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# Estrogen-Related Receptors and the control of bone cell fate

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Abstract: Bone loss is naturally occurring in ageing 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  $\alpha$  and/or  $\gamma$  could promote bone formation and compensate for bone loss induced by ageing or estrogen-deficiency.



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Lyon May 25th 2015,

Dear Editor

Please find a review entitled “Estrogen-Related Receptors and the control of bone cell fate” by Julie Carnesecchi and JM Vanacker, that is our tentative contribution to the special issue “Bone disorders: mechanisms and targets” of *Molecular and Cellular Endocrinology*.

In this review we tried to describe as exhaustively as possible the data concerning the effects of Estrogen-Related Receptors on the pathways and mechanisms of differentiation of mesenchymal cells. Although we focus on bone cells (osteoblasts and osteoclasts) as requested for this special issue, we indeed chose to extend the data we reviewed to mesenchymal cells, feeling a broader point of view would be useful to the reader.

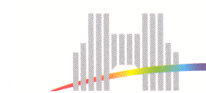
Hoping our review will be considered in a favorable manner.

Sincerely,

JM Vanacker

Possible reviewers of our manuscript include

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***Estrogen-Related Receptors and the control of bone cell fate.***

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keywords: mesenchymal cells; Estrogen-Related Receptors; bone cells; bone loss.

1 **ABSTRACT**

2

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

10

11 **MAIN TEXT**

12 ***The ERRs as constitutively acting nuclear receptors***

13

14 Nuclear receptors form a gene/protein family of 48 members in the human and have  
15 been defined as ligand-dependent transcription factors (Laudet and Gronemeyer, 2002;  
16 Perissi and Rosenfeld, 2005). With few exceptions, they share a common organization  
17 comprising two conserved, highly structured domains. A centrally located DNA-binding  
18 domain (DBD) encompassing two zinc finger modules mediates direct contact with DNA  
19 response elements located in the enhancer/promoter of their target genes. A C-terminal  
20 domain, containing up to 12  $\alpha$ -helices arranged in a globular structure, is responsible for  
21 ligand fixation (and is hence referred to as ligand-binding domain, LBD) as well as ligand-  
22 dependent transcriptional activity. These two domains are linked together by a poorly  
23 conserved, so-called “hinge” domain. In addition, some nuclear receptors comprise an  
24 additional N-terminally located domain that can mediate ligand-independent transcriptional  
25 activation. Schematically, the activity of nuclear receptors such as the Estrogen- or Thyroid  
26 hormone Receptors (ERs and TRs, respectively) is regulated *in vivo* by the fixation of a  
27 hormone (17 $\beta$ -estradiol or tri-iodothyronine [T3], respectively) in a hydrophobic ligand-  
28 binding pocket, located in the heart of the LBD. This induces a conformational change in the  
29 LBD that relocates its C-terminal-most helix, thereby forming a contact surface for the  
30 recruitment of transcriptional co-factors that will ultimately trigger the expression of  
31 downstream target genes. In addition the recruitment of some NRs (such as ERs, but not  
32 TRs) to DNA can be induced by ligand-binding.

33 The existence and (at least part) of the physiological activities of hormones such as  
34 17 $\beta$ -estradiol or thyroid hormone had been explored for decades before the identification, in  
35 the mid 80s, of their corresponding receptors (Evans and Mangelsdorf, 2014; Gustafsson,  
36 2015). In contrast, other nuclear receptors have been later isolated (starting in the late 80s),

37 based on their sequence similarity to “classical” receptors. Since no hormone had been  
38 previously identified that promoted their activities, these newly isolated receptors were  
39 referred to as “orphan” (Giguère, 1999). Subsequent research efforts have allowed to “de-  
40 orphanize” a number of these receptors, by identifying natural compounds that specifically  
41 bind to them and regulate their activities *in vivo*. However some orphan nuclear receptors are  
42 still reluctant to adoption. Strikingly this is the case of the first orphan nuclear receptors  
43 identified in 1988 (Giguère et al.), namely the Estrogen-Related Receptor  $\alpha$  and  $\beta$  (ERR $\alpha$   
44 and  $\beta$ ), which, together with ERR $\gamma$  (identified in 1999, Hong et al.), form a defined subfamily  
45 (Tremblay and Giguère, 2007). Interestingly these receptors display the above-mentioned  
46 domain organization, with a DBD that displays an elevated level of sequence identity to the  
47 one of the ERs, and a more moderately conserved LBD (Horard and Vanacker, 2003). As  
48 evidenced by structural analysis, the latter includes a putative ligand-binding pocket, which is  
49 much smaller than that of the ERs and is crossed by the side-chain of a phenylalanine  
50 residue (Chen et al., 2001; Greschik et al., 2002; Kallen et al., 2004). This is thought to  
51 confer a certain level of rigidity to the LBD and to lock the receptor in an active conformation  
52 where the helix 12 is constitutively positioned so as to contact co-activators, even in the  
53 absence of compound filling the ligand-binding pocket (Takacs et al., 2013). In other terms  
54 (and although structural data have so far only been obtained for ERR $\alpha$  and  $\gamma$ , not for  $\beta$ ), it is  
55 likely that the ERRs regulate transcription in constitutive, ligand-independent manner,  
56 thereby betraying the current definition of nuclear receptors. However, one cannot exclude  
57 the existence of *bona fide* ligands that could over-promote or reduce the activities of these  
58 receptors *in vivo*, by inducing conformational changes. In this respect, synthetic ligands have  
59 been identified that modulate the activities of the ERRs. For instance 4-OH-tamoxifene (a  
60 selective estrogen receptor modulator [SERM] that is widely used in breast cancer therapy)  
61 decreases the activities of ERR $\gamma$  (Coward et al., 2001; Tremblay et al., 2001) whereas  
62 DY131 increases the activities of both ERR $\beta$  and  $\gamma$  (Yu and Forman, 2005). A special  
63 mention should be made of Bisphenol A (BPA, an endocrine disruptor) that, on its own,  
64 appears innocuous toward ERR $\gamma$  but prevents the inactivation of the receptor by compounds  
65 such as 4-OH-tamoxifene in cell culture based assays (Takayanagi et al., 2006; Matsushima  
66 et al., 2007). In contrast to ERR $\beta$  and  $\gamma$ , only deactivating compounds have been identified  
67 for ERR $\alpha$  to date (Yang and Chen, 1999; Willy et al., 2004; Chisamore et al., 2009a;  
68 Chisamore et al., 2009b; Duellman et al., 2010). This is for instance the case of XCT790  
69 which enters the ligand binding pocket, disrupt the organization of the LBD and prevents co-  
70 activator recruitment, in addition to inducing proteasome-dependent degradation of the  
71 receptor (Willy et al., 2004; Kallen et al., 2007; Lanvin et al., 2007). Few of these compounds  
72 have been evaluated *in vivo* for a specific ERR-dependent effect, although recent data show

73 that BPA induces otolith aggregation in the zebrafish embryo in an ERR $\gamma$ -dependent manner  
74 (Tohmé et al., 2014). In spite of the lack of clear *in vivo* data, the identification of  
75 (de)activating ligands for the ERRs suggests that they could be targeted through a  
76 pharmacological approach in order to modulate their activities. The identification of ERR-  
77 ligands would open promising avenues for the treatment of given pathologies.

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

89

## 90 ***Functions of the ERRs***

91

92 A number of physiopathological functions have been ascribed to the ERR receptors.  
93 ERR $\beta$  is mainly expressed in the embryo and regulates placental formation, the maintenance  
94 of pluripotency of embryonic stem cells, and the specification of epithelial cells in the inner  
95 ear (Luo et al., 1997; Chen and Nathans, 2007; Chen et al., 2008). ERR $\alpha$  and  $\gamma$  are strongly  
96 expressed in tissues with a high energy demand (*e.g.* liver, heart, muscle and fat) where they  
97 govern energetic metabolism, regulating processes such as lipid uptake, fatty acid oxidation,  
98 neoglucogenesis, tricarboxylic acid cycle, oxidative phosphorylation, and mitochondrial  
99 biogenesis and activities (Villena and Kralli, 2008; Giguère, 2008; Deblois and Giguère,  
100 2011). These functions strongly depend on the PGC-1 co-activators and are also exerted  
101 during somatic reprogramming to pluripotency (Kida et al., 2015) as well as in cancer cells  
102 (reviewed in Chang and McDonnell, 2012; Bianco et al., 2012; Deblois and Giguère, 2013;  
103 Deblois et al., 2013). For instance, ERR $\alpha$  induces, in advanced breast cancers and in a  
104 PGC-1 $\beta$ -dependent manner, a transcriptional cascade leading to the switch from oxidative to  
105 glycolytic metabolism referred to as the Warburg effect which is instrumental in cancer  
106 progression (Eichner et al., 2010; Chang et al., 2011; Cai et al., 2013). ERR $\gamma$  apparently  
107 displays converse activities, promoting oxidative rather than glycolytic metabolism thus  
108 potentially reducing cancer aggressiveness (Eichner et al., 2010). The activities of the ERRs

109 in cancer cells however extend beyond the regulation of metabolism. Opposite activities of  
110  $ERR\alpha$  and  $\gamma$  have been documented in cancer-related processes such as cell proliferation  
111 and epithelial-to-mesenchymal transition (EMT), which are promoted by  $ERR\alpha$  and reversed  
112 by  $ERR\gamma$  (Yu et al., 2007; Tiraby et al., 2011; Bianco et al. 2012; Lam et al., 2014). In  
113 addition  $ERR\alpha$  promotes cell migration and invasion as well as the establishment of distant  
114 metastasis in a manner that apparently does not directly rely on energy metabolism (Dwyer  
115 et al., 2010, Fradet et al., 2011; Sailland et al., 2014). In line with these functions, high  
116 expression of  $ERR\alpha$  is considered as a factor of poor prognosis in diverse cancers (including  
117 from the breast), whereas  $ERR\gamma$  constitutes a favorable biomarker (Ariazi et al., 2002; Suzuki  
118 et al., 2004; reviewed in Bianco et al., 2012).

119

### 120 ***ERRs and mesenchymal cell fate***

121

122 Mesenchymal stem cells can differentiate into various lineages including myocytes,  
123 adipocytes, chondrocytes and osteoblasts. Strikingly an array of data indicates that  $ERR\alpha$   
124 and/or  $\gamma$  modulate the differentiation of all these lineages, suggesting that they may be key  
125 regulators of mesenchymal stem cell fate.  $ERR\alpha$  expression increases during the transition  
126 from myoblasts to myotubes (Bonnelye et al., 1997) and stimulates the expression of  
127 *Dusp1/MKP1*, which in turn dephosphorylates ERK1/2 (Murray and Huss, 2011). In the  
128 absence of  $ERR\alpha$ , ERK1/2 signaling is therefore aberrantly increased and results in  
129 sustained proliferation and delayed differentiation. Interestingly  $ERR\gamma$  expression is also  
130 strongly enhanced when myoblasts differentiate into myotubes (Murray and Huss, 2011).  
131 The receptor increases mitochondrial content and promotes oxidative programs *in vivo*,  
132 including in mouse model of muscular dystrophy suggesting that it may constitute a  
133 promising target against muscle wasting (Narkar et al., 2011; Murray et al., 2013; Matsakas  
134 et al., 2013).

135 Human and mouse mesenchymal cells in which  $ERR\alpha$  has been inactivated display  
136 reduced adipocytic differentiation capacities (Ijichi et al., 2007; Delhon et al., 2009; Rajalin et  
137 al., 2010; Carnesecchi and Vanacker, 2013). This is consistent with the reduced fat deposit  
138 and the decreased number and size of adipocytes as well as reduced marrow fat volumes  
139 that have been observed in  $ERR\alpha^{-/-}$  mice (Luo et al., 2003; Delhon et al., 2009).  $ERR\alpha$   
140 expression increases during human adipocyte differentiation supporting the hypothesis of a  
141 contribution of the receptor to this process (Delhon et al., 2009). Interestingly, it has recently  
142 been shown that miR125a directly targets  $ERR\alpha$  to inhibit its expression in porcine  
143 preadipocytes (Ji et al., 2014). Furthermore, miR125a expression decreases during  
144 adipocytic differentiation, suggesting a possible mechanism for the regulation of  $ERR\alpha$



145 expression during this phenomenon. ERR $\gamma$  expression also increases during preadipocyte  
146 differentiation *in vitro* and the receptor promotes the expression of markers of adipocytic  
147 differentiation as well as intracellular fat accumulation (Kubo et al., 2009).

148 ERR $\alpha$  is expressed in rat cartilage *in vivo* and chondrogenic cell lines *in vitro* and has  
149 been suggested to positively modulate chondrocytic differentiation *in vitro* through a possible  
150 effect on the expression of Sox9 (the master gene for chondrocyte differentiation) (Bonnelye  
151 et al., 2007). A recent report addressed the *in vivo* effect of ERR $\gamma$ , which was specifically  
152 overexpressed in the cartilage of transgenic mice (Cardelli et al., 2013). The resulting  
153 animals displayed a mild dwarfism attributed to a reduced thickness of the proliferative zone  
154 of long bones. This is likely due to a disequilibrium of the balance between proliferation and  
155 differentiation of the chondrocytes of the growth plate. Indeed, ERR $\gamma$  overexpression was  
156 found to result in increased expression of the p27 cell cycle inhibitor thereby reducing  
157 chondrocyte proliferation. It is possible that this reduced proliferation is the primary event  
158 leading to increased differentiation, accompanied by enhanced expression of functional  
159 chondrocyte markers such as Pth1 receptor and Aggrecan.

160

### 161 ***ERRs and bone cell fate***

162

163 ERR $\alpha$  is strongly expressed in the ossification zones during mouse embryonic  
164 development (Bonnelye et al., 1997). This observation is valid for the long bones as well as  
165 for the flat bones of the skull, suggesting that the receptor may contribute to both  
166 endochondral and intramembranous ossification. ERR $\alpha$  is also highly expressed in  
167 osteoblastic cell lines and normal human bones. The receptor has initially been thought to  
168 promote osteoblast differentiation when overexpressed in *in vitro* experiments (Bonnelye et  
169 al., 2001). However analysis of young (8 weeks old) ERR $\alpha^{-/-}$  mice showed that these animals  
170 did not display an overt bone phenotype (Delhon et al., 2009; Teyssier et al., 2009; reviewed  
171 in Gallet and Vanacker, 2010). Nevertheless, no bone loss was observed in ERR $\alpha^{-/-}$  mice  
172 upon ageing or when females were ovariectomized, in sharp contrast to wild type littermates.  
173 Consistently *in vivo* markers of osteoblastic activity as well as bone formation rate were  
174 found increased in ERR $\alpha^{-/-}$  animals as compared to wild type counterparts. This altogether  
175 suggests that bone loss induced by ageing or estrogen-deficiency is compensated for by a  
176 maintained or increased osteoblast activity in ERR $\alpha^{-/-}$  mice. Pre-osteoblasts from originating  
177 from these animals indeed displayed an enhanced differentiation capacity *ex vivo*, as  
178 evidenced by increased expression of Runx2 (the master gene for osteoblast differentiation)  
179 and downstream functional markers (e.g. alkaline phosphatase, collagen I and osteocalcin).  
180 Cotransfection studies have suggested a complex effect of ERR $\alpha$  on Runx2 promoter activity

181 (Kammerer et al., 2013). Indeed the receptor may act positively or negatively depending on  
182 the presence of PGC-1 $\alpha$  or  $\beta$  as well as on the expression and activity of the Estrogen  
183 Receptor. A similar non-monotonous effect of ERR $\alpha$  has been proposed on Wnt signaling,  
184 which could be repressed or activated, depending on the expression of co-factors (e.g. PGC-  
185 1 family members) (Auld et al., 2012). Although these results have not been verified *in vivo*,  
186 they together suggest a bimodal effect of the receptor which could fine-tune osteoblast  
187 differentiation (far from an all-or-nothing effect). This might depend on the specific expression  
188 and/or mode of co-factor recruitment that will drive the target-specific activity of ERR $\alpha$ . In  
189 addition, it is possible that the *in vivo* consequences of these subtle effects are to be  
190 observed only in the long term, consistent with the appearance of a bone phenotype in  
191 ageing, but not young, ERR $\alpha$ <sup>-/-</sup> mice.

192 The above results were obtained using “complete” knock out models, *i.e.* where  
193 ERR $\alpha$  is inactivated in all cells including in mesenchymal stem cells and progenitors,  
194 suggesting an effect of the receptor during the early phases of osteoblast differentiation. The  
195 role of ERR $\alpha$  in late osteoblast differentiation has also been addressed using a conditional  
196 knock out (cKO) model in which the receptor is only inactivated after the onset of osteoblast  
197 maturation (Gallet et al., 2013). *Ex vivo*, Runx2 activity (but not expression) is enhanced in  
198 differentiating ERR $\alpha$ cKO pre-osteoblasts as compared to wild type, resulting in increased  
199 terminal differentiation, including in terms of mineralizing capacities. Interestingly ERR $\alpha$ cKO  
200 females resist to bone loss induced by estrogen-deficiency but not by ageing. The molecular  
201 mechanisms through which osteoblasts maturation is affected may also involve osteopontin  
202 (*opn*), a positive direct target of ERR $\alpha$  in various cell types, including osteoblasts (Vanacker  
203 et al., 1998, 1999; Delhon et al., 2009; Teyssier et al., 2009; Zirnbigl et al., 2008, 2013;  
204 Boudjadi et al., 2013). Indeed *opn* inhibits mineralization and its inactivation *in vivo* leads to  
205 resistance to the bone loss induced by estrogen deficiency (Yoshitake et al., 1999; Boskey et  
206 al., 2002).

207 Together these data show that ERR $\alpha$  negatively impacts osteoblast differentiation at  
208 two different defined stages (onset and maturation), resulting in two distinct modes of  
209 resistance to bone loss (ageing and estrogen-deficiency). However, future studies are  
210 needed to precisely tackle the molecular mechanisms driving these time-specific activities in  
211 osteoblasts.

212

213 Due to perinatal death ERR $\gamma$ <sup>-/-</sup> mice cannot be studied for their post-embryonic bone  
214 phenotype (Alaynick et al., 2007). However a recent publication showed that ERR $\gamma$ <sup>+/-</sup>  
215 displayed increased trabecular bone as compared to wild type animals (Cardelli and Aubin,  
216 2014). Strikingly this phenotype only affected males but not females. Intriguingly an opposite

217 gender-dependent effect has been observed in the case of  $ERR\alpha^{-/-}$  mice, where only  
218 females, but not males, are resistant to ageing (Teyssier et al., 2009). Although these  
219 phenotypic differences have not been confirmed by an independent study using a different  
220  $ERR\alpha^{-/-}$  mouse strain (Delhon et al., 2009), these data altogether suggest intricate levels of  
221 interferences between ERRs and sex-hormone signaling. Increased trabecular bone in  
222  $ERR\gamma^{+/-}$  mice was readily observed in 8 week-old animals (in contrast to  $ERR\alpha^{-/-}$ ) and  
223 aggravated with age (Cardelli and Aubin, 2014). Similar to  $ERR\alpha^{-/-}$  this phenotype is  
224 associated with increased osteoblast number and activity *in vivo* and enhanced  
225 differentiation capacities of  $ERR\gamma^{+/-}$  *ex vivo*. Interestingly the negative effects of  $ERR\gamma$  on  
226 osteoblast differentiation appear to rely on inhibition of Runx2 signaling. Indeed  $ERR\gamma$   
227 physically interacts with Runx2 and represses its transactivation capacities (Jeong et al.,  
228 2009; Cardelli and Aubin, 2014). An alternative mechanism of action has also been  
229 suggested where  $ERR\gamma$  stimulates the expression of miR-433 (at least in *in vitro* cell  
230 cultures), which in turn, induces the degradation of Runx2 mRNA (Kim et al., 2013).

231

232 In addition to osteoblasts as bone-forming cells, bone remodeling involves another  
233 major cellular component, the osteoclasts, which are bone-resorbing cells of hematopoietic  
234 origin. Data have been published concerning the functions of the  $ERR\alpha$  on osteoclasts. *In*  
235 *vitro*  $ERR\alpha$  does not appear to exert any effect on osteoclast differentiation but promotes  
236 spreading and migration of the cells (Bonnelye et al., 2010). However analysis of  $ERR\alpha^{-/-}$   
237 mice provided contradictory results: deletion of  $ERR\alpha$  *in vivo* may or may not reduce  
238 osteoclast differentiation and activity (Delhon et al., 2009; Teyssier et al., 2009; Wei et al.,  
239 2010). The reason of these discrepancies is unknown. However  $ERR\alpha$  may also exert  
240 indirect effects on osteoclasts. Indeed overexpression of  $ERR\alpha$  in human breast cancer cells  
241 induces the expression of osteoprotegerin (an inhibitor of osteoclastogenesis). Xenografting  
242 these cells in immunodeficient mice results in decreased osteoclast differentiation (Fradet et  
243 al., 2011). No study has, to date, addressed the potential role of  $ERR\gamma$  on osteoclasts, but it  
244 should be mentioned that  $ERR\gamma^{+/-}$  mice do not show any variation in osteoclast activity, as  
245 compared to wild type animals (Cardelli and Aubin, 2014).

246

### 247 **Conclusions and future prospects.**

248

249 In cancer cells  $ERR\alpha$  and  $\gamma$  appear to act in an opposite manner, respectively  
250 promoting and repressing traits of cancer progression. In contrast, the above-mentioned  
251 results strongly suggest that  $ERR\alpha$  and  $\gamma$  exert rather similar effects on cells from  
252 mesenchymal origin, which are summarized on Figure 1. Both receptors indeed promote

253 adipocyte differentiation and inhibit osteoblast differentiation. This suggests that they may act  
254 as switch factors in the choice of differentiation exerted by progenitor cells to engage into  
255 one or the other lineage. The inhibition of osteoblastic commitment appears to be exerted in  
256 a Runx2-dependent manner, impacting on the expression and/or the activity of this  
257 transcription factor. However, this view is challenged by data indicating a possible co-factor  
258 dependent effect and non-monotonous effect of  $ERR\alpha$  on Runx2 and/or Wnt-targets  
259 expression. This last explanation possibly accounts for the delay with which  $ERR\alpha$   
260 inactivation in mice leads to an observable bone phenotype. Nevertheless, a general  
261 hypothesis that may be proposed from published data is that inactivating  $ERR\alpha$  and/or  $ERR\gamma$   
262 should increase bone mass *in vivo* and therefore protect from bone loss, whether originating  
263 from ageing or from estrogen-deficiency (*i.e.* after menopause in humans). However, it  
264 should be noted that the majority of these data (outside few studies using cultures of human  
265 cells) were obtained using rodent models. In the present state of the art it would be therefore  
266 hazardous to claim that deactivation of ERR receptors would be beneficial in human bones.  
267 Nonetheless it is worth noting that deactivating  $ERR\alpha$ , in contrast to  $ERR\gamma$ , is unlikely to lead  
268 to adverse effects in cancers, given that receptor rather promotes cancer aggressiveness.

269 Most studies on the functions of the ERRs on bone have, up to now, focused on the  
270 investigating of autonomous effects. The ERRs are however well known as instrumental  
271 regulators of energy metabolism. How the metabolic activities of the ERRs in “energetic  
272 tissues” impact on bone status and how potential metabolic activities of ERRs within bone  
273 cells influence bone physiology remain to be investigated although hypotheses have started  
274 to emerge (Bonnelye and Aubin, 2013).

275 The vast majority of the data describing the effects of the ERR receptors in bone have  
276 used a genetic approach to overexpress or inhibit the expression of the receptors and  
277 examine the consequences of such a modification. However, if ERR functions in bone  
278 formation are well described, how these effects are exerted needs to be understood,  
279 including in a temporal manner. Targeting the receptors using a pharmacological approach  
280 would however be more convenient. Such a strategy can be envisioned since small  
281 compounds have been identified that deactivate or overactivate the ERRs. The effects of  
282 such compounds in bone *in vivo* remain to be evaluated.

283

284

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286

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290

291

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#### **LEGEND TO FIGURE**

644 **Figure 1: ERRs and mesenchymal cell fate, a summary.**

645 ERR $\alpha$  and  $\gamma$  positively or negatively contribute to the differentiation of mesenchymal cells into  
646 the myocyte, adipocyte, chondrocyte and osteoblast lineages. Green and red arrows  
647 represent positive and negative actions, respectively, on the considered process or gene.

648 See text for details and references.

649

