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Sex-Specific Effects of Estrogen and Androgen on Gene Expression in Human Monocyte-Derived Osteoclasts

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ABSTRACT

Estrogen and androgen are both critical for the maintenance of bone, but the target cells, mechanisms, and responses could be sex-specific. To compare sex-specific actions of estrogen and androgen on osteoclasts, human peripheral blood mononuclear precursor cells from adult Caucasian males ($n = 3$) and females ($n = 3$) were differentiated into osteoclasts and then treated for 24 h with 17β-estradiol (10 nM) or testosterone (10 nM). Gene expression was studied with a custom designed qPCR-based array containing 94 target genes related to bone and hormone action. In untreated osteoclasts, 4 genes showed significant gender differences. 17β-estradiol significantly affected 12 genes in osteoclasts from females and 6 genes in osteoclasts from males. Fifteen of the 18 17β-estradiol-responsive genes were different in the cells from the two sexes; 2 genes affected by 17β-estradiol in both sexes were regulated oppositely in the two sexes. Testosterone significantly affected 6 genes in osteoclasts from females and 2 genes in osteoclasts from males; all except one were different in the two sexes. 17β-estradiol and testosterone largely affected different genes, suggesting that conversion of testosterone to 17β-estradiol had a limited role in the responses. The findings indicate that although osteoclasts from both sexes respond to 17β-estradiol and testosterone, the effects of both 17β-estradiol and testosterone differ in the two sexes, highlighting the importance of considering gender in the design of therapy. J. Cell. Biochem. 112: 3714–3721, 2011. © 2011 Wiley Periodicals, Inc.

KEY WORDS: GENDER; OSTEOCLAST; GENE; ESTROGEN; ANDROGEN

Estrogen and androgen are both critical for the maintenance of bone. The estrogen effect is seen most dramatically at menopause, when the decline in estrogen results in accelerated bone loss and an increased probability of the development of osteoporosis [Raisz, 2005]. However, it is apparent that men require estrogen for normal bone health, as osteoporosis occurs in men who lack aromatase [Morishima et al., 1995], the enzyme that converts testosterone to estradiol. Also, it is established that the absence of the estrogen receptor ERα in the male leads to osteoporosis [Smith et al., 1994]. Several recent reviews have directed attention to the complexity of the effects and the probable role for both direct androgen receptor-mediated and estradiol-mediated effects in the actions of androgen on bone [Ebeling, 2010; Frenkel et al., 2010; Khosla, 2010; Nicks et al., 2010]. Findings largely obtained in animal models suggest that the cellular mechanisms by which androgens and estrogens decrease bone resorption may be dissimilar. These findings suggest that androgens directly inhibit osteoclasts [Pederson et al., 1999; Michael et al., 2005], whereas estrogens inhibit osteoclasts both directly through induction of the apoptotic mediator Fas ligand (FasL) [Nakamura et al., 2007] and indirectly through osteoblast expression of FasL, which then activates Fas death receptors on osteoclasts and decreases their survival [Krum et al., 2008]. Also, estrogenic agents increase osteoblast expression of osteoprotegerin (OPG) [Hofbauer et al., 1999], a RANKL decoy receptor/RANKL antagonist that could reduce RANKL-induced osteoclast differentiation, activity, and survival. Although these findings provide possible mechanisms for differential effects of estrogens and androgens, some were carried out in rodent or murine species, and might not fully apply to human cells. Since both hormones are present in both sexes, it was of interest to determine whether they have the same or different effects in bone cells from the two sexes. To address these questions, we have investigated effects of 17β-estradiol and testosterone on genes in human osteoclasts derived from precursor cells (human peripheral blood monocytic cells, HPBMC) from males and females. The findings indicate that the two hormones have different patterns of

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effects on genes that could mediate actions on bone, and strikingly, that there are differences in response of cells from males and females that could have important implications for the design and effectiveness of therapy.

METHODS

CELLS AND TREATMENTS

Six lots of adult HPBMC, three from females and three from males were purchased from Lonza (Basel, Switzerland). All samples were from de-identified Caucasian donors. Ages of the female donors were 24, 48, and 58 years (av: 43.3 ± 10.1 years); ages of the male donors were 36, 41, and 62 years (av: 46.3 ± 8.0 years). The cells were maintained in phenol red-free, serum-free, X-VIVO culture medium (Lonza). To induce osteoclastic differentiation, the cells were treated with 25 ng/ml macrophage colony stimulating factor (M-CSF) (R&D Systems, Minneapolis, MN) and 40 ng/ml RANKL (Peprotech, Rocky Hill, NJ) for 10 days from the day after seeding. Osteoclastogenesis was confirmed by assay of tartrate-resistant acid phosphatase (TRAP). The differentiated cells were treated with 17β-estradiol (10 nM) or testosterone (10 nM) for 24 h.

GENE EXPRESSION

Total RNA was extracted using a RNeasy kit (Qiagen, Valencia, CA) Genomic DNA contamination was eliminated and first strand cDNA was synthesized from 2 μg total RNA using a RT2 first strand kit (SABiosciences/Qiagen). Gene expression profiles were studied using a custom designed qPCR-based gene array (Lonza), which contained 94 target genes related to bone metabolism and to hormone action and a loading control (Hs 18s) for normalization (Supplementary Table 1). The cDNA samples mixed with qPCR master mix (containing SYBR green) were loaded in 96-well PCR arrays. qPCR reactions were performed on a BioRad real-time PCR detection system. After denaturing the template and activating the HotStart DNA polymerase at 95°C for 10 min, the two-step cycling program was run for 40 cycles at 95°C for 15 s, 60°C for 60 s. The PCR array data were analyzed using web-based software. ΔΔCt based fold-change calculations were carried out from raw threshold cycle data. Pair-wise comparisons between samples from male and female donors or between hormone-treated samples and control samples were performed. Genes that were changed ≥2-fold with $P \leq 0.05$ (two-tailed *t*-test) were considered to be significantly modified.

RESULTS

CHARACTERISTICS OF THE RANKL AND M-CSF TREATED HPBMC

The differentiated cells used in this investigation had a marked osteoclastic phenotype, as determined both by the expression of tartrate-resistant acid phosphatase (TRAP) in their characterization before treatment and the genes that were expressed or not expressed in the differentiated cells prior to treatment. Of the 94 genes examined in the gene array, the 10 most highly expressed genes in the osteoclasts are shown in Table IA. Several recognized osteoclast differentiation-related genes were on this list, including osteoclast-associated, immunoglobin-like receptor (*OSCAR*), acid phosphatase 5, TRAP (*ACP5*), TNF-receptor superfamily member 11a, NF κB activator, RANK (*TNFRSF11A*), tumor necrosis factor (ligand) superfamily, member 10, APO2L (*TNFSF10*), and TNF receptor-associated factor 2 (*TRAF2*). *OSCAR*, the most highly expressed gene, has been shown to be involved in osteoclast differentiation and activity [Kim et al., 2002], and *ACP5*, the second most highly expressed gene, encodes expression of TRAP [Grimes et al., 1993]. Another highly expressed gene was *TNFRSF11A*, the gene for RANK, the receptor for the osteoclastogenic cytokine, receptor activator of NF κB ligand (RANKL) [Nakagawa et al., 1998]. TRAF2 has been found to be an essential factor for TNF-α-induced osteoclastogenesis [Kanazawa and Kudo, 2005]. The protein encoded by *TNFSF10* is involved in the activation of c-Jun N-terminal kinase (JNK) [Muhlenbeck et al., 1998], which regulates osteoclast apoptosis [Ikeda et al., 2008].

Forty-nine genes were undetectable in the untreated cells (Table IB). It is notable that many genes characteristic of the osteoblast phenotype, such as liver/bone kidney alkaline phosphatase (*ALP1*) and collagen type I, alpha 1 and collagen type I, alpha 2 (*COL1A1*, *COL1A2*), were undetectable. As shown in tables, several of the undetectable genes, that is, growth arrest and DNA-damage-inducible, alpha (*GADD45A*), low density lipoprotein receptor-related protein 5 (*LRP5*), mitogen-activated protein kinase kinase kinase 7 (*MAP3K7*), matrix metallopeptidase 8 (*MMP8*), and transforming growth factor beta 3 (*TGFB3*), increased to detectable levels with treatment.

SEX DIFFERENCES IN BASAL EXPRESSION OF GENES IN UNTREATED HPBMC-DERIVED OSTEOCLASTS

Only four genes showed a significant sex difference in the untreated osteoclasts (Table II). Most notably, there was a striking sex difference in expression of the tumor necrosis factor (ligand) superfamily member 10, *APO2L*, whose expression was 776 times greater in the cells from the males than in the cells from the females. This may reflect an effect of endogenous androgen, as increases in *TNF* were elicited with testosterone treatment, as will be described below. In the case of three other genes, matrix Gla protein (*MGP*), lamin B1 (*LMNB1*), and mitogen-activated protein kinase-8 (*MAPK8*), the differences between the two genders were not large, although they were statistically significant, cells from males having higher expression of these genes than cells from females for *MGP* and *LMNB1*, and cells from females having higher expression of *MAPK8* than cells from males. The overall male/female pattern of expression of detectable genes is illustrated in Figure 1, in which the ratios are represented on a volcano plot. Symbols for genes expressed at significantly higher or lower levels in the males than in the females appear above the horizontal line that represents the $P \leq 0.05$ level of significance.

GENE RESPONSES TO 17β-ESTRADIOL TREATMENT IN THE HPBMC-DERIVED OSTEOCLASTS FROM FEMALE AND MALE DONORS

The responses of the cells to 17β-estradiol are shown in Figure 2 and Table III. As can be seen from the volcano plots in Figure 2, there was a trend for many genes to be up-regulated by 17β-estradiol treatment in cells from both males and females. Cells from the

TABLE I. Characteristics of the Osteoclasts Derived From Male and Female HPBMC

Gene symbol	Gene name	Average Ct
A: The ten most highly expressed genes in the osteoclasts derived from male and female HPBMC		
OSCAR	Osteoclast-associated, immunoglobin-like receptor	17.5
ACP5	Acid phosphatase 5, TRAP	20.5
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	22.2
ITGB2	Integrin beta 2	22.5
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10, APO2L	25.7
TNFRSF11A	TNF-receptor superfamily member 11a, NF κB activator, RANK	26.7
TRAF2	TNF receptor-associated factor 2	29.7
NRIP1	Nuclear receptor interacting protein 1	32.5
MAPK8	Mitogen-activated protein kinase 8	32.9
IGF1	Insulin-like growth factor 1 (somatomedin C)	33.6
B: Genes undetectable in the osteoclasts derived from male and female HPBMC		
ALPL	Alkaline phosphatase, liver/bone/kidney	
AR	Androgen receptor	
BAG4	BCL2-associated athanogene 4	
BGLAP	Bone gamma-carboxyglutamate (gla) protein	
BGN	Biglycan	
BMP2	Bone morphogenetic protein 2	
BMP4	Bone morphogenetic protein 4	
COL1A1	Collagen type I, alpha 1	
COL1A2	Collagen type I, alpha 2	
CTSK	Cathepsin K	
DKK1	Dickkopf homolog 1 (*Xenopus laevis*)	
EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	
ESR1	Estrogen receptor 1	
ESR2	Estrogen receptor 2 (beta)	
FADD	Fas (TNFRSF6)-associated via death domain	
GADD45A	Growth arrest and DNA-damage-inducible, alpha	
IGFBP3	Insulin-like growth factor binding protein 3	
IGFBP5	Insulin-like growth factor binding protein 5	
IL10	Interleukin 10	
IL1A	Interleukin 1 alpha	
IRAK1	Interleukin-1 receptor-associated kinase 1	
IRAK3	Interleukin-1 receptor-associated kinase 3	
ITGA1	Integrin alpha 1	
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	
LMNB2	Lamin B2	
LRP5	Low density lipoprotein receptor-related protein 5	
MAP3K1	Mitogen-activated protein kinase kinase kinase 1	
MAP3K7	Mitogen-activated protein kinase kinase kinase 7	
MMP8	Matrix metallopeptidase 8 (neutrophil collagenase)	
MMP9	Matrix metalloproteinase 9 (gelatinase B, 92 kDa type IV collagenase)	
MSX1	Msh homeobox 1	
NFATC1	Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1	
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	
PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	
PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	
PTH1R	Parathyroid hormone receptor 1	
SFRP4	Secreted frizzled-related protein 4	
SOST	Sclerostin	
SOX9	SRY (sex determining region Y)-box 9	
SPP1	Secreted phosphoprotein 1	
TGFB1	Transforming growth factor beta 1	
TGFB3	Transforming growth factor, beta 3	
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a	
TNFRSF11B	TNF-receptor superfamily member 11b	
TNFRSF1B	TNF-receptor superfamily member 1b	
TNFRSF25	Tumor necrosis factor receptor superfamily, member 25	
TNFSF11	TNF (ligand) superfamily member 11	
VDR	Vitamin D (1,25-dihydroxyvitamin D3) receptor	

TABLE II. Genes Differentially Expressed in Osteoclasts Derived From Male and Female HPBMC

Gene symbol	Gene name	Male vs. female	*P*-value
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10, APO2L	776.0	0.002
MGP	Matrix Gla protein	7.6	0.027
LMNB1	Lamin B1	5.9	0.049
MAPK8	Mitogen-activated protein kinase 8	0.1	0.038

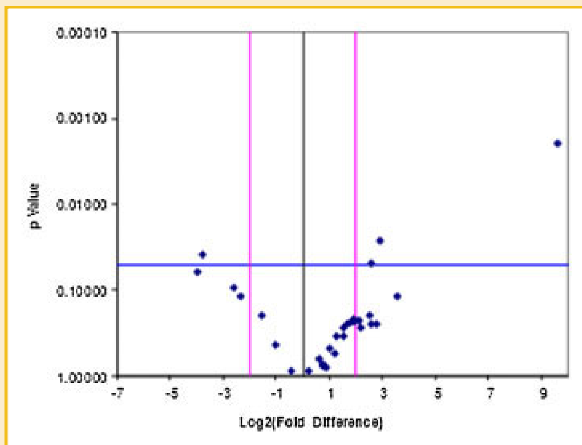

Fig. 1. Volcano plot showing fold differences in expression of genes in osteoclasts differentiated from HPBMC from male donors and osteoclasts differentiated from HPBMC from female donors. The center vertical line indicates no difference between the expression of the gene in male and female donor-derived osteoclasts. The side vertical lines (pink in color version) indicate a four-fold difference in either direction. The horizontal line (blue in color version) indicates a P-value = 0.05. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

females were more sensitive to estrogen, with more genes and larger effects noted than in the cells from the males (Table III). Twelve genes, integrin alpha 1 (*ITGA1*), *MMP8*, *GADD45A*, *LRP5*, secreted protein, acidic, cysteine-rich/osteonectin (*SPARC*), mitogen-activated protein kinase kinase 4 (*MAP2K4*), caspase 2 (*CASP2*), *MAP3K7*, *TNFRSF11A*, Fas ligand (*FASLG*), fibronectin 1 (*FN1*), and catenin beta 1 (*CTNNB1*), were significantly regulated by estrogen in HPBMC-derived osteoclasts from the females, and six genes (*FAS*, *FN1*, *NFATC1*, *LRP5*, *ITGA1*, and *CTNNB1*) were significantly changed in the cells from the males. *LRP5* and *FN1* were up-regulated in the cells from both sexes, although the effects on *LRP5* were greater in the cells from the female donors. *ITGA1* and *CTNNB1* were up-regulated in the cells from the female donors, but down-regulated in the cells from the males.

GENE RESPONSES TO TESTOSTERONE TREATMENT IN THE HPBMC-DERIVED OSTEOCLASTS FROM FEMALE AND MALE DONORS

As shown in Figure 3 and Table IV, treatment with testosterone also resulted in significant changes in genes from both female and male donors, although fewer genes were affected by testosterone than had been affected by 17β-estradiol. Six genes, *TGFB3*, phosphatase and tensin homolog (PTEN), intercellular adhesion molecule 1 (*ICAM1*), *LRP5*, tumor necrosis factor (*TNF*), and *MAP2K4*, were significantly up-regulated by testosterone treatment in the cells from the female donors, whereas only two genes, decorin (*DCN*) and *TNF* were significantly up-regulated by testosterone in the cells from the males. Only *TNF* was up-regulated by testosterone in cells from both sexes. Interestingly, there was almost no overlap between the genes activated by testosterone and by 17β-estradiol, the only identical genes being *LRP5*, which was increased by 17β-estradiol in the cells from both sexes, and by testosterone in the cells from the females, and *MAP2K4*, which was increased by both 17β-estradiol and testosterone in the cells from the females.

DISCUSSION

Investigation of sex differences in responses of osteoclasts from males and females yielded some interesting and unexpected findings. Although the number of genes in the array was small, it is interesting that a larger number of genes were affected in the cells from the females by the 17β-estradiol treatment, an effect that could be postulated to be related to the decline in endogenous estrogen in the adult women. In the female-derived cells, there was a marked 17β-estradiol-stimulated (greater than 500-fold) increase in *ITGA1*, which encodes the integrin α1 chain. Integrin α1 is important for fracture healing [Ekholm et al., 2002]. Genetic analyses have shown an association of *ITGA1* polymorphisms and bone mineral density [Lee et al., 2007; Richards et al., 2009]. It is interesting that the effect to increase *ITGA1* was not elicited by estrogen in the cells from the males, rather there was a decrease in the expression of the *ITGA1* gene. A similar dichotomy was seen with the adherence protein *CTTNB1* which encodes β-catenin, with the mRNA being increased by estrogen in osteoclasts derived from the females and decreased by estrogen in the osteoclasts from the males. The expression of two genes associated with apoptosis, *CASP2* and *FASLG*, was increased by estrogen in the cells from the females. The effect on *FASLG* is consistent with studies in mice, in which the ERα-dependent induction of osteoclast apoptosis was associated with up-regulation of FasL by estrogen [Nakamura et al., 2007]. In a contrasting finding, there was no increase in FasL mRNA in RAW 264.7 osteoclastic cells differentiated with RANKL and incubated with 17β-estradiol for 5 days, although there was an increase in caspase-3, -8, and -9 activities, and enhancement of Fas-induced apoptosis [Saintier et al., 2006]. In male donor-derived cells, 17β-estradiol increased *FAS* more than 2,000-fold. Thus, estrogen had apoptosis-augmenting effects in cells from the two sexes, but by effects on different genes, and in part by different pathways.

In the current study, estrogen increased mRNA expression of *SPARC*. Increased SPARC results in loss of focal adhesions and partial detachment of cells from a substrate [Young et al., 1998]. Estradiol inhibits the adhesion of murine osteoclasts in vitro [Saintier et al., 2006]. Conceivably the effect on *SPARC* could contribute to this decreased adhesion and in this way promote apoptosis. One unexpected finding from our study was an increase in *TNFRSF11A* (RANK) with estrogen treatment, an effect seen only in the cells from the female donors. There is limited literature on estrogen effects on RANK, although in a long-term study of postmenopausal women treated with estrogen, RANK gene expression was decreased at six months [Bashir et al., 2005]. In ovariectomized mice, expression of RANK increased through the chemokine receptor CCR2 [Binder et al., 2009]. Polymorphisms in RANK have been associated with bone mineral density in Korean postmenopausal women [Choi et al., 2005]. The unexpected finding of an increase in RANK with 17β-estradiol treatment raises the possibility of the involvement of this effect in the osteolytic effect of

TABLE III. Genes Regulated by Estrogen in Osteoclasts Derived From HPBMC

Gene symbol	Gene name	Fold	*P*-value
A: From female donor-derived cells			
ITGA1	Integrin alpha 1	562.5	0.007
MMP8	Matrix metallopeptidase 8 (neutrophil collagenase)	291.0	0.00004
GADD45A	Growth arrest and DNA-damage-inducible, alpha	284.4	0.00004
LRP5	Low density lipoprotein receptor-related protein 5	265.3	0.00006
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	229.7	0.012
MAP2K4	Mitogen-activated protein kinase kinase 4	202.0	0.011
CASP2	Caspase 2, apoptosis-related cysteine peptidase	202.0	0.011
MAP3K7	Mitogen-activated protein kinase kinase kinase 7	184.2	0.010
TNFRSF11A	TNF-receptor superfamily member 11a, NF κB activator	173.0	0.009
FASLG	Fas ligand (TNF superfamily, member 6)	163.3	0.002
FN1	Fibronectin 1	40.6	0.023
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa	19.8	0.051
B: From male donor-derived cells			
FAS	Fas (TNF receptor superfamily, member 6)	2119.0	0.0001
FN1	Fibronectin 1	67.7	0.001
NFATC1	Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1	18.3	0.014
LRP5	Low density lipoprotein receptor-related protein 5	7.4	0.035
ITGA1	Integrin alpha 1	0.1	0.014
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa	0.1	0.014

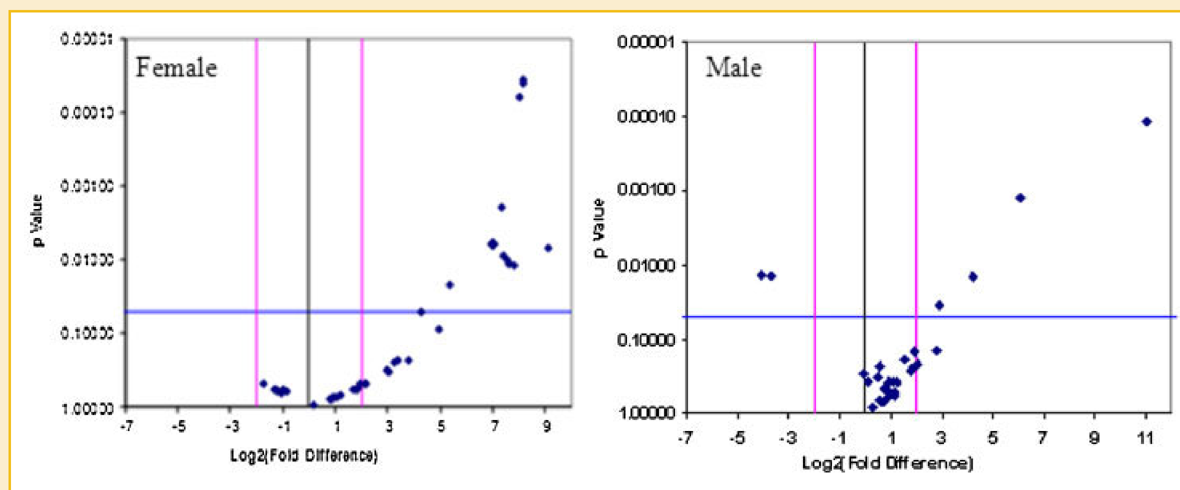

Fig. 2. Volcano plots showing the fold differences in expression of genes between 17β-estradiol (10 nM)—treated and untreated osteoclasts differentiated from HPBMC from female (left) and male (right) donors. The center vertical line indicates no difference between the expression of the gene in the 17β-estradiol—treated and the untreated osteoclasts. The side vertical lines (pink in color version) indicate a four-fold difference in either direction. The horizontal line (blue in color version) indicates a *P*-value = 0.05. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

TABLE IV. Genes Regulated by Testosterone in Osteoclasts Derived From HPBMC

Gene symbol	Gene name	Fold	*P*-value
A: From female donor-derived cells			
TGFB3	Transforming growth factor, beta 3	1880.2	0.0005
PTEN	Phosphatase and tensin homolog	645.8	0.016
ICAM1	Intercellular adhesion molecule 1	122.5	0.003
LRP5	Low density lipoprotein receptor-related protein 5	79.5	0.005
TNF	Tumor necrosis factor (TNF superfamily member 2)	79.5	0.005
MAP2K4	Mitogen-activated protein kinase kinase 4	45.9	0.014
B: From male donor-derived cells			
DCN	Decorin	1717.2	0.022
TNF	Tumor necrosis factor (TNF superfamily member 2)	577.0	0.033

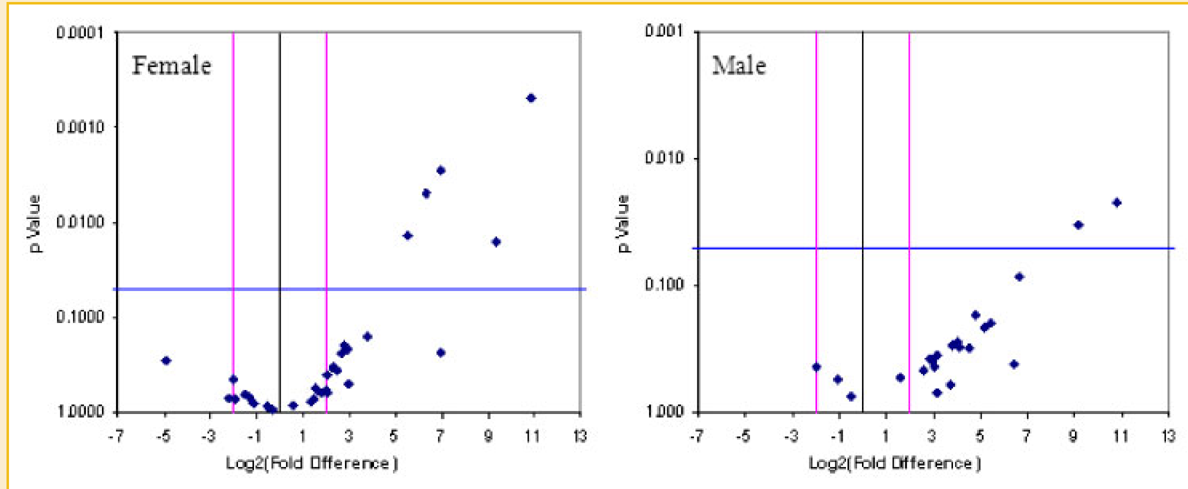

Fig. 3. Volcano plots showing the fold differences in expression of genes between testosterone (10 nM)—treated and untreated osteoclasts differentiated from HPBMC from female (left) and male (right) donors. The center vertical line indicates no difference between the expression of the gene in the testosterone-treated and the untreated osteoclasts. The side vertical lines (pink in color version) indicate a four-fold difference in either direction. The horizontal line (blue in color version) indicates a *P*-value = 0.05. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

breast cancer metastases. Interestingly, both *SPARC* and *FN1*, which were increased by estrogen, have been associated with reduced metastasis-free survival in breast cancer patients [Helleman et al., 2008]. *NFATC1*, a transcription factor that effects the differentiation, fusion, and activity of osteoclasts [Takayanagi et al., 2002] was significantly increased by estrogen in the male donor derived cells, but not in the female donor derived cells. It is interesting in this regard that the phosphatase inhibitor cyclosporine, which blocks the activation of NFAT, exhibits gender specific effects in rats, with bone loss only seen in the males [Erben et al., 2003].

Testosterone increased a number of genes in the cells from the female donors. Two of the genes, *LRP5* and *MAP2K4*, were also increased by 17β-estradiol, and thus may have been activated as a result of conversion of the testosterone to estrogen through aromatase activity. *LRP5* encodes a low-density lipoprotein receptor that is involved in endocytosis. *LRP5* is an established osteoporosis-associated gene [Ichikawa et al., 2010; Li et al., 2010], with the focus of the bone effects being on bone formation mediated through its action as a co-receptor for wnt signaling. In patients with autosomal dominant osteopetrosis type I, there is a mutation in *LRP5* that results in decreased resorption [Henriksen et al., 2005]. This effect on osteoclasts was likely indirectly mediated through production by osteoblasts of an inhibitor of osteoclastogenesis or osteoclast survival. *MAP2K4* is an activator of MAP kinases of the JNK and p38 families [Derijard et al., 1995].

Four genes that were affected by testosterone in the female-derived cells, *TGFβ3*, *PTEN*, *ICAM 1*, and *TNF*, were not affected by 17β-estradiol in either male or female-derived cells, and thus it seems likely that these effects of testosterone were not due to its conversion to estrogen. TGFβ is increased by dihydrotestosterone, a testosterone product with antiresorptive effects in isolated avian osteoclast cells [Pederson et al., 1999]. TGFβ promotes osteoclast differentiation through p38 MAP kinase [Karsdal et al., 2003; Tiedemann et al., 2009] and ERK1/2 [Tiedemann et al., 2009] but also decreases osteoclast RANK expression and leads to osteoclast apoptosis [Karsdal et al., 2003]. Although *TGFβ3* was not significantly affected by 17β-estradiol in the human cells in the current study, TGFβ mediates apoptotic effects of estrogen in murine osteoclasts [Hughes et al., 1996]. *PTEN* encodes phosphatidylinositol-3,4,5-trisphosphate-3-phosphatase, resulting in negative regulation of AKT and hence decreased cell proliferation and tumor suppression. In RAW 264.7 osteoclastic cells, PTEN overexpression antagonizes RANKL-stimulated osteoclast survival and osteopontin-stimulated cell migration [Sugatani et al., 2003]. *ICAM1*, which encodes a glycoprotein that binds to several integrin classes, is important in osteoclastogenesis [Kurachi et al., 1993; Okada et al., 2002; Garcia-Palacios et al., 2007; Bloemen et al., 2010]. Thus, the currently observed stimulatory effect of testosterone on *ICAM1* is paradoxical, and merits further investigation as to its physiological or pathophysiological significance. TNF-α has a well-established role in inducing osteoclast differentiation [Azuma et al., 2000; Kobayashi et al., 2000]. The significance of an effect of testosterone to increase expression of a gene that leads to osteoclastogenesis is unclear, but could be involved in the resorption component of prostate cancer metastases to bone [Zhang et al., 2001]. The reported effect of TNF-α to stimulate the production by osteoblasts of an inhibitor of osteoclastogenesis [Balga et al., 2006] could mitigate the osteoclastogenic effect. In renal cortical tissue, stimulation of TNF-α production by testosterone leads to proapoptotic signaling [Metcalfe et al., 2008]. The effects of testosterone on *TNF* and *ICAM* could be related, as TNF-α can increase expression of *ICAM1* [Zhang et al., 2002].

Testosterone had few significant effects on cells from the males. Perhaps exposure of the cells to testosterone in vivo might have limited further response to in vitro exposure. There were only two significant responses to testosterone in the cells from the males,

these being large increases in the expression of the gene for the matrix protein decorin and in that for *TNF*. There is little information on the possible role of decorin in osteoclasts. Mice deficient in decorin and biglycan had greater osteopenia than mice deficient in biglycan alone, however it is likely that this was an effect on osteogenic precursors [Young et al., 2006].

In summary, the current studies clearly demonstrate that both 17β-estradiol and testosterone affect gene expression in osteoclasts derived from human peripheral blood mononuclear cells. However, not only do the two hormones elicit different effects, but the cells from the two sexes respond differently to each of the hormones. Both hormones elicit effects that can lead to osteoclast apoptosis, but the mechanisms by which the two hormones act appear to be different. The differences between the effects of testosterone and 17β-estradiol support the conclusion that testosterone probably acts by aromatase-independent as well as aromatase-dependent pathways. Finally, the results suggest that skeletal responses to other hormones and a range of endogenous and exogenous stimuli, including therapeutic agents, could be sex-specific or show a differential response in males and females. Studies from other systems reveal important findings in this regard. Sex differences in response to therapeutic agents based on both pharmacokinetic and pharmacodynamic factors are being increasingly recognized [Chen et al., 2007; Franconi et al., 2007]. There are sexually dimorphic effects of glucocorticoids on gene expression in rat liver, effects potentially relevant to sex differences in susceptibility to inflammatory disease [Duma et al., 2010]. These findings indicate that a more complete understanding of the mechanisms of sex differences of responses at the gene level will be an important step in developing the most effective personalized therapies.

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