BoneKEy-Osteovision. 2007 October;4(10):267-272
http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/10/267
DOI: 10.1138/20070275

COMMENTARIES

Estrogen and the Death of Osteoclasts: A Fascinating Story

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Commentary on: Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, Yamamoto Y, Nishina H, Takeda S, Takayanagi H, Metzger D, Kanno J, Takaoka K, Martin TJ, Chambon P, Kato S. Estrogen prevents bone loss via estrogen receptor α and induction of Fas ligand in osteoclasts. *Cell*. 2007 Sep 7;130(5):811-23.

Estrogen deficiency is the major cause of postmenopausal bone loss in women, and decreases in biologically available estrogen levels also contribute to age-related bone loss in men (1). While estrogen treatment (typically combined with a progestin in women with an intact uterus in order to prevent increases in uterine cancer risk) had been widely used to prevent and treat osteoporosis, growing concerns about the effects of estrogen/progestin treatment on the risk of breast cancer, cardiovascular disease, and thromboembolic events (2) have led to a marked decrease in estrogen use. This, in turn, has increased the urgency to better identify the cellular and molecular pathways responsible for the protective effects of estrogen on bone, with the eventual goal of using non-estrogenic compounds (or better selective estrogen receptor modulators, SERMS) to target these pathways. In this context, the elegant study by Nakamura *et al*. (3), published in *Cell*, unequivocally demonstrates that the osteoclast is a major target for estrogen action, at least in trabecular bone. In addition, the finding that the osteoclast apoptosis induced by estrogen may be principally mediated via activation of the Fas/Fas ligand (FasL) system provides a potential pathway that may be modulated by non-estrogenic compounds to prevent or treat osteoporosis.

Effects of estrogen on bone are often considered in the context of estrogen deficiency in postmenopausal women or following ovariectomy in rodents. However, it is important to recognize that the major physiological effects of estrogen, or estrogen deficiency, are likely manifest during skeletal growth and during pregnancy and lactation. For example, girls with gonadal dysgenesis due to Turner's syndrome have reduced bone mass and an increased risk of fractures (4), and the description of estrogen receptor α (ERα)- and aromatase-deficient males who also had reduced bone mass provided compelling evidence for the importance of estrogen during growth of the skeleton, even in males (5-7). During pregnancy, the high circulating levels of estrogen likely have important effects not only on bone, but rather also on extra-skeletal calcium homeostasis, such as intestinal and renal calcium handling (1). Moreover, studies in rodents and humans have now convincingly demonstrated that the suppression of estrogen levels during lactation, combined with increases in PTHrP levels, are responsible for mobilization of calcium from skeletal stores into breast milk (8-10). From an evolutionary perspective, the provision of adequate calcium in breast milk for the needs of the neonatal skeleton is probably the major reason why estrogen deficiency leads to increased bone resorption and skeletal calcium mobilization, and postmenopausal bone loss may simply be an unforeseen consequence of this physiological mechanism as a result of humans now outliving their reproductive usefulness.

Based on these considerations, the findings of Nakamura *et al*. (3) begin to make eminent physiological sense. Reasoning that

the skeletal phenotype of global ERα or ERβ knock out (KO) mice was confounded by changes in circulating sex steroid levels as well as by alterations in concentrations of other hormones, such as IGF-1, leptin, and FSH (11), these investigators used *Cre-lox* technology to specifically (using the *Cathepsin K* promoter to drive the *Cre* recombinase) delete ERα in differentiated osteoclasts, since these cells had been shown by Oursler and colleagues a number of years ago to express ERs (12;13). As expected, and in contrast to mice with global deletion of ERα, the *ERα*(ΔOc/ΔOc) mice had normal circulating levels of testosterone, 17β-estradiol, IGF-1, leptin, and FSH. Thus, the consequences of the deleted ERα specifically in mature osteoclasts could be analyzed *in vivo* without these potential hormonal confounders. A detailed skeletal phenotyping of these mice revealed 1) osteopenia, with increased bone turnover in trabecular bone in female, but not male ERα(ΔOc/ΔOc) mice; 2) no apparent alterations in cortical bone mass in either sex; and 3) in contrast to *ERα*(+/+) females, who lost trabecular bone following 2 weeks of ovariectomy, the *ERα*(ΔOc/ΔOc) mice failed to lose trabecular bone, at least over this time period. Further, using microarray analysis, the investigators found that the activated ERα induced expression of the important mediator of apoptosis, Fas L (which binds to Fas on the cell surface, triggering apoptotic pathways (14)), in osteoclasts in *ERα*(+/+), but not in *ERα*(ΔOc/ΔOc) mice.

To put the findings of this study in physiological context, Figure 1 provides a working model of changes following estrogen deficiency, as well as the potential time course of these changes (probably most evident following acute estrogen withdrawal, as occurs during lactation or following ovariectomy). An important caveat is that while this figure is based on a compilation of findings from rodent and human studies, its main utility is as a working model with clearly testable hypotheses.

Studies from a number of groups in rodents (15) and in humans (16;17) have demonstrated that estrogen stimulates osteoclast apoptosis and suppresses osteoblast/osteocyte apoptosis. Conversely, as depicted in Figure 1A, acute estrogen withdrawal is associated with a decrease in osteoclast apoptosis and an increase in osteoblast apoptosis. Given the role of estrogen deficiency in lactation noted above, this is exactly what the mother (rodent or human) needs in the early days post-partum – a stimulation of bone resorption due to prolongation of the lifespan of the osteoclast (leading to a release of calcium from bone), and a decrease in bone formation due to shortening of the osteoblast lifespan (leading to less calcium returning to the skeleton). Combined with the actions of PTHrP, these changes lead to mobilization of calcium, largely from trabecular bone (8; 18), for the needs of the newborn through the breast milk. Concomitant with these effects, as shown in Figure 1B, a number of studies, principally in rodents (1), have found increases in bone marrow levels of pro-resorptive cytokines (TNF-α, IL-1α, and others). This was also found in the peripheral blood of both *ERα*(+/+) and *ERα*(ΔOc/ΔOc) mice by Nakamura *et al*. (3) at 2 weeks following ovariectomy. These cytokines expand the pool of osteoclast precursors and also increase RANKL expression by osteoblastic and T-cells (1). Finally, as shown in Figure 1C, estrogen deficiency upregulates osteoblastogenesis (19) and expands the numbers of T-cells (20); both osteoblastic and T-cells produce RANKL (21), which leads to enhanced osteoclast development. The changes in Figure 1C likely occur over a longer time frame than the very acute changes in osteoclast and osteoblast apoptosis depicted in Figure 1A. Based on this working model, it is apparent why the *ERα*(ΔOc/ΔOc) mice in the study by Nakamura *et al*. (3) did not lose bone over the first 2 weeks following ovariectomy: this phase is likely largely driven by changes in osteoclast lifespan, and this was not altered by ovariectomy in the mice with deletion of ERα in differentiated osteoclasts. However, a prediction of this model would be that the *ERα*(ΔOc/ΔOc) mice would lose bone if examined 4-8 weeks following ovariectomy, since the other consequences of estrogen

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deficiency depicted in Panels 1B and 1C would still be present in these mice. This prediction should easily be testable by the authors and by other investigators developing similar models.

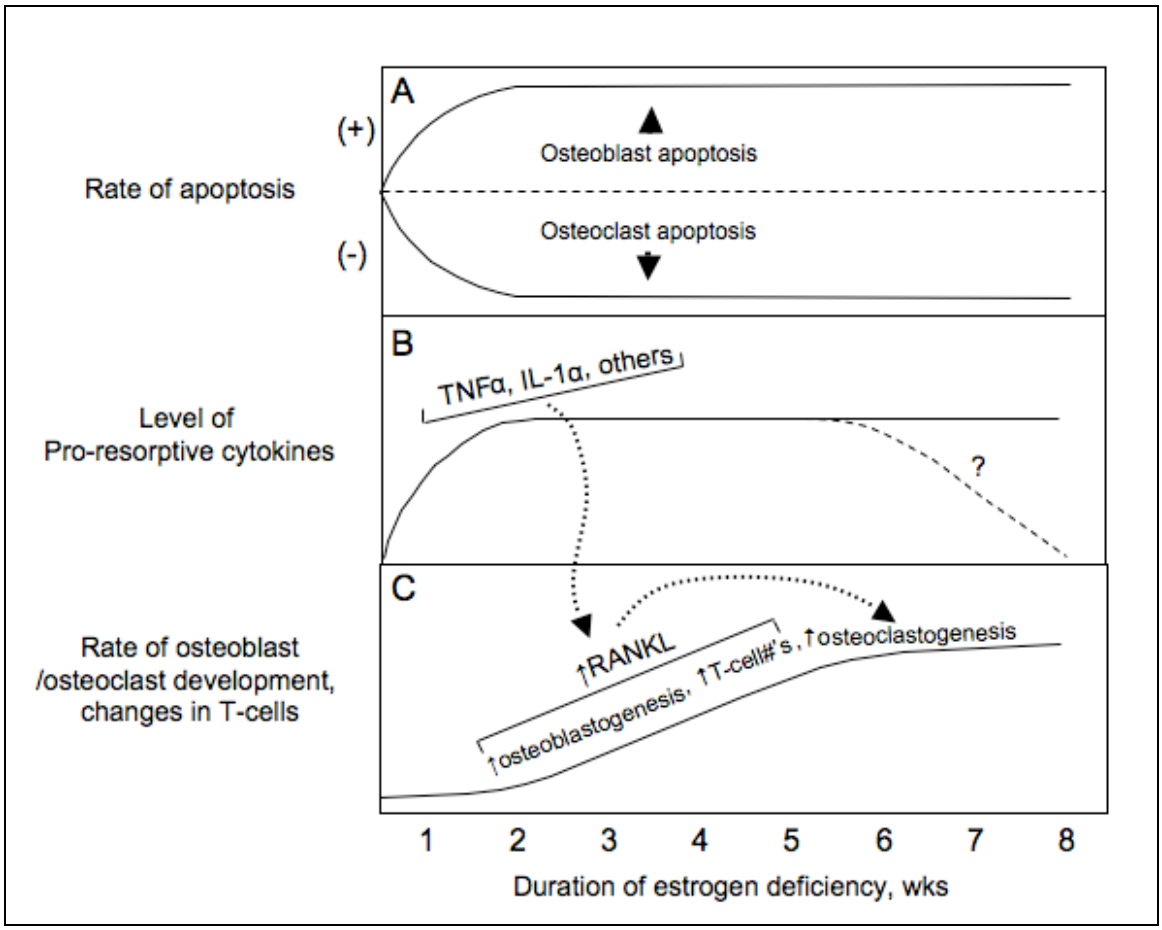

Figure 1. Working model of changes and potential time course of these changes in osteoclast/osteoblast apoptosis (Panel A), in pro-resorptive cytokines (Panel B), and in osteoblastogenesis, increases in T-cells, and osteoclastogenesis (Panel C) following the induction of estrogen deficiency. Please see text for further discussion.

At a conceptual level, the changes depicted in Figure 1 likely apply to both rodents and humans, but a number of species differences may be present. First, the period of lactation prior to weaning is relatively short (~3 weeks) in rodents as compared to the often prolonged phase of breast feeding (up to several years) in humans. Thus, the rapidity and relative importance of the changes depicted in Figure 1 may be quite different in rodents versus humans. Second, while aromatase is widely expressed in humans, with human males having serum estradiol levels of 30-40 pg/ml (22), aromatase expression in rodents is much more limited; male mice typically have serum estradiol levels of < 5 pg/ml (23). This may explain why even acute estrogen deficiency despite maintenance of normal testosterone levels is associated with increased bone resorption in men (17), while male *ERα*(ΔOc/ΔOc) mice had no skeletal abnormalities. Specifically, there may be important species differences in the relative contributions of estrogen versus testosterone towards bone metabolism in rodents as compared to humans. Finally, the data of Nakamura *et al*. (3) indicate that

cortical bone was not affected by deletion of ERα in mature osteoclasts. This suggests that, at least in rodents, who lack intra-cortical remodeling (in contrast to humans, who do have extensive remodeling within cortical bone (24)), ERα may not play an important regulatory role in osteoclasts in cortical bone, with estrogen regulation of cortical bone in rodents primarily dependent on ERα expressed by osteocytes or osteoblasts. As noted by the authors, further studies using mice with selective deletion of ERα in these cells are needed to examine this issue.

Thus, while a number of important questions regarding estrogen action on bone remain to be answered, the paper by Nakamura *et al*. (3) is a landmark contribution that clearly demonstrates a critical role for ERα action in differentiated osteoclasts in mediating skeletal effects of estrogen. However, as depicted in Figure 1 (which is undoubtedly an over-simplification), estrogen action on bone is far too complex to be attributed solely to a single cell type, cytokine, or pathway; indeed, in the immortal words of a famous radio newscaster, stay tuned for “the rest of the story.”

Conflict of Interest: The author reports that no conflict of interest exists.

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