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Expression of estrogen receptor-alpha in cells of the osteoclastic lineage

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Abstract Estrogen deficiency at the menopause is associated with an increased rate of bone loss and subsequent risk of skeletal fracture. Whilst cells of the osteoblastic lineage are known to express estrogen receptors, the presence of estrogen receptors in osteoclasts remains controversial. We have examined expression of the classic estrogen receptor, estrogen receptor-alpha (ERα), during osteoclast differentiation. In situ mRNA hybridisation with a digoxygenin-labelled riboprobe to ERα mRNA, together with immunocytochemical analysis using a human ERα-specific monoclonal antibody demonstrated similar findings and confirmed the expression of ERα in chondroblasts and osteoblasts from human fetal bone and mineralising human bone marrow cultures. ERα expression was detected in human bone marrow cultures treated with $1,25(OH)_2D_3$ and macrophage colony-stimulating factor and in macrophage cultures treated with $1,25(OH)_2D_3$. However, in an in vitro model of human osteoclast formation, no ERα expression was observed in the osteoclasts that developed. The human preosteoclast TCG 51 cell line showed strong expression of ERα in contrast to the low levels observed in the more mature bone resorptive TCG 23 cell line. No expression was detectable in osteoclasts cultured from giant cell tumour of bone (GCTB) tissue or in osteoclasts in Pagetic, GCTB, or hyperparathyroid bone tissues. In conclusion, preosteoclasts express detectable levels of ERα, but osteoclast maturation and bone resorption is associated with loss of ERα expression. This indicates that ERα expression and regulation may play a role in osteoclast formation.

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Introduction

Postmenopausal osteoporosis, a major health problem in the developed world, is associated with estrogen deficiency (Prince 1994). A number of studies have shown that estrogen is essential for the development, growth and maturation of the skeleton. It plays a central role in the maintenance of optimal bone mass through the suppression of bone remodelling and by maintaining a balance between bone formation and bone resorption in both women and men (Parfitt 1979; Lindsay and Cosman 1992; Girasole et al. 1992; Prince 1994). Furthermore, estrogen deficiency at menopause is associated with an increased rate of bone loss due to increased osteoclast number and activity, resulting in increased risk of skeletal fracture which can be prevented with hormone replacement therapy (Lindsay et al. 1976; Ettinger et al. 1985; Vedi and Comptson 1996). However, the mechanism of action of estrogen in bone and, in particular, the presence of estrogen receptors (ERs) in osteoclasts remains controversial.

The identification of nuclear ERs in osteoblasts (Eriksson et al. 1988; Ernst et al. 1991), osteoblast-like cells (Komm et al. 1988; Davis et al. 1994; Huo et al. 1995; Kassem et al. 1996; Lajeunesse 1994) and reports in the literature of reduced bone density in: (1) estrogen receptor knock-out (ERKO) compared to wild-type mice (Korach 1994) and (2) a young adult male with functional disruption of the ER, support a direct (receptor-mediated) effect of estrogen in bone (Smith et al. 1994). This and other studies suggest that estrogen plays a key role in the modulation of osteogenic cell differentiation, development and turnover (Suda et al. 1997; Robinson et al. 1997). However, studies on the localisation and identification of ERs and the effect of estrogen on cells of the osteoclast lineage have proved conflicting. Receptors have been identified in impure cell populations of avian

osteoclasts (Oursler et al. 1991; Brubaker and Gay 1994), rabbit osteoclasts (Mano et al. 1996) and multinucleated cells from human osteoclast-like giant cell tumours of bone (Oursler et al. 1994; Kaneda et al. 1997) and a human preosteoclastic cell line (Fiorelli et al. 1995). Recently Hoyland and coworkers (1997), using in situ reverse transcriptase polymerase chain reaction (RT-PCR), detected ER mRNA expression in osteoblasts, osteocytes and some osteoclasts. However, other studies have failed to detect ERs in avian or human osteoclasts (Colston et al. 1989; Ohashi et al. 1991; Zheng et al. 1995; Kusec et al. 1996, 1998; Collier et al. 1998). In a two-phase human bone marrow culture model of osteoclastogenesis, Sarma et al. (1998) showed estrogen (17β-estradiol) addition to phase I inhibited bone resorption by reducing osteoclast formation, whilst addition in phase II had no effect on bone resorption, suggesting estrogen does not interfere with bone resorption occasioned by mature osteoclasts. Furthermore, Arnett and coworkers (1996) observed no significant effects of estrogen on resorption pit formation by rat osteoclasts, even at supraphysiological concentrations (10 μM). Tamura et al. (1993) also reported a lack of effect of estrogen on bone resorption using mouse osteoclast-like cells formed in vitro. Hughes et al. (1996) have reported that estrogen promotes apoptosis of osteoclasts in vitro and in vivo and this process is possibly mediated by TGFβ. However, in the absence of pure osteoclast populations it was not possible to confirm if these effects were direct or indirect.

In a recent study (Kusec et al. 1998), using skeletal tissue from rabbits and from young human patients undergoing corrective surgery for leg growth, we have observed ERα expression in chondrocytes of the growth plate and subarticular epiphyseal growth centre as well as in osteoblasts and lining cells of trabecular bone. However, no clear evidence for ERα expression was observed in mature osteocytes or osteoclasts in these tissues. We postulate that the conflicting reports of ER expression in osteoclasts and the lack of effect of estrogen on isolated osteoclast populations may be attributable to a variation in receptor expression with osteoclast differentiation and maturation. Thus, the aim of the current study was to test the hypothesis that ERα expression is dependent on osteoclast maturation. We have used a variety of in vitro models of osteoclastogenesis, together with a number of tissues rich in osteoclasts, derived from Pagetic, hyperparathyroid bone and giant cell tumour of bone (GCTB) patients, to investigate this question. The current studies indicate that osteoclast maturation and bone resorption is associated with loss of ERα expression.

Materials and methods

Tissue culture reagents were obtained from Gibco/BRL (Paisley, UK). Acid phosphatase kits and other reagents were from Sigma (Poole, UK). $1,25(OH)_2D_3$ was a generous gift from Dr. L. Binderup (Leo Pharmaceutical Products, Ballerup, Denmark). Antibody to the vitronectin receptor (23C6) was kindly provided by Prof. M.A. Horton (ICRF, London, UK).

Cell culture

Bone marrow cells were harvested from samples obtained from haematologically normal patients undergoing routine total hip replacement surgery or corrective surgery as previously described (Oreffo et al. 1997). Marrow cells were plated out on glass slides in square petri dishes at 2×10^7 nucleated cells/dish or in T80 cm^2 flasks in αMEM supplemented with 10% ($^v/_v$) fetal calf serum alone or human serum or 10% ($^v/_v$) human serum containing dexamethasone (10^{-8} M) and $1,25(OH)_2D_3$ (10^{-8} M). Cultures were fed after 6 days and thereafter every 3 days for up to 15 days. One group of cultures were trypsinised after 10–14 days and replated in αMEM supplemented with 10% ($^v/_v$) fetal calf serum, $1,25(OH)_2D_3$ (10^{-8}M) and macrophage colony-stimulating factor (M-CSF; 25 ng/ml) for a further 10–15 days. At appropriate time points cultures were analysed for mRNA expression of estrogen receptor by in situ hybridisation or immunocytochemistry and stained for alkaline phosphatase activity/tartrate-resistant acid phosphatase (TRAP) non-specific esterase and vitronectin receptor expression.

Monocyte-macrophage populations were isolated from renal dialysate preparations by centrifugation at 500 *g* for 5 min at 4°C. Monocyte preparations were washed twice in αMEM supplemented with 10% ($^v/_v$) fetal calf serum and cultured on glass slides (2×10^4 cells/slide) in αMEM supplemented with 10% ($^v/_v$) fetal calf serum and $1,25(OH)_2D_3$ (10^{-8} M) for up to 7 days.

Multinucleated cells derived from explant cultures of GCTB tissue, obtained after surgery, were cultured in αMEM supplemented with 10% ($^v/_v$) fetal calf serum in 25cm^2 flasks. The multinucleated cells observed in culture after 24–48 h were used for in situ hybridisation, immunocytochemistry and examination of calcitonin-induced shape change. For studies to examine cell contraction in response to calcitonin, salmon calcitonin was added to cultures of these cells at a final concentration of 0.01–1 nM. Cells were inspected for change in shape in situ by phase contrast microscopy over a 2-h period.

TCG 23 and TCG 51 cells (2×10^4 cells/glass coverslip) were cultured on glass coverslips in αMEM supplemented with 10% ($^v/_v$) fetal calf serum (Grano et al. 1994). Cells were fixed in 95% ($^v/_v$) ethanol or 4% paraformaldehyde, after washing twice in phosphate-buffered saline (PBS), and stored in PBS until examined for ERα expression.

All cultures were maintained at 37°C in a gassed incubator, 5% CO_2 in air. At the completion of cell culture, the medium was removed, the cell layer washed in PBS and cultures fixed in 95% ($^v/_v$) ethanol or 4% paraformaldehyde.

Tissue preparation

Tissue samples including GCTB, Paget's, hyperparathyroid bone, human fetal bone and human bone obtained during epiphyseodesis for corrective treatment of leg growth by curettage of the growth plate, were fixed in 4% ($^w/_v$) paraformaldehyde in PBS. Only human tissues that otherwise would have been discarded were used, with the approval of the hospital medical committee. Specimens were processed for paraffin embedding following decalcification in EDTA [15% ($^w/_v$)] for 3–7 days and dehydration in alcohol and xylene. Sections (5 μm) were placed on 3-amino propylene triethoxy silane-coated slides, and adjacent sections evaluated by in situ hybridisation, immunocytochemistry or routine histology.

In situ hybridisation

Preparation of riboprobes

The cDNA plasmid used for detecting ERα was a generous gift from P. Chambon (IGBMC-LGME-U.184-ULP, Strasbourg, France). The original plasmid contained a 1.8 kb fragment of the human ERα coding region in pSG5 vector. Probes were labelled with digoxigenin using a kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions.

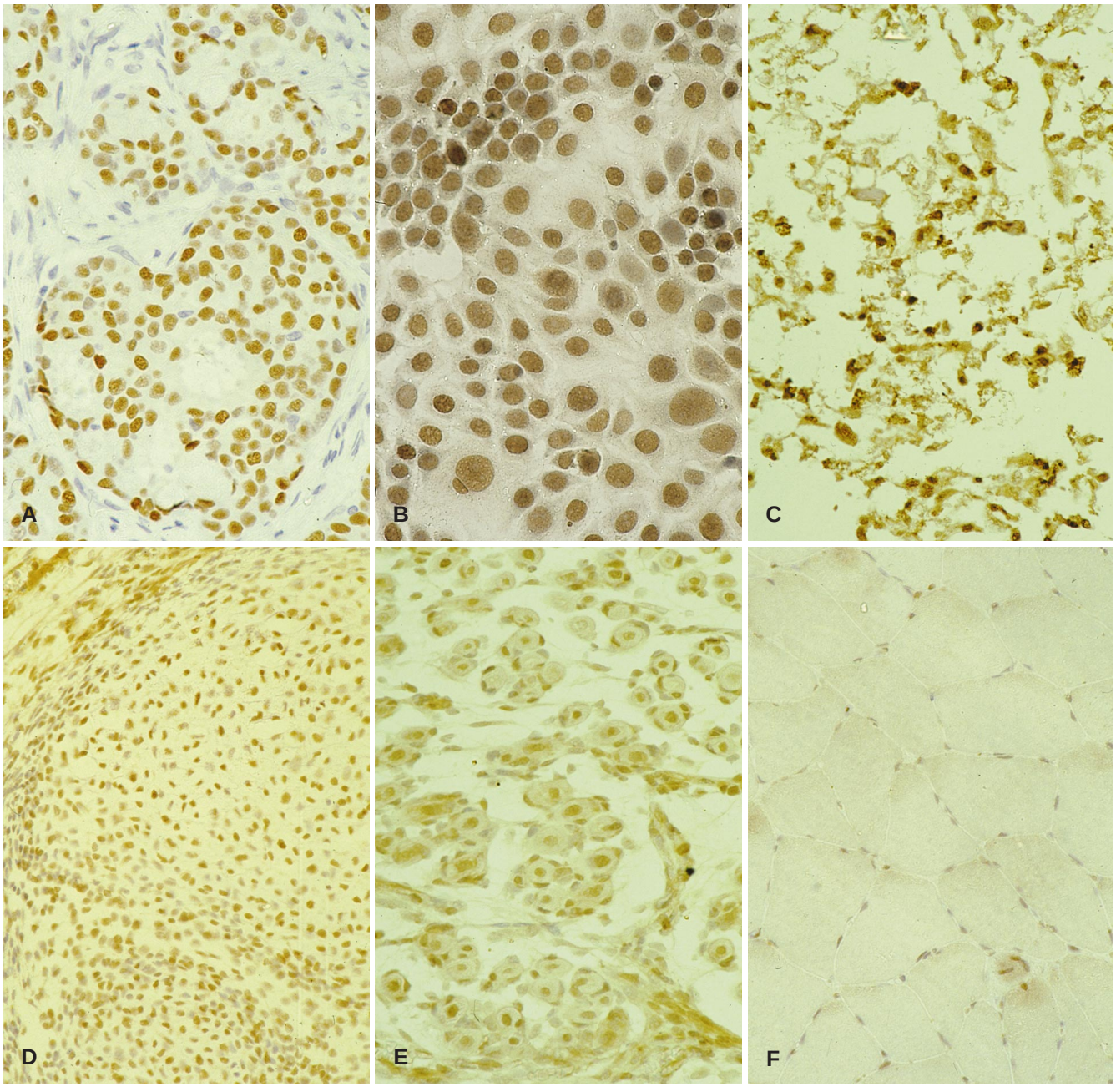

Fig. 1A–F Immunolocalisation of estrogen receptor (ER) expression. **A** Human breast carcinoma (×100). **B** MCF-7 breast cancer cells (×400). **C** Human bone marrow (×100). **D** Human chondrogenic tissue (digit; ×100). **E** Human fetal bone (×400). **F** Muscle (×400). negative control

Hybridisation procedure

Antisense or sense probe to ERα mRNA was applied to one of two histological sections on each slide and hybridisation was performed overnight at 60°C. Posthybridisation treatment included digestion of unbound probe with RNase, and washes with SSC (1×, 0.5×, 0.1×). Detection of hybridised probe was carried out with alkaline phosphatase-coupled anti-digoxigenin antibody according to the manufacturer's instructions (Boehringer Mannheim). Human breast tissue and the MCF-7 breast cancer cell line were used as positive controls for ERα mRNA. The breast cancer cell line, MDA, and muscle tissue were used as negative controls. Tissue preservation of RNA was assessed by in situ hybridisation with an oligo d(T) probe (R&D Systems, Abingdon, UK). Controls included hybridisation with sense probes. All photographs were taken under differential interference contrast microscopy [Axiophot; Carl Zeiss (Oberkochen), Welwyn Garden City, UK] to emphasise cellular morphology.

Immunohistochemistry procedure

Localisation of ERα was analysed by immunohistochemistry on demineralised paraffin-embedded specimens. Paraffin-embedded breast tissue, human fetal tissue and MCF-7 breast cancer cells were used as positive control tissues. A standard indirect peroxidase procedure recommended by the manufacturer was followed using concentrated monoclonal mouse antibody to human ERα and reagents from Biogenex (San Ramon, USA) as previously described (Kusec et al. 1998).

Table 1 Estrogen receptor-alpha (*ERα*) expression in breast tissue/cell lines, fetal and chondrogenic tissues (– Absent, +++ strong expression of ERα)

Specimen	Characteristics	ERα
Muscle	ERα negative	–
MDA cells	ERα negative	–
MCF-7 cells	ERα positive	+++
Breast carcinoma	ERα positive	+++
Human fetal bone	Osteoblastic and chondrogenic tissue	+++
Tibial growth plate (epiphyseodesis)	Osteoblastic and chondrogenic tissue	+++

Histochemical staining – alkaline phosphatase/TRAP and vitronectin receptor activity

Cultures were rinsed 3 times in PBS, fixed in 95% ($^{v}/_{v}$) ethanol and stained for alkaline phosphatase and acid phosphatase using Sigma kit numbers 387 and 85, respectively, according to the manufacturer's instructions. Vitronectin receptor (αvβ3) expression on multinucleated cells was determined using a monoclonal antibody to the vitronectin receptor complex on human osteoclasts (23C6) (Davies et al. 1989) and a vectastain ABC kit (Vector laboratories, Peterborough, UK) according to manufacturer's instructions.

Results

ERα expression in skeletal and breast control tissues and cells

Immunocytochemistry confirmed the expression of ERα in control samples of breast carcinoma tissue and MCF-7 cells (Fig. 1A, B, Table 1), in human bone marrow cultures (Fig. 1C) and in osteoblasts and chondrogenic cells in human fetal bone (Fig. 1D, E). Expression was predominantly localised to the nuclei with negligible cytoplasmic staining. In situ hybridisation together with immunocytochemical analysis demonstrated similar findings and confirmed the strong expression of ERα observed in chondrogenic and osteoblastic cells from tibial growth plate as previously reported (not shown). Expression was observed in all regions of the growth plate, and in all breast carcinoma sections examined as well as fetal bone samples. Sections hybridised with the sense probe or in specimens in which the primary ERα monoclonal antibody was omitted showed no staining (not shown). The specificity of the ERα antisense riboprobe was confirmed using northern analysis of mRNA from MCF-7 cells showing major hybridisation with a single band of approximately 6.6 kb (not shown). No expression of ERα was observed in the ER-negative cell line, MDA, or in the ERα-negative tissue, skeletal muscle (Fig. 1F, Table 1).

ERα expression in preosteoclastic cells and in vitro osteoclast formation models

ERα expression was readily detected in the human preosteoclast cell line TCG 51 (Fig. 2A, Table 2). Negligible expression was observed in the bone resorptive human osteoclast cell line TCG 23 (Fig. 2B).There was no ERα staining of paired sections run in the absence of primary ERα antibody (not shown). Macrophage cultures established from peritoneal dialysate fractions and treated with $1,25(OH)_2D_3$ yielded TRAP- positive, non-specific esterase-positive and vitronectin receptor-positive cells (not shown), which strongly expressed ERα (Fig. 2C). Similarly, in human bone marrow cells treated with $1,25(OH)_2D_3$ and M-CSF (25 ng/ml) for 21–28 days, strong expression of ERα, as shown by in situ hybridisation, was observed (Fig. 2D). These cells were found to express the early markers of the osteoclast phenotype, including TRAP activity, non-specific esterase activity and to express the vitronectin receptor (not shown). Cells from both of these culture models failed to contract in response to exogenously added calcitonin (0.01–1 nM; not shown).

Human bone marrow cultures pretreated with dexamethasone, trypsinised and grown in the presence of M-CSF and $1,25(OH)_2D_3$ for 10–21 days on bone slices, form bone-resorbing osteoclasts (Sarma and Flanagan 1996). In parallel cultures, ERα expression could be observed in the first culture stage but could not be detected in the later second stage of culture following the addition of M-CSF and $1,25(OH)_2D_3$ in human osteoclast preparations (Fig. 2E, Table 2).

ERα expression by osteoclasts ex vivo

In freshly isolated osteoclasts from GCTB tissue, TRAP-positive, non-specific esterase-positive and vitronectin receptor-positive cells were observed which contracted within 15–20 min when exposed to exogenously added calcitonin at concentrations as low as 10^{-12}M (not shown). ERα expression could not be detected in these osteoclast cells (Fig. 2F, Table 2).

ERα expression by osteoclasts from metabolic bone disease sites

ERα mRNA or protein could not be detected in the numerous multinucleated TRAP-positive osteoclasts pres-

Fig. 2 **A** Human preosteoclast cell line TCG 51 showing positive immunolocalisation for ERα (×100). **B** Preosteoclast cell line TCG 23 demonstrating an absence of ERα immunostaining (×100). **C** Immunolocalisation of ERα expression in human macrophage cultures grown in the presence of $1,25(OH)_2D_3$ (×400). **D** In situ mRNA hybridisation using ERα riboprobe in human bone marrow cultures treated with $1,25(OH)_2D_3$ and macrophage colony-stimulating factor (M-CSF; ×400). **E** Absence of ERα mRNA expression in osteoclasts generated from human bone marrow cultures treated with human serum, $1,25(OH)_2D_3$ and M-CSF for 17 days (×400). **F** Absence of ERα mRNA expression in osteoclasts grown from a giant cell tumour of bone (GCTB) sample (×400) using in situ mRNA hybridisation. **G** Absence of ERα immunostaining in osteoclasts from human GCTB (×400). **H** Osteoclasts from Pagetic tissue (×400). **I** Osteoclasts from hyperparathyroid tissue (×400). Note the positive staining of ERα positive marrow stromal cells in the GCTB Pagetic and hyperparathyroid tissues (*arrows* in **G–I**) ▶

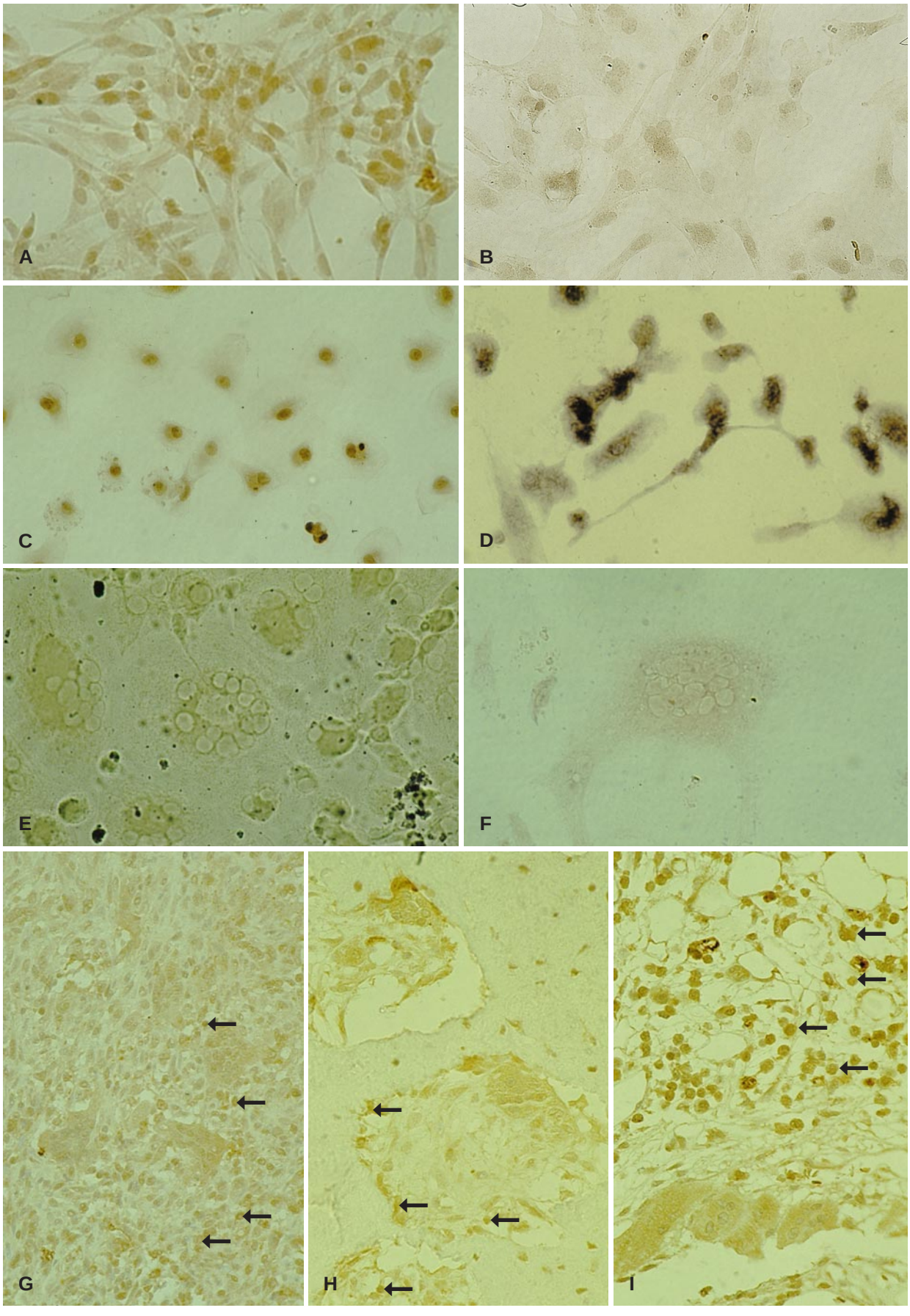
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Table 2 ERα expression in human preosteoclast cell lines and in in vitro models of osteoclast formation (*nd* Not determined, – absent, –/+ weak, ++ present, ✓ present, *BMDC* bone narrow derived cells, *MNC* multinucleated cells, *HuS* human serum, *FCS* fetal calf serum, *TRAP* tartrate-resistant acid phosphatase, *NSE* non-specific esterase, *VNR* vitronec-tin receptor, *M-CSF* macrophage colony-stimulating factor)

Specimen	Characteristics/ culture details	ERα	TRAP	NSE	VNR	Calcitonin response
TCG 23	Passaged cells	–/+	✓	✓	✓	–
TCG 51	Passaged cells	++	✓	✓	✓	–
Macrophage	Dialysate, HuS D_3	++	✓	✓	✓	–
BMDC-MNC	HuS/D_3/M-CSF	++	✓	✓	✓	–
Monocytes	D_3, UMR d10	–	✓	✓	nd	–
BMDC-2 stage model	FCS/Dex, FCS/D_3+M-CSF	–	✓	✓	✓	nd

Table 3 ERα expression in human osteoclasts (*N/A* Not applicable, – absent, ✓ present)

Osteoclast specimen	ERα	TRAP	VNR	Calcitonin response
Giant cell tumour of bone, isolated osteoclasts	–	✓	✓	✓
Giant cell tumour of bone tissue	–	✓	✓	N/A
Pagetic bone	–	✓	✓	N/A
Hyperparathyroid bone	–	✓	✓	N/A

ent in tissue taken from Pagetic, GCTB or hyperparathyroid patients (Fig. 2G–I, Table 3). However, within sections of hyperparathyroid tissue, GCTB and Pagetic tissue, ERα-positive stromal cells within the bone marrow and osteoblasts lining the bone trabeculae were observed (Fig. 2G–I).

Discussion

This study demonstrates that ERα expression is detected in cells of the osteoclastic lineage, specifically preosteoclastic cells, but not in mature osteoclast cell populations. This expression was observed only in a preosteoclastic cell line, TCG 51 and in precursor cells generated by a variety of in vitro models of osteoclastogenesis. We observed that TRAP -positive, non-specific esterase-positive, vitronectin receptor-positive multinucleated cells could be generated from human bone marrow cultures treated with $1,25(OH)_2D_3$. These cells, typical of immature osteoclasts in the absence of bone resorption or calcitonin contraction, showed strong ERα expression. Similarly, monocyte/macrophage populations, which also displayed the phenotype of early osteoclast lineage cells, all expressed the receptor whether analysed by immunocytochemistry or by in situ hybridisation. In contrast, ERα expression could not be detected in osteoclasts from fresh GCTB or in osteoclasts from a variety of tissues known to contain increased osteoclast numbers including hyperparathyroid, GCTB and Pagetic bone. Similar results indicating an absence of ERα as well as ERβ expression whether examined by RT-PCR or fluorescence in situ hybridisation in pure osteoclast populations obtained by microisolation of osteoclasts from human GCTB preparations have recently been reported by Collier and coworkers (1998).

Expression of the receptor was negligible in the TCG 23 osteoclast line, which has been shown to resorb bone particles, compared to the TCG 51 preosteoclast cell line, which contains highly motile mononucleated cells with insignificant resorptive capacity. These results are in agreement with the findings of Fiorelli *et al*. (1995) who reported the presence of functional ERs in the bone marrow-derived human preosteoclastic cell line FLG 29.1. Furthermore, in the same study, the authors found ER expression was lost in FLG 29.1 cells that were induced to differentiate towards the osteoclastic phenotype by phorbol ester treatment and suggested this may reflect a phorbol ester-dependent downregulation of ER mRNA and ER protein. The current results offer an alternative explanation, namely the loss of ER expression observed in mature osteoclasts.

In support of this hypothesis, ERα expression could not be detected in osteoclasts derived from a two-stage culture model of human bone marrow cells treated with dexamethasone followed by $1,25(OH)_2D_3$ and M-CSF, known to resorb bone, further demonstrating osteoclast maturation is associated with loss of ER expression. In this and other studies this two-stage model of osteoclastogenesis has been shown to produce osteoclast populations that resorb bone (Sarma and Flanagan 1996; Lader and Flanagan 1998; Sarma et al. 1998).

The current results appear to differ from the observation of ER mRNA detection in osteoclasts (and osteocytes) using in situ RT-PCR reported by Hoyland and colleagues (1997). However, in the latter studies successful demonstration of ERα in some and not all of the osteoclasts relied on amplification of the extremely low copy number of the ER mRNA. Furthermore, it cannot be excluded that the expression of ERα observed was attributable to preosteoclast cells, as suggested by the current data. In separate studies using human skeletal tissues from patients undergoing epiphyseodesis for corrective treatment of leg growth, ERα expression was localised to chondrocytes of the growth plate, the subarticular epiphyseal centre, within osteoblasts and lining cells on

trabecular surfaces by in situ mRNA hybridisation and immunocytochemistry (Kusec et al. 1998). However, no evidence of ERα expression (mRNA or functional protein) was observed in osteocytes or osteoclasts. Expression of ERα in osteoclasts has been the subject of several conflicting articles. ERα receptors have been identified in impure cell populations of avian osteoclasts (Oursler et al. 1991), rabbit osteoclasts (Mano et al. 1996) and multinucleated cells from human osteoclast-like giant cell tumours of bone (Oursler et al. 1994) whilst other groups have failed to detect ERs in avian or human osteoclasts (Colston et al. 1989; Ohashi et al. 1991; Zheng et al 1995; Collier et al. 1998; Kusec et al. 1998). In the current studies, ERα was observed in a range of osteoclastic cell lines and in preosteoclast populations. However, expression of ERα could not be detected in isolated mature osteoclast populations or in osteoclasts from metabolic diseases with high osteoclastic activity. These results may explain, in part, the apparent discrepancies in ER expression reported in the literature.

The dramatic increase in the rate of bone loss, as much as tenfold, which accompanies the menopause or loss of ovarian function, is indicative of the central role of estrogen in the maintenance of bone mass in females (Richelson et al. 1984; Jilka et al. 1992; Turner et al. 1992; Smith et al. 1994). Evidence to substantiate such a direct role in bone physiology of both males and females came from work using ERKO mice (Korach 1994) in which ERKO male and female mice showed a 20–25% decrease in bone density compared to wild-type mice, suggesting a direct role for estrogen in bone. The significance of ERα in bone metabolism has been demonstrated in the case report of a 28-year-old man with a disruptive mutation in the ERα gene leading to estrogen resistance and resulting in decreased bone mineral density, increased bone turnover and incomplete epiphyseal closure (Smith et al. 1994). However, the recent cloning of a new ER subtype, ERβ, indicates alternative mechanisms for the mediation of the hormonal effects of estrogen, other than via the ERα receptor (Kuiper et al. 1996). The ERβ is capable of binding 17β-estradiol with an affinity similar to that of ERα (Mosselman et al. 1996; Tremblay et al. 1997) and ERβ transcripts have been detected, in the human, in the testis, ovary and thymus (Kuiper et al. 1997; Onoe et al. 1997). The presence and role of this receptor in bone is unclear, but a recent report (Arts et al. 1997) indicates ERβ is expressed in a human osteoblast cell line, and that ERα and ER-β are differentially expressed during human osteoblast differentiation. Couse and coworkers (1977) have shown from studies using the ERKO mouse that the ERβ mRNA levels are slightly decreased in ovary, epididymis and prostate whereas ERβ expression is unaltered in wild-type littermates suggesting the biological functions of ERβ may be dependent on the presence of ERα in various tissues.

The current results echo a point recently made by Jemtland and coworkers (1998), who observed significant heterogeneity among cells that express osteoclast-associated genes (TRAP, type IV collagenase and c-fms, the receptor for M-CSF) in developing bone from fetal mouse hind limbs (embryonic days 15–17). The authors suggested that the distinct expression patterns exhibited by these markers may represent different stages of osteoclastogenesis or distinct subpopulations of the osteoclast lineage. The differential expression of ERs within bone cell populations has been observed among cells of the osteoblast lineage. Recently, Bodine and coworkers (1998), using fetal rat calvarial-derived osteoblasts, have shown that ERα expression is developmentally regulated during osteoblast differentiation, with a biphasic increase in ERα expression that peaks in mature osteoblasts. Furthermore, selective differences were observed in 17β-estradiol regulation of type I procollagen, TGFβ1, osteocalcin, alkaline phosphatase and ERα expression in late mineralisation cultures from these cells indicative, in osteoblasts, of a functional relationship between ERα expression and bone differentiation.

The current results indicate that the levels of ERα expression vary with osteoclast maturation. The expression of ERβ in osteoclast cell populations remains, at present, unknown, but it is likely that expression and regulation of these receptors, as for the "classic" ER, ERα, will prove equally complex within bone. The present results may explain, in part, the apparent discrepancies in ER expression observed in the literature and, furthermore, suggest that estrogen receptor expression and its regulation may prove key in osteoclast formation and bone turnover.

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