Molecular and Cellular Endocrinology xxx (2015) 1–7

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Estrogen-Related Receptors and the control of bone cell fate

Julie Carnesecchi [1], Jean-Marc Vanacker*

Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Université Lyon I, CNRS UMR5242, Ecole Normale Supérieure de Lyon, Lyon, France

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form
23 June 2015
Accepted 17 July 2015
Available online xxx

Keywords:
Mesenchymal cells
Estrogen-Related Receptors
Bone cells
Bone loss

ABSTRACT

Bone loss is naturally occurring in aging males and females and exacerbated in the latter after menopause, altogether leading to cumulative skeleton fragility and increased fracture risk. Two types of therapeutic strategies can be envisioned to counteract age- or menopause-associated bone loss, aiming at either reducing bone resorption exerted by osteoclasts or, alternatively, promoting bone formation by osteoblasts. We here summarize data suggesting that inhibition of the Estrogen-Related Receptors α and/or γ could promote bone formation and compensate for bone loss induced by ageing or estrogen-deficiency.

Schematically, bone is composed of two main cell types: the osteoclasts are bone-resorbing cells of hematopoietic origin, whereas the osteoblasts (and their bone-embedded derivatives, the osteocytes) are bone-forming cells that derive from mesenchymal stem cells. Bone remodeling is life-lasting equilibrium between bone formation and resorption (see Frenkel et al., 2010; Manolagas and Parfitt, 2010; Khosla, 2013; Manolagas et al., 2013 for reviews) (Fig. 1). Nevertheless, this equilibrium can be disrupted either as a result of pathologies or of naturally occurring processes such as aging or menopause, leading to a relative increase in bone resorption. Eventually, this results in cumulative bone loss (osteoporosis) and in enhanced fracture risk. Despite leading to similar consequences, aging and menopause appear to act through unrelated mechanisms, with the former decreasing osteoblast differentiation and life span, whereas the latter results in derepression of osteoclast differentiation. Treatments against osteoporosis have long aimed at inhibiting bone resorption (anti-catalytic treatments). However, there is an increasing interest in the development of drugs that would promote bone formation (anabolic treatments; reviewed in Marie and Kassem (2011)). In this review we summarize results suggesting that members of Estrogen-Related Receptor subfamily might be appropriate bone anabolic targets.

1. The ERRs as constitutively acting nuclear receptors

Nuclear receptors form a gene/protein family of 48 members in the human and have been defined as ligand-dependent transcription factors (Laudet and Gronemeyer, 2002; Perissi and Rosenfeld, 2005). With few exceptions, they share a common organization comprising two conserved, highly structured domains. A centrally located DNA-binding domain (DBD) encompassing two zinc finger modules mediates direct contact with DNA response elements located in the enhancer/promoter of their target genes. A C-terminal domain, containing up to 12 α-helices arranged in a globular structure, is responsible for ligand fixation (and is hence referred to as ligand-binding domain, LBD) as well as ligand-dependent transcriptional activity. These two domains are linked together by a poorly conserved, so-called “hinge” domain. In addition, some nuclear receptors comprise an additional N-terminally located domain that can mediate ligand-independent transcriptional activation. Schematically, the activity of nuclear receptors such as the Estrogen- or Thyroid hormone Receptors (ERs and TRs, respectively) is regulated *in vivo* by the fixation of a hormone (17β-estradiol or tri-iodothyronine [T3], respectively) in a hydrophobic ligand-binding pocket, located in the heart of the LBD. This induces a conformational change in the LBD that relocates its C-terminal-most helix, thereby forming a contact surface for the recruitment of transcriptional co-factors that will ultimately trigger the expression of downstream target genes (see examples in Wagner et al., 1995; Shiau et al., 1998). In addition, the translocation from the cytoplasm to the nucleus as well as the direct recruitment of some NRs

* Corresponding author.
E-mail address: jean-marc.vanacker@ens-lyon.fr (J.-M. Vanacker).
[1] Present address: Department of Developmental Biology, Centre for Organismal Studies, University of Heidelberg, Germany.

http://dx.doi.org/10.1016/j.mce.2015.07.019
0303-7207/

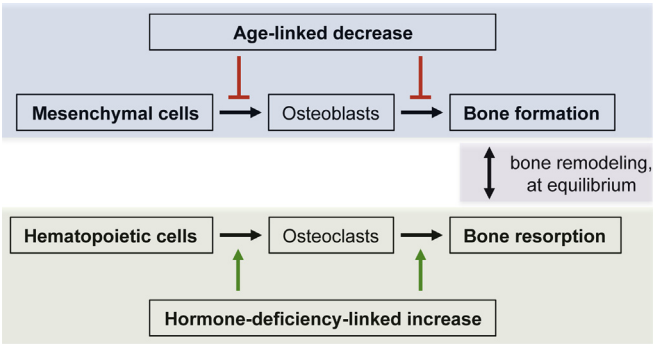

Fig. 1. General mechanisms of bone loss. Age- and hormone-deficiency affect different cellular compartments and eventually result in disruption of equilibrated bone remodeling. Green and red arrows indicate positive and negative actions, respectively. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

(such as the steroid receptors, *e.g.* ERs or Glucocorticoid Receptor) to DNA can be induced by ligand-binding (see for instance Picard and Yamamoto, 1987; Guiochon-Mantel et al., 1991; Métivier et al., 2003).

The existence and (at least part) of the physiological activities of hormones such as 17β-estradiol or thyroid hormone had been explored for decades before the identification, in the mid 80s, of their corresponding receptors (Evans and Mangelsdorf, 2014; Gustafsson, 2015). In contrast, other nuclear receptors have been later isolated (starting in the late 80s), based on their sequence similarity to "classical" receptors. Since no hormone had been previously identified that promoted their activities, these newly isolated receptors were referred to as "orphan" (Giguère, 1999). Subsequent research efforts have allowed to "de-orphanize" a number of these receptors, by identifying natural compounds that specifically bind to them and regulate their activities *in vivo*. However some orphan nuclear receptors are still reluctant to adoption. Strikingly this is the case of the first orphan nuclear receptors identified in Giguère et al. (1988), namely the Estrogen-Related Receptor α and β (ERRα and β), which, together with ERRγ (identified in Hong et al. 1999), form a defined subfamily (Tremblay and Giguère, 2007). Interestingly these receptors display the above-mentioned domain organization, with a DBD that displays an elevated level of sequence identity to the one of the ERs (≈70%), and a more moderately conserved LBD (≈35%) (Horard and Vanacker, 2003). ERRs can bind directly as homo- or heterodimers (*e.g.* ERRα/γ) on specific DNA response elements that are distinct from those mediating the classical estrogen response (Vanacker et al., 1999a, 1999b; Dufour et al., 2007; Deblois et al., 2009; Takacs et al., 2013). As evidenced by structural analysis, their LBD includes a putative ligand-binding pocket, which is much smaller than that of the ERs and is crossed by the side-chain of a phenylalanine residue (Chen et al., 2001; Greschik et al., 2002; Kallen et al., 2004). This is thought to confer a certain level of rigidity to the LBD and to lock the receptor in an active conformation where the helix 12 is constitutively positioned so as to contact co-activators, even in the absence of compound filling the ligand-binding pocket (Devarakonda et al., 2011; Takacs et al., 2013). In other terms (and although structural data have so far only been obtained for ERRα and γ, not for β), it is likely that the ERRs regulate transcription in constitutive, ligand-independent manner, thereby betraying the current definition of nuclear receptors. However, one cannot exclude the existence of *bona fide* ligands that could regulate the activities of these receptors *in vivo*, by inducing conformational changes.

In this respect, synthetic ligands have been identified that modulate the activities of the ERRs. For instance 4-OH-tamoxifene (the active metabolite of tamoxifene, a selective estrogen receptor modulator [SERM] that is widely used in breast cancer therapy) and DY131 respectively decreases or increases the activities of both ERRβ and γ (Coward et al., 2001; Tremblay et al., 2001; Yu and Forman, 2005). A special mention should be made of Bisphenol A (BPA, an endocrine disruptor) that, on its own, appears inactive toward ERRγ but prevents the inactivation of the receptor by compounds such as 4-OH-tamoxifene in cell culture based assays (Takayanagi et al., 2006; Matsushima et al., 2007). With the exception of pyrido[1,2-α]pyrimidine-4-ones derivatives that have recently been shown to increase ERRα transcriptional activities (Peng et al., 2011), mainly deactivating compounds have been identified to impact on this receptor (Yang and Chen, 1999; Willy et al., 2004; Chisamore et al., 2009a, 2009b; Duellman et al., 2010). This is for instance the case of XCT790 which enters the ligand binding pocket, disrupt the organization of the LBD and prevents co-activator recruitment, in addition to inducing proteasome-dependent degradation of the receptor (Willy et al., 2004; Kallen et al., 2007; Lanvin et al., 2007). Recently the C29 compound, a demonstrated ERRα inverse agonist *in vitro* has been shown to exert *in vivo* effects that resemble those resulting from the absence of the receptor (Patch et al., 2011; Chaveroux et al., 2013). This suggests a specific effect through ERRα, although a lack of effect of C29 in ERRαKO mice has not been presented. In the zebrafish embryo, BPA induces, in an ERRγ-dependent manner, an aggregation of the otoliths (mineralized structures of the inner ear that are involved in the maintenance of equilibrium) (Tohmé et al., 2014). In spite of the partial lack of clear *in vivo* data, the identification of (de)activating ligands for the ERRs suggests that they could be targeted through a pharmacological approach in order to modulate their activities. The identification of ERR-ligands would open promising avenues for the treatment of given pathologies.

In addition, diverse levels of possible regulation of ERRs' activities have been suggested. For instance, ERK8 relocates ERRα in the cytoplasm, thereby inhibiting its DNA binding and transactivation capabilities (Rossi et al., 2011). Moreover, ERRα and γ are subjected to post translational modifications (sumoylation, phosphorylation, acetylation), some which having been shown to impact on the receptor activity (Barry and Giguère, 2005; Vu et al., 2007; Tremblay et al., 2008; Wilson et al., 2010; Kim et al., 2014). One of the key levels of regulations seems however to be represented by the capacity of contacting given co-activators, which can be expressed in a cell-type specific manner. For instance members of the PGC-1 family of transcriptional co-activators have been shown to be instrumental in promoting ERR-based response (at least ERRα and γ), in particular in metabolism-related processes (Villena and Kralli, 2008; Giguère, 2008).

2. Functions of the ERRs

A number of physiopathological functions have been ascribed to the ERR receptors. ERRβ is mainly expressed in the embryo and analysis of knock out mice showed that the receptor regulates placental formation, the maintenance of pluripotency of embryonic stem cells, and the specification of epithelial cells in the inner ear (Luo et al., 1997; Chen and Nathans, 2007; Chen et al., 2008). ERRα and γ are strongly expressed in tissues displaying a high energy demand (*e.g.* liver, heart, muscle and fat) where they govern energetic metabolism, regulating processes such as lipid uptake, fatty acid oxidation, neoglucogenesis, tricarboxylic acid cycle and oxidative phosphorylation (Villena and Kralli, 2008; Giguère, 2008; Deblois and Giguère, 2011). Analyses of knock out mice combined

Please cite this article in press as: Carnesecchi, J., Vanacker, J.-M., Estrogen-Related Receptors and the control of bone cell fate, Molecular and Cellular Endocrinology (2015), http://dx.doi.org/10.1016/j.mce.2015.07.019