synthesize estrogen, both in vivo and in vitro [8–10], which is in agreement with the observation that the P450 aromatase is expressed in growth plate cartilage of both humans [8] and rats [10, 11]. Moreover, local estrogen production has been reported to be essential for chondrocyte proliferation and protecting these cells from undergoing spontaneous cell death and thereby the maintenance of longitudinal bone growth [11].

Effects of Estrogen Deficiency on Bone Growth

The basic assumption that estrogen is the sex steroid primarily responsible for regulating pubertal growth in girls, while in boys this regulation is mediated primarily by androgens was challenged by Smith and his colleagues in the 1990s. These investigators described the unique case of a 28-year-old male patient referred to the surgeon for progressive genu valgum, whose radiological examination revealed unfused epiphyses, a bone age of 15 years and a lumbar spine BMD that was 3 SD below the appropriate control mean. He was 204 cm tall, had an arm span of 213 cm and he was still growing slowly during the third decade of his life. Transdermal treatment with high doses of ethinyl E2 for 6 months neither improved his total BMD nor advanced his bone age [12].

DNA analysis revealed a homozygous mutation in exon 2 of this patient's ERα gene, resulting in a substitution of thymine for cytosine. Despite his normal serum levels of androgen, this genetic estrogen resistance was associated with a delay in skeletal maturation and continuing increase in height into adulthood [12]. Histomorphometrical analysis of this patient's bones revealed that disruption of ERα actions markedly affected their mineral content and structure, but not the periosteal circumference [13].

In 1995, a male and a female sibling with a similar phenotype appearance were described, but in these cases the genetic defect was due to a homozygous mutation in exon IX of the aromatase P450 (CYP19) gene that led to high levels of androgens, but unlike the patient described by Smith et al. [12, 13], this patient had undetectable levels of serum E2 [14, 15] and responded to therapy with conjugated estrogen. These observations indicate that androgen alone is not sufficient to promote skeletal

maturation and maintain bone mass and that estrogens play a pivotal role in bone mineralization in both males and females. The local expression of ERs by growth plate chondrocytes of various animal species [16, 17] indicates that estrogens exert direct effects in this context.

Estrogen Induces Fusion of the Growth Plate in Humans

Clinical cases of estrogen deficiency due to a defect in the synthesis of estrogen or function of its receptor clearly illustrate the significance of this sex hormone in regulating the pubertal growth spurt and growth plate fusion in both girls and boys [12, 14, 18]. When a child approaches the end of his or her period of growth, the growth plate thins and the rate of growth gradually declines, and finally ceases when the growth plate has vanished completely. High-dose treatment of children with E2 promotes early fusion of the growth plate via the same processes that normally occur late in puberty [19].

The mechanism by which estrogens promote epiphyseal fusion is not well understood, but there is an alternative hypothesis based on a study in rabbits. Unlike what happens in small rodents, the growth plate in rabbits fuses at the time of sexual maturation or upon estrogen treatment [20]. This hypothesis proposes that the growth plate undergoes programmed senescence, involving reductions in the rates of growth and chondrocyte proliferation, as well as the number and size of chondrocytes [20]. Moreover, growth plate fusion appears to be triggered when the proliferative capacity of the chondrocytes located there is fully exhausted.

Estrogen Treatment for Modulation of Bone Growth

When administered during adolescence, high-dose estrogen treatment has been reported to effectively reduce adult height in constitutionally tall girls [21, 22]. However, the long-term effects of such treatment are now being recognized. Today, it is known that high-dose estrogen therapy may negatively influence fertility later in life [23], increase the risk for deep vein thrombosis [24], as well as, possibly, increase the risk for breast and gynecological cancers [25]. In contrast, treatment of idiopathic short stature by blocking estrogen biosynthesis successfully delays skeletal maturation and enhances adult height without serious side effects [26]. However, a recent study in prepubertal boys treated for 6 months with an aromatase inhibitor to improve their adult final height revealed that the elevated bone resorption caused by this drug was not paralleled by an increase in bone formation [27]. Moreover, there is a strong correlation between serum levels of E2 and BMD in males [28], once again emphasizing the importance of this sex hormone for bone health in both females and males.

Lessons from Mice Lacking ERα and/or ERβ

To gain further insight into the role played by each individual ER, ERKO, BERKO or DERKO mice strains have been generated [29–31]. ERKO mice generated in the laboratory of Dr. Smithies [32–37] express a truncated form of ERα produced by alternative splicing [38], whereas BERKO mice generated in Dr. Smithies' laboratory express no ERβ at all. An ERKO mouse strain generated in the laboratory of Dr. Chambon [39, 40] is considered to be ERα-null, with no ER activity, whereas BERKO mice from the same group express isoforms of ERβ.

The femur length of adult or elderly female ERKO mice has been reported to be either normal [39, 40] or reduced [33, 41]. On the other hand, male ERKO mice show decreased bone growth, both as adults [35] and upon aging [41]. In male BERKO mice bone growth is unaffected throughout development [35–37, 39], while in adult female BERKO mice bone length is increased [32–33, 37]. Interestingly, in male DERKO mice bone growth is decreased to the same extent as in the ERKO strain [35], while in females, DERKO animals have longer bones than ERKO [32–33]. Altogether, these observations suggest that ERβ has a negative impact on longitudinal bone growth. The difference between the phenotype of the unique clinical case with a mutated ERα and the knockout mouse models might reflect species differences with regard to the functions of ER isoforms. Moreover, the diverse phenotypes of male and female ER-knockout mice indicate the existence of sex-related differences in ER functions.

Direct Effects of Estrogens on Chondrocytes

Indeed, the expression of ERα [42], ERβ [16] and GPR30 [5] by human epiphyseal chondrocytes supports direct actions of estrogens on these cells. However, at physiological levels E2 does not influence cell proliferation and viability, synthesis of type X collagen, alkaline phosphatase activity, or matrix calcification in primary cultures of resting, proliferating, and prehypertrophic chondrocytes derived from the bovine fetal epiphyseal growth plate [43]. The effect of estrogens on longitudinal bone growth in vivo, but not in vitro suggests the presence of a cofactor in vivo which is lacking in cultures or, alternatively, that local production of estrogen in cultures masks the effects of exogenous estrogens [11].

Selective Estrogen Receptor Modulators

Thanks to their selective effects, certain SERMs can be used to prevent or treat diseases caused by estrogen deficiency, such as osteoporosis, without most of the undesirable side effects of estrogens. Conversely, other SERMs act as selective estrogen