Extensive bone metabolism studies have shown that the rate at which osteoclasts resorb bone and osteoblasts replace the lost bone with new bone are tightly coupled, creating a scenario in which osteoclasts and osteoblasts communicate to maintain a balance of bone resorption and formation. Although it has been recognized for many years that estrogen depletion at menopause results in a net loss of bone and that this can be prevented by estrogen replacement therapy, the mechanisms by which estrogen has this impact have remained somewhat elusive. This breakdown in the balance of resorption and formation implies that estrogen loss uncouples the resorption and formation phases of bone metabolism. Discoveries of the mechanisms by which resorption and formation are coupled and how estrogen loss disrupts this coupling have been complicated by a number of factors including the fact that the tissue is mineralized, there is cellular heterogeneity of the tissue, a relatively small number of cells are available and they are difficult to recover intact from the tissue, and there is a lack of suitable cell lines for all stages of differentiation. In addition, the most powerful method for studying bone metabolism, dynamic histomorphometry, is invasive and cannot be accomplished on live tissue. Recent advances in all of these areas have allowed for a more complete picture of how estrogen modulates bone balance by directly and indirectly targeting bone resorbing osteoclasts. This summary will outline current knowledge of both of these mechanisms by which estrogen impacts bone resorption.

Estrogen targets osteoclast numbers

It is generally acknowledged that the rate of bone resorption over the long term is modulated effectively by controlling the number of osteoclasts present at the site of bone resorption. Since cell numbers are influenced by the rate at which they differentiate from precursors and the rate at which they are eliminated, in this case through apoptosis, both of these parameters are discussed.

Indirect estrogen effects on osteoclasts

Effects on differentiation: It is apparent that many of the paracrine and endocrine mechanisms that regulate bone metabolism do so, at least in part, by targeting management of osteoclast differentiation. A wealth of data document that receptor activator of nuclear factor kappa B ligand (RANKL) is critical for osteoclast differentiation. Interactions between RANKL and its cognate receptor, RANK, are regulated by production of a decoy receptor, osteoprotegerin (OPG). Many paracrine factors influence osteoclast differentiation by regulating RANKL and OPG expression by stromal support cells. Saika et al. have documented that the mouse ST-2 stromal cell line responds to estrogen treatment with increased OPG expression. Since OPG is a decoy receptor for RANKL, this suggests that at least one mechanism by which estrogen regulates osteoclast differentiation would be by repressing RANKL binding to RANK on osteoclast precursors. Studies by Bord et al. documenting that human osteoblasts respond to low dose estrogen treatment with repression of RANKL while maintaining OPG expression, support that modulating this mechanism may be key in estrogen effects on osteoclast differentiation in human cells as well. Whether these changes in the RANKL/OPG ratio are the direct result of estrogen modulation of either or both gene expressions or through estrogen modulation of cytokines and other growth factors that then impact this ratio is not resolved. Indeed, a number of other studies, both in vivo and in vitro, have implicated multiple cytokines and other growth factors as being involved in estrogen effects on osteoclast differentiation. These studies have highlighted IL-6, IL-1 and/or TNF-α, IL-7, as potential mediators of osteoclast differentiation. Evidence that estrogen also modulates TGF-β production by osteoblasts, coupled with evidence that TGF-β regulates osteoclast differentiation, suggests that this growth factor may also...
play a critical part in osteoblast-mediated estrogen effects on osteoclast differentiation.\textsuperscript{6,21-24}

**Effects on apoptosis:** Modulation of osteoclast survival may also be important in controlling the number of osteoclasts present at sites of bone resorption. Hughes et al. have shown that estrogen induces osteoclast apoptosis, a process that was inhibited by antibodies to TGF-ß\textsuperscript{25,26}. Murakami et al. have demonstrated that TGF-ß treatment of co-cultures of osteoclasts and support cells causes apoptosis by increasing OPG\textsuperscript{27}. Stromal cells respond to TGF-ß with increased OPG expression and this is consistent with that observation\textsuperscript{27}. Since estrogen increases osteoblast TGF-ß production, estrogen-induced osteoclast apoptosis is likely, at least in part, to be mediated by osteoblast TGF-ß production\textsuperscript{28}.

**Direct estrogen effects on osteoclasts**

**Effects on differentiation:** There has not been extensive study of direct estrogen effects on osteoclast precursor differentiation. Shevde et al.\textsuperscript{29} have documented that estrogen directly targets RANKL-induced osteoclast differentiation by repressing c-Jun activation and that this response was dependent on the estrogen receptor. Studies of Srivastava et al.\textsuperscript{30} have shown that estrogen treatment of osteoclast precursors results in down regulation of the c-Jun activating kinase JNK. These data support a direct action of estrogen on osteoclast precursors to repress differentiation and, therefore, bone resorption levels.

**Effects on apoptosis:** The above discussion highlighted that estrogen effects on osteoclast apoptosis may be mediated, at least in part, by estrogen stimulation of osteoblast TGF-ß production causing OPG production. Interestingly, when stromal cells are removed from the mature osteoclasts in these studies, OPG was not involved in TGF-ß-induced osteoclast apoptosis\textsuperscript{31}. Thus, it appears that stromal or osteoblast OPG production is not the sole mechanism by which estrogen causes osteoclast apoptosis. This intriguing observation raises the important question of how osteoclast TGF-ß-induced apoptosis is driven.

**Estrogen targets osteoclast activity**

Much research has focused on estrogen regulation of the number of osteoclasts as this is a likely major mechanism by which bone resorption in controlled \textit{in vivo} over the span of many years. However, there is a body of data that support that, over the short term, modulation of osteoclast activity may also result from changes in estrogen levels. \textit{In vivo} data examining the effects of estrogen withdrawal on young women support that decreased estrogen exposure may result in increased activity levels of individual osteoclasts\textsuperscript{32}. In these studies, therapeutic lowering of serum estrogen levels caused increased bone loss and elevated TRAP activity of individual osteoclasts. \textit{In vitro} studies of this possibility support this concept as well. Studies of mixed cell cultures consisting of osteoclasts and stromal or osteoblastic cells have shown that estrogen also modulates mature osteoclast bone resorption activity. In studies of this nature, the target cell for estrogen effects are impossible to discern due to the multiple cell types present in the cultures. Because of this, these studies are discussed below under the category of potential indirect effects. In studies using highly purified osteoclasts or studies comparing purified and co-culture responses, it is possible to attribute the responses to direct effects on osteoclasts and these studies are considered separately.

**Potential indirect estrogen effects on osteoclasts**

Studies have shown that RANKL can activate mature osteoclasts, so studies discussed above of estrogen modulation of osteoblast RANKL and/or OPG have relevance here in that increased RANKL and/or decreased OPG would activate mature osteoclasts. Moreover, an interesting study by Parikka et al.\textsuperscript{33} examined mixed cultures plated on bone and found that estrogen treatment resulted in shallower pits that were filled with non-degraded collagen. Further examination of this collagen led to the conclusion, on the basis of enzyme specificity, that estrogen repressed cathepsin K activity. As noted above, the mixed nature of these cultures means that one cannot discern the estrogen responding cell. However, studies outlined below document that estrogen targets osteoclast cathepsin expression in highly purified cultures, supporting that at least some of this observed effect is likely due to direct estrogen effects on osteoclast cathepsin production.

**Direct estrogen effects on osteoclasts**

Pensler et al.\textsuperscript{34} and Hoyland et al.\textsuperscript{35} provided early evidence of estrogen receptors in human osteoclasts. Studies of chicken, mouse, and rabbit osteoclasts have confirmed that mature osteoclasts express estrogen receptors.\textsuperscript{36-39} Human, mouse, rabbit, and avian osteoclasts have been shown to respond to estrogen treatment with decreased resorption activity.\textsuperscript{35,39} Studies of the mechanisms of estrogen’s impact on osteoclast activity can be divided into two categories: rapid effects that presumably are non-genomic in nature and more delayed effects that appear due to estrogen effects on osteoclast gene expression. Each of these will be considered separately.

**Rapid non-genomic effects:** Rapid estrogen responses include increased acidification and modulation of Src kinase activity.\textsuperscript{40-42} Moreover, studies of rat osteoclasts have documented rapid inhibition of superoxide anion generation and inward rectifying K+ channel-mediated depolarization of the plasma membrane.\textsuperscript{43,44} Although studies of non-genomic estrogen effects on mature osteoclasts are in their infancy, it appears that these cells are among those that exhibit estrogen responses too rapid to be genomic in nature.

**Genomic effects:** Direct estrogen effects on osteoclast gene expression include rapid stimulation of c-fos and c-jun expression, a response observed within 15 minutes of treatment. More long-term responses include repression of
TRAP as well as cathepsins B, L and K\textsuperscript{37,39,45}. These data support that estrogen modulation of mature osteoclast activity may be mediated, at least in part, by direct repression of the genes for proteins involved in degrading bone matrix, the cathepsins.

**Conclusions and future directions**

Recent advances in technology, coupled with advances in model systems and our fundamental knowledge of osteoclast biology are creating an environment where we will be rapidly accumulating knowledge that, hopefully, will enable us to therapeutically address fundamental issues relating to controlling pathological bone loss. Undoubtedly, future therapies will focus on the regulation of both osteoclast numbers through targeting differentiation and survival as well as repressing activity of mature osteoclasts to control bone resorption.

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