J Musculoskel Neuron Interact 2003; 3(4):363-366

Perspective Article

Hylonome

Direct and indirect effects of estrogen on osteoclasts

M.J. Oursler

Departments of Biology, Medical Microbiology and Immunology,
and Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA

Keywords: Osteoclasts, Osteoblasts, Estrogen, Genomic Responses, Non-genomic Responses

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[1]. 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)[1]. Many paracrine factors influence osteoclast differentiation by regulating RANKL and OPG expression by stromal support cells[1-9]. Saika et al.[10] 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.[11] 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[12,13], IL-1 and/or TNF-α[14-17], IL-11[18], and IL-7[19,20] as potential mediators of osteoclast differentiation. Evidence that estrogen also modulates TGF-ß production by osteoblasts, coupled with evidence that TGF-ß regulates osteoclast differentiation, suggests that this growth factor may also

The author has no conflict of interest.

Corresponding author: Merry Jo Oursler, Ph.D., Endocrine Research Unit, Mayo Clinic and Foundation, 200 First St. SW, Rochester, MN 55905, USA
E-mail: oursler.merryjo@mayo.edu

Accepted 1 August 2003

play a critical part in osteoblast-mediated estrogen effects on osteoclast differentiation[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[25,26]. Murakami et al. have demonstrated that TGF-ß treatment of co-cultures of osteoclasts and support cells causes apoptosis by increasing OPG[27]. Stromal cells respond to TGF-ß with increased OPG expression and this is consistent with that observation[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[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.[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.[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[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 *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. *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[32]. In these studies, therapeutic lowering of serum estrogen levels caused increased bone loss and elevated TRAP activity of individual osteoclasts. *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.[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.[34] and Hoyland et al.[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[36-39]. Human, mouse, rabbit, and avian osteoclasts have been shown to respond to estrogen treatment with decreased resorption activity[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[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[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[36]. More long-term responses include repression of

TRAP as well as cathepsins B, L and K[37,39,45]. These data support that estrogen modulation of mature osteoclast activity may be mediated, at least in part, by direct repression of expression 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.

Acknowledgment

Dr. Oursler is supported by the Department of the Army Grant DAMD17-00-1-0346 and the National Institutes of Health Grant DE14680.

References

1. Khosla S. Minireview: the OPG/RANKL/RANK system. Endocrinology 2001; 142:5050-5055.
2. Atkins GJ, Haynes DR, Geary SM, Loric M, Crotti TN, Findlay DM. Coordinated cytokine expression by stromal and hematopoietic cells during human osteoclast formation. Bone 2000; 26:653-661.
3. Atkins GJ, Haynes DR, Graves SE, Evdokiou A, Hay S, Bouralexis S, Findlay DM. Expression of osteoclast differentiation signals by stromal elements of giant cell tumors. J Bone Miner Res 2000; 15:640-649.
4. Mancino AT, Klimberg VS, Yamamoto M, Manolagas SC, Abe E. Breast cancer increases osteoclastogenesis by secreting M-CSF and upregulating RANKL in stromal cells. J Surg Res 2001; 100:18-24.
5. Massey HM, Scopes J, Horton MA, Flanagan AM. Transforming growth factor-beta1 (TGF-β) stimulates the osteoclast-forming potential of peripheral blood hematopoietic precursors in a lymphocyte-rich microenvironment. Bone 2001; 28:577-582.
6. Quinn JM, Itoh K, Udagawa N, Häusler K, Yasuda H, Shima N, Mizuno A, Higashio K, Takahashi N, Suda T, Martin TJ, Gillespie MT. Transforming growth factor beta affects osteoclast differentiation via direct and indirect actions. J Bone Miner Res 2001; 16:1787-1794.
7. Suda T, Kobayashi K, Jimi E, Udagawa N, Takahashi N. The molecular basis of osteoclast differentiation and activation. Novartis Found Symp 2001; 232:235-247; discussion 247-250.
8. Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, Nakagawa N, Yasuda H, Goto M, Tsuda E, Higashio K, Gillespie MT, Martin TJ, Suda T. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone 1999; 25:517-523.
9. Wei S, Wang MW, Teitelbaum SL, Ross FP. Interleukin-4 reversibly inhibits osteoclastogenesis via inhibition of NF-kappa B and mitogen-activated protein kinase signaling. J Biol Chem 2002; 277:6622-6630.
10. Saika M, Inoue D, Kido S, Matsumoto T. 17β-estradiol stimulates expression of osteoprotegerin by a mouse stromal cell line, ST-2, via estrogen receptor-alpha. Endocrinol 2001; 142:2205-2212.
11. Bord S, Ireland DC, Beavan SR, Compston JE. The effects of estrogen on osteoprotegerin, RANKL, and estrogen receptor expression in human osteoblasts. Bone 2003; 32:136-141.
12. Jilka RL, Hangoc C, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 1992; 257:88-91.
13. Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, MAnolagas SC. 17β-estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts *in vitro*: a potential mechanism for the anti-osteoporotic effect of estrogens. J Clin Invest 1992; 89:883-891.
14. Pacifici R, Rifas L, McCracken R, Vered I, McMurty C, Avioli LV, Peck WA. Ovarian steroid treatment blocks a postmenopausal increase in blood monocyte interleukin-1 release. Proc Natl Acad Sci USA 1989; 86:2398-2402.
15. Pacifici R, Brown C, Puscheck E, Friedrich E. Slatopolsky E, Maggio D, McCracken R, Avioli LV. Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Natl Acad Sci USA 1991; 88:5134-5138 .
16. Kimble RB, Bain S, Pacifici R. The functional block of TNF but not of IL-6 prevents bone loss in ovariectomized mice. J Bone Miner Res 1997; 12:935-941.
17. Kimble RB, Matayoshi AB, Vannice JL, Kung VT, Williams C, Pacifici R. Simultaneous block of interleukin-1 and tumor necrosis factor is required to completely prevent bone loss in the early post-ovariectomy period. Endocrinology 1995; 136:3054-3061.
18. Shaughnessy SG, Walton KJ, Deschamps P, Butcher M, Beaudin SM. Neutralization of interleukin-11 activity decreases osteoclast formation and increases cancellous bone volume in ovariectomized mice. Cytokine 2002; 20:78-85.
19. Toraldo G, Roggia C, Qian WP, Pacifici R, Weitzmann MN. IL-7 induces bone loss *in vivo* by induction of receptor activator of nuclear factor kappa B ligand and tumor necrosis factor alpha from T cells. Proc Natl Acad Sci USA 2003; 100:125-130.
20. Weitzmann MN, Roggia C, Toraldo G, Weitzmann L,

Pacifici R. Increased production of IL-7 uncouples bone formation from bone resorption during estrogen deficiency. J Clin Invest 2002; 110:1643-1650.
21. Shinar DM, Rodan GA. Biphasic effects of transforming growth factor-β on the production of osteoclast-like cells in mouse bone marrow cultures: the role of prostaglandins in the generation of these cells. Endocrinology 1990; 126:3153-3158.
22. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, Matsumoto K. Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. Science 1995; 270:2008-2011.
23. Mundy GR. The effects of TGF-β on bone. Ciba Found Symp 1991; 157:137-143.
24. Heino TJ, Hentunen TA, Vaananen HK. Osteocytes inhibit osteoclastic bone resorption through transforming growth factor-β: enhancement by estrogen. J Cell Biochem 2002; 85:185-197.
25. Hughes DE, Wright KR, Mundy GR, Boyce BF. TGFβ1 induces osteoclast apoptosis *in vitro*. J Bone Miner Res 1994; 9:S138.
26. Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR, Boyce BF. Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-β. Nat Med 1996; 2:1132-1136.
27. Murakami T, Yamamoto M, Ono K, Nishikawa M, Nagata N, Motoyoshi K, Akatsu T. Transforming growth factor-β 1 increases mRNA levels of osteoclastogenesis inhibitory factor in osteoblastic/stromal cells and inhibits the survival of murine osteoclast-like cells. Biochem Biophys Res Commun 1998; 252:747-752.
28. Oursler MJ, Cortese C, Keeting P, Anderson MA, Bonde SK, Riggs BL, Spelsber TC. Modulation of transforming growth factor-β production in normal human osteoblast-like cells by 17 β-estradiol and parathyroid hormone. Endocrinology 1991; 129:3313-3320.
29. Shevde NK, Bendixen AC, Dienger KM, Pike JW. Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci USA 2000; 97:7829-7834.
30. Srivastava S, Toraldo G, Weitzmann MN, Cenci S, Ross FP, Pacifici R. Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-kappa B ligand (RANKL)-induced JNK activation. J Biol Chem 2001; 276:8836-8840.
31. Akatsu T, Murakami T, Nishikawa M, Ono K, Shinomiya N, Tsuda E. Mochizuki S, Yamaguchi K, Kinosaki M, Higashio K, Yamamoto M, Motoyoshi K, Nagata N. Osteoclastogenesis inhibitory factor suppresses osteoclast survival by interfering in the interaction of stromal cells with osteoclast. Biochem Biophys Res Commun 1998; 250:229-234.
32. Bell KL, Loveridge N, Lindsay PC, Lunt M, Garrahan N, Compston JE, Reeve J. Cortical remodeling following suppression of endogenous estrogen with analogs of gonadotrophin releasing hormone. J Bone Miner Res 1997; 12:1231-1240.
33. Parikka V, Lehenkari P, Sassi ML, Halleen J, Risteli J, Harkonen P, Vaananen HK. Estrogen reduced the depth of resorption pits by disturbing organic bone matrix degradation activity of mature osteoclasts. Endocrinology 2001; 142:5371-5378.
34. Pensler JM, Radosevich JA, Higbee R, Langman CB. Osteoclasts isolated from membranous bone in children exhibit nuclear estrogen and progesterone receptors. J Bone Miner Res 1990; 5:797-802.
35. Hoyland JA, Mee AP, Baird P, Braidman IP, Mawer EB, Freemont AJ. Demonstration of estrogen receptor mRNA in bone using *in situ* reverse-transcriptase polymerase chain reaction. Bone 1997; 20:87-92.
36. Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL, Spelsberg TC. Avian osteoclasts as estrogen target cells. Proc Natl Acad Sci USA 1991; 88:6613-6617.
37. Oursler MJ, Pederson L, Fitzpatrick L, Riggs BL, Spelsberg T. Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. Proc Natl Acad Sci USA 1994; 91:5227-5231.
38. Mizuno Y, Hosoi T, Inoue S, Ikegami A, Kaneki M, Akedo Y, Nakamura T, Ouchi Y, Chang C, Orimo H. Immunocytochemical identification of androgen receptor in mouse osteoclast-like multinucleated cells. Calcif Tissue Int 1994; 54:325-326.
39. Mano H, Yuasa T, Kameda T, Miqazawa K, Nakamaru Y, Shiokawa M, Mori Y, Yamada T, Miyata Km Shindo H, Azuma H, Hakeda Y, Kumegawa M. Mammalian mature osteoclasts as estrogen target cells. Biochem Biophys Res Commun 1996; 223:637-642.
40. Brubaker KD, Gay CV. Specific binding of estrogen to osteoclast surfaces [published erratum appears in Biochem Biophys Res Commun 1994 15;202:643]. Biochem Biophys Res Commun 1994; 200:899-907.
41. Pascoe D, Oursler MJ. The Src signaling pathway regulates osteoclast lysosomal enzyme secretion and is rapidly modulated by estrogen. J Bone Miner Res 2001; 16:1028-1036.
42. Brubaker KD, Gay CV. Estrogen stimulates protein tyrosine phosphorylation and Src kinase activity in avian osteoclasts. J Cell Biochem 1999; 76:206-216.
43. Berger CE, Horrocks BR, Datta HK. Direct non-genomic effect of steroid hormones on superoxide anion generation in the bone resorbing osteoclasts. Mol Cell Endocrinol 1999; 149:53-59.
44. Okabe K, Okamoto F, Kajiya H, Takada K, Soeda H. Estrogen directly acts on osteoclasts via inhibition of inward rectifier K+ channels. Naunyn Schmiedebergs Arch Pharmacol 2000; 361:610-620.
45. Kremer M, Judd J, Rifkin B, Auszmann J, Oursler MJ. Estrogen modulation of osteoclast lysosomal enzyme secretion. J Cell Biochem 1994; 58:1-9.