

A Novel Member of the Leukocyte Receptor Complex Regulates Osteoclast Differentiation

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Abstract

Osteoclasts (OCs) are multinucleated cells that resorb bone and are essential for bone homeostasis. They develop from hematopoietic cells of the myelomonocytic lineage. OC formation requires cell-to-cell interactions with osteoblasts and can be achieved by coculturing bone marrow precursor cells with osteoblasts/stromal cells. Two of the key factors mediating the osteoblast-induced osteoclastogenesis are macrophage-colony stimulating factor (M-CSF) and the tumor necrosis factor (TNF) family member TNF-related activation-induced cytokine (TRANCE) that are produced by osteoblasts/stromal cells in response to various bone resorbing hormones. In addition, other factors produced by osteoblasts/stromal cells further influence osteoclastogenesis. Here we report the identification and characterization of OC-associated receptor (OSCAR), a novel member of the leukocyte receptor complex (LRC)-encoded family expressed specifically in OCs. Genes in the LRC produce immunoglobulin (Ig)-like surface receptors and play critical roles in the regulation of both innate and adaptive immune responses. Different from the previously characterized members of the LRC complex, OSCAR expression is detected specifically in preosteoclasts or mature OCs. Its putative-ligand (OSCAR-L) is expressed primarily in osteoblasts/stromal cells. Moreover, addition of a soluble form of OSCAR in coculture with osteoblasts inhibits the formation of OCs from bone marrow precursor cells in the presence of bone-resorbing factors, indicating that OSCAR may be an important bone-specific regulator of OC differentiation. In addition, this study suggests that LRC-encoded genes may have evolved to regulate the physiology of cells beyond those of the immune system.

Key words: osteoclast • Ig-like receptor • TRANCE • differentiation • costimulation

Introduction

Bones provide rigid support for the body, mechanical integrity of movement and protection, and serve as a site of mineral homeostasis. Additionally, bone is an indispensable connective tissue as well as the primary site for hematopoiesis. Bone is continuously remodeled through new bone formation by osteoblasts and the resorption of old bone by osteoclasts (OCs)* which provides the renewal of the skeletal

with structural and functional integrity. Normal skeletal formation and maintenance, and mineral homeostasis are thus maintained by the coupled actions of two principal cell types in bone, osteoblasts, and OCs (1, 2).

OCs, the only cells capable of resorbing bone, are derived from the same bone marrow precursor cells of the monocyte-macrophage lineage that give rise to macrophages and dendritic cells (DCs) (1, 2). For OC differentiation and activation of mature OCs, osteoblasts play an obligatory role by providing essential osteoclastogenesis factors in response to bone resorbing hormones (1, 2).

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*Abbreviations used in this paper: DC, dendritic cell; LRC, leukocyte receptor complex; OC, osteoclast; OSCAR, OC-associated receptor; PIR, paired

Ig-like receptor; RH, radiation hybrid; TRANCE, TNF-related activation-induced cytokine; TRAP, tartrate-resistant acid phosphatase.

There are at least two essential factors provided by osteoblasts to support osteoclastogenesis that are macrophage-colony stimulating factor (M-CSF) and TNF-related activation-induced cytokine (TRANCE). Spontaneous osteopetrotic mice (op/op) which are deficient in M-CSF show defective OC development. Osteoblasts derived from M-CSF-deficient mice failed to support osteoclastogenesis, indicating that M-CSF produced by osteoblasts is essential for differentiation of OCs from bone marrow precursors (1–4). The TNF family member TRANCE, which is expressed in osteoblasts in response to various bone-resorbing hormones, is also required for osteoclastogenesis (1, 2, 5–8). Similar to the case of M-CSF-deficient osteoblasts, TRANCE-deficient osteoblasts failed to support osteoclastogenesis. These results clearly indicate that M-CSF and TRANCE are required for OC differentiation. TRANCE or M-CSF expression on osteoblasts is not constitutive but regulated by various bone-resorbing hormones (e.g., the active form of vitamin D₃, 1 α ,25-dihydroxyvitamin D₃, 1 α ,25(OH)₂D₃ and parathyroid hormone) or cytokines (e.g., IL-1 and TNF), suggesting that a number of cytokines and hormones indirectly regulate osteoclastogenesis in part by controlling the expression of TRANCE or M-CSF on osteoblasts (1, 2, 9, 10). Bone marrow cells or spleen cells can differentiate into bone-resorbing mature OCs in the absence of osteoblasts/stromal cells when recombinant M-CSF and TRANCE are added in the culture. Thus, M-CSF and TRANCE together are not only necessary but also sufficient factors for OC development, and they are likely to be responsible for most of the obligatory roles provided by osteoblast for osteoclastogenesis (1, 2).

Although it is clear that M-CSF and TRANCE are two most important factors regulating osteoclastogenesis, other cytokines (e.g., IL-1, INF- γ , TGF- β) have also been shown to regulate OC differentiation or activity directly (1, 2, 11–17). It is not clear how those cytokines modulate the OC differentiation or activity, but they are likely to act in conjunction with M-CSF and/or TRANCE.

Most of factors described to date, including M-CSF and TRANCE, that affect the fate of OCs (differentiation, activation, and survival) also influence macrophages or DCs which share their bone marrow precursors during development (18). Therefore, it appears that there are considerable molecular overlaps between OCs and cells of the immune system such as macrophages and DCs. In this paper, we have attempted to further investigate the commonality and uniqueness of OCs compared with their relatives such as macrophages or DCs by exploring mRNA expression profiles in these cells. Here we have identified a novel member of leukocyte receptor complex (LRC)-encoded protein which is expressed specifically on OC-lineage cells and show that this novel LRC-family member regulates osteoclastogenesis.

Materials and Methods

Cell Culture and Poly A⁺ RNA Preparation. OCs: murine (OCs) were derived as described previously (19). In brief, bone mar-

row-derived cells (10⁷/100 mm dish) from C57BL/6 mice (4–6 wk of age) were incubated with human M-CSF (5 ng/ml) in α -MEM/10% FCS for 16 h. After incubation, the floating cells were collected and further cultured with 30 ng/ml of M-CSF, 1 μ g/ml of soluble murine TRANCE, and PGE₂ (10⁻⁶ M) for 4 d. On day 3, the culture was changed with fresh media. On day 4, the cells were washed with PBS, and harvested. Human OCs are generated as described previously (20). Total RNA was prepared by TRIZOL reagent (GIBCO BRL). 1 mg of total RNA was subjected into Oligotex poly A⁺ RNA column (QIAGEN) to purify poly A⁺ RNA. Macrophages: murine macrophages were generated from bone marrow progenitors, using human M-CSF (30 ng/ml; reference 19). On day 4, the cells were washed with PBS, harvested, and subjected to poly A⁺ RNA purification as described above. DCs: murine bone marrow-derived DCs were generated from mouse bone marrow progenitors, using GM-CSF as described previously (21). On day 8, the mature bone marrow-derived DCs were washed with PBS, and harvested. RNA was prepared as described above. RAW264.7: the murine myeloid RAW264.7 cell line (American Type Culture Collection) was maintained with DMEM/10% FCS containing 1 mM sodium pyruvate and 10 mM Hepes. To generate OCs, the cells (5 \times 10⁵ cells per 100-mm dish) were cultured with 1 μ g/ml of recombinant soluble TRANCE for 4 d. On day 3, the culture was changed with fresh media. To prepare poly A⁺ RNA, the cells were washed with PBS, harvested, and subjected to poly A⁺ RNA purification as described above.

OC-enriched cDNA Library, PCR-Subtraction, and Full-Length cDNA Cloning. An OC-enriched cDNA library was generated by using poly A⁺ RNA from OCs and macrophages (M ϕ s) as described previously (22). In brief, 2 μ g of poly A⁺ RNA from OCs and M ϕ s was used to make tester and driver cDNAs, respectively. Subtractive PCR was performed using the PCR-select cDNA subtraction kit according to the manufacturer's protocol (CLONTECH). Around 250 clones from this cDNA library were tested in this study and OCL178 encoding amino acid residues 161–265 of murine OC-associated receptor (OSCAR) was isolated. To clone full-length OSCAR cDNA, cDNA fragments of OSCAR derived from OC-enriched cDNA library were used to screen a λ ZAP cDNA library derived from mouse OCs and human OCs. Two mRNA species for OSCAR seen in Northern blot analysis are from alternatively spliced forms of OSCAR mRNAs. Two forms differ only in the 3' untranslated region.

OC Formation Assay and Pit Formation Assay. For OC formation culture, primary calvarial osteoclasts were obtained from calvariae of newborn C57BL/6 mice by conventional method using collagenase, and bone marrow cells were obtained from the femora and tibiae of 4–7-wk-old C57BL/6 male mice (23). Calvarial osteoblasts (10⁴ cells) and bone marrow cells (10⁵ cells) were cocultured in the presence or absence of 10⁻⁸ M 1,25(OH)₂D₃ in 0.2 ml minimum essential medium α medium (α -MEM) containing 10% FBS in 96-well culture plates. After 5, 6, or 7 d of culture, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) as described previously (23). TRAP-positive multinucleated cells were counted as OC-like multinucleated cells. To determine the pit-forming activity of OCs, calvarial osteoblasts (10⁴ cells) and bone marrow cells (10⁵ cells) were cocultured in the presence or absence of 10⁻⁸ M 1,25(OH)₂D₃ on dentine slices (0.2–0.3 mm in thickness, 4 mm in diameter) placed in 96-well culture plates for 6 d as described previously (23). The slices were then recovered, cleaned by ultrasonication in 0.5 M NH₄OH to remove adherent cells, and stained with Mayer's hematoxylin (Sigma-Aldrich) to visualize resorption pits. The numbers of pits on slices

were counted by microscope observation. Some cultures were treated with OSCAR-Fc, human IgG or TRANCE-R-Fc (24). Media of each culture were replaced with fresh media every 3 d.

A Soluble Form of OSCAR and Anti-OSCAR mAb. A soluble form of OSCAR was produced as an OSCAR-Fc fusion protein, in which the entire extracellular domain (amino acid residues 1–228) was fused in frame to the constant region of human IgG1. OSCAR-Fc protein was produced in insect cells, and purified by Protein A affinity chromatography as described previously (24). Purified OSCAR-Fc was then used to immunize rats to generate mAbs against murine OSCAR as described previously (8).

Northern Blot Analysis. Total RNA (10 µg) or poly A⁺ RNA (0.5 µg) was separated and transferred to nylon membranes as described previously (22). The membranes were hybridized with ³²P]dCTP probe prepared using Ready-to-Go labeling kit and ProbeQuant G-50 purification kit (Amersham Pharmacia Biotech).

Chromosome Mapping of OSCAR. The contiguous map of mouse OSCAR was identified using radiation hybrid (RH) mapping kit (Research Genetics) following the manufacturer's instructions. In brief, two types of primer sets were designed from 3' untranslated region of mouse OSCAR cDNA. The primers used were as follows: primer set #1 (5'AGA TGG ACA GAG AAG CTG GGATCC3' and 5'TTA GGA GCC AGC CAG ATG GCT CAG3'), and primer set #2 (5'AAG GGT AAT ACC CAG GCC TCA GCC3' and 5'AAG AGT ACT GGC TAC TCT TCT AGA3'). Genomic DNA PCR was performed for 30 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 30 s). RH data of murine OSCAR genomic PCRs were analyzed by The Jackson Laboratory (<http://www.jax.org/>). The RH data showed mouse OSCAR location in chromosome 7 near paired Ig-like receptor (PIR) genes. Human BAC clone (AC009968.6) containing human OSCAR genomic sequences was identified in the human genome database by blast search. Chromosome location was identified in the NCBI Entrez browser.

Results and Discussion

OCs are the only cells capable of resorbing bone, although they are derived from the same bone marrow precursor cells of the monocyte-macrophage lineage that give rise to macrophages and DCs (1, 2). To provide insight into how OCs differentiate and function to control bone homeostasis, we have compared mRNA expression profiles between mature OCs and macrophages (MØ), two closely related cell types, by PCR-select cDNA subtraction. A subtraction cDNA library (OC-MØ) was constructed and analyzed as described previously (22), and one clone (OCL178 that contains a partial cDNA fragment of the gene that we have called OSCAR) was chosen for further analysis in this study.

To test whether OSCAR is derived from a gene specifically expressed in OCs, mRNA from OCs and MØs was used for Northern blot analysis (Fig. 1 A). OSCAR fragment detected two distinct mRNA species (1.8 kb and 1.0 kb) that were specifically expressed in bone marrow-derived OCs but not in bone marrow-derived MØs. Moreover, OSCAR mRNA expression was not detected in bone marrow-derived DCs which differentiate from the same bone marrow precursor cells of the monocyte-macrophage lineage that give rise to MØs and OCs. As reported previously, TRAP or cathepsin K expression was detected in OCs but not in MØs (1, 2). However, unlike OSCAR, TRAP, or cathepsin K was also detected in DCs, albeit at low levels. Thus OSCAR mRNA expression is much more specific for OCs than TRAP or cathepsin K.

In addition to bone marrow-derived OCs, OSCAR expression was also detected in OC-like cells derived from

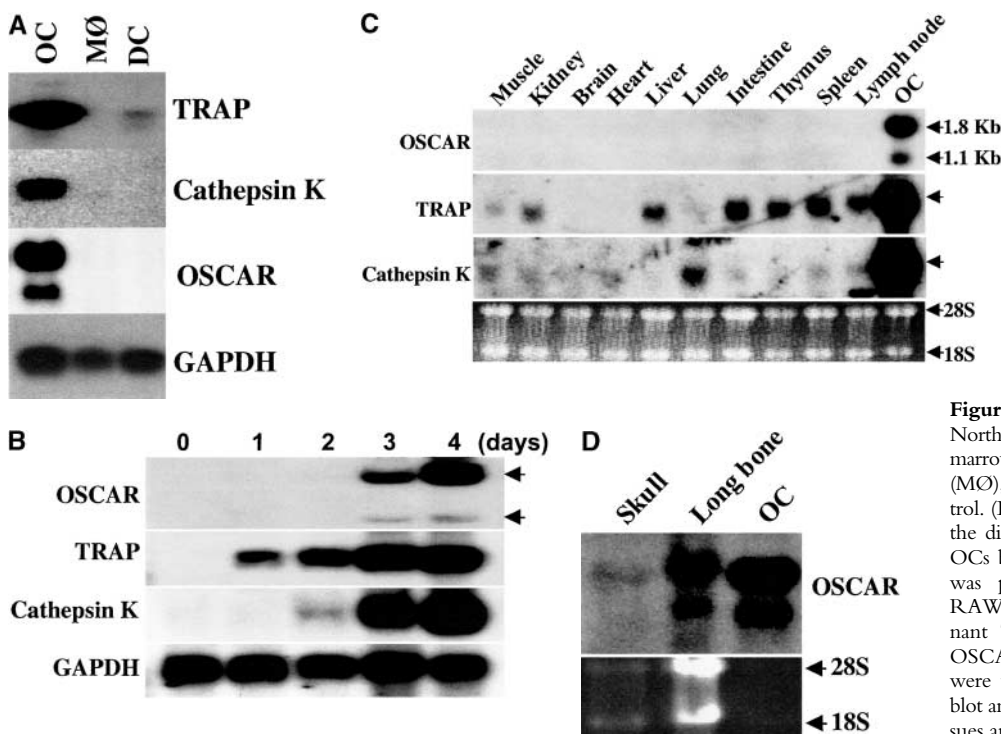


Figure 1. OSCAR mRNA expression. (A) Northern blot analysis of RNA from bone marrow-derived OCs, macrophages (MØ), and DCs. GAPDH was used as control. (B) OSCAR mRNA expressions during the differentiation of RAW264.7 to mature OCs by TRANCE. Northern blot analysis was performed using total RNA from RAW264.7 cells stimulated with recombinant TRANCE (200 ng/ml) for 0–4 d. OSCAR, TRAP, Cathepsin K, and GAPDH were used for probes. (C and D) Northern blot analysis of OSCAR in various mouse tissues and bone marrow-derived OCs.

the RAW264.7 cell line. As reported, RAW264.7 cells can be differentiated into OC-like cells by treatment of the TNF family member TRANCE (25). Here we showed that OSCAR expression could be easily detected 72 h after stimulation of RAW264.7 cells with recombinant TRANCE, and its expression was highest when RAW264.7 cells were completely differentiated into OCs (4 d after TRANCE stimulation) (Fig. 1 B). However, under more sensitive conditions, OSCAR mRNA was also detected during OC differentiation before no mature OCs were

visibly detected in the culture (48 h after stimulation with TRANCE; data not shown), suggesting that some OC differentiation intermediates might express OSCAR.

To further determine the specificity of OSCAR mRNA expression, mRNAs from various tissues were analyzed by Northern blot analysis. OSCAR mRNA expression was not detected in any soft tissues tested (Fig. 1 C). However, OSCAR mRNA could be detected when mRNA was prepared from OC-rich tissues such as skull or long bones (Fig. 1 D). In comparison, TRAP or cathepsin K mRNA, which

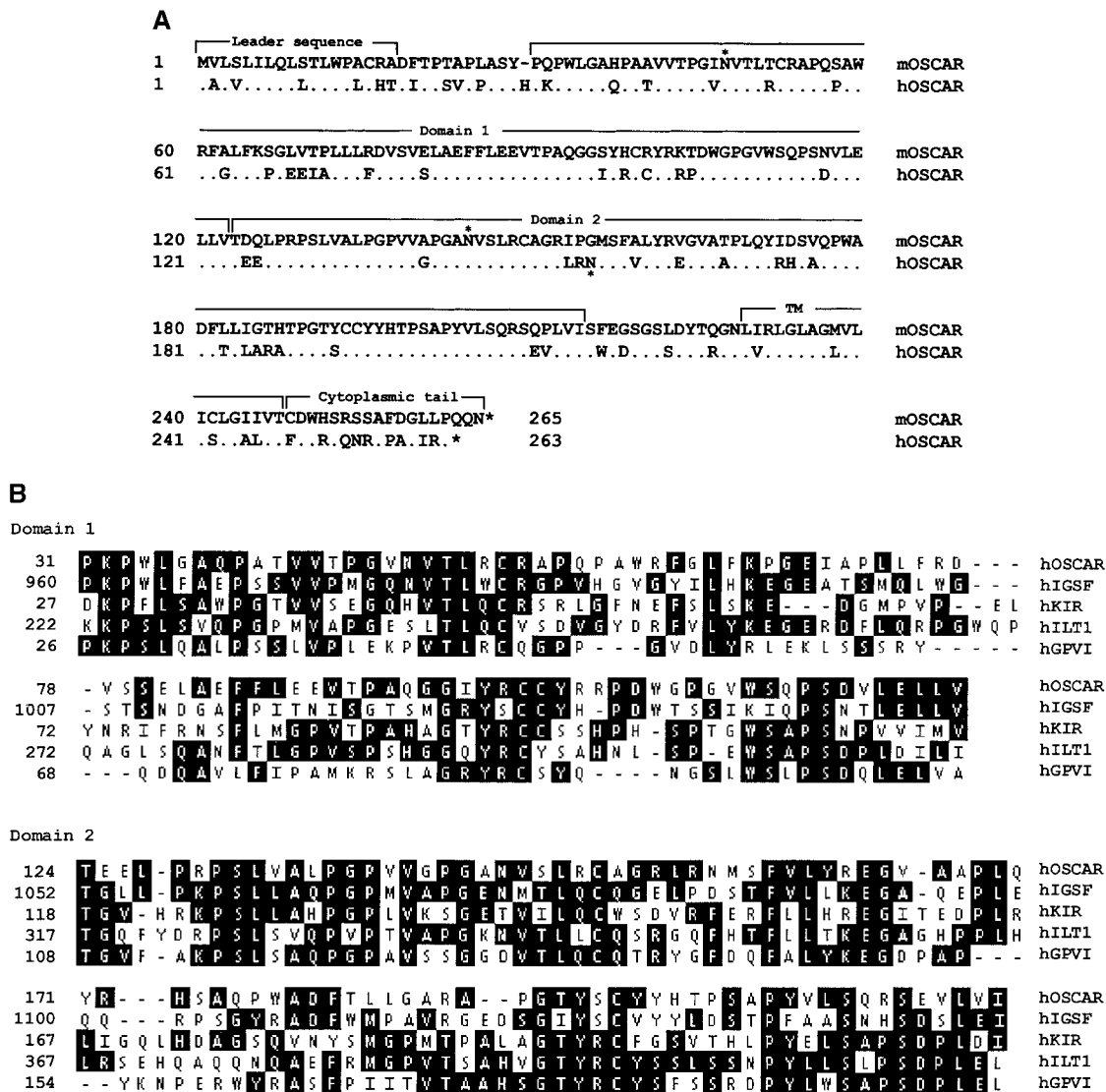


Figure 2. Sequence analysis of OSCAR. (A) The predicted amino acid sequence of the full-length murine OSCAR protein (mOSCAR) compared with that of human OSCAR (hOSCAR). Dots indicate shared identities between mouse and human protein, and dashes indicate gaps between regions of homology. The boldface letters indicate the position of the conserved cysteine in the Ig-fold. Residues labeled with an asterisk (*) indicate potential NH₂-linked glycosylation sites. The numbers in left-hand column indicate the amino acid residue positions in the mOSCAR and hOSCAR protein. Between L12 and W13 of mOSCAR, six amino acids (CELSLP) are inserted by alternative splicing in the minor population of mOSCAR cDNA clones. Also, 1 of 15 clones of hOSCAR has a four amino acid (AIIV) insertion between V24 and P25 of hOSCAR and 2 of 15 clones of hOSCAR have a deletion of 11 amino acids (residues 13 through 24, WPLCHTDITPSV, are replaced with F13) by alternative splicing. GenBank accession nos. for mOSCAR are AF391159, AF391160, and AF391161 and for hOSCAR are AF391162, AF391163, and AF391164. (B) The sequence comparison of the two Ig-like domains of hOSCAR with other members of the LRC-encoded genes: hIGSF(AF034198), hKIR(AF072410), hIL1T1(U82275), and hGFVI(AB035073). Gaps in alignment are indicated by dashes and identical amino acids appear on a dark background. The numbers in the left-hand column indicate the residue positions from the full-length protein sequences.

were considered to be markers for OCs, could be detected in mRNA derived from tissues other than bone. These results thus show that OSCAR is specifically expressed in OCs, both bone marrow–derived OCs and OCs found *in vivo*.

Since OSCAR expression follows TRAP or cathepsin K during OC differentiation by two independent experimental systems (bone marrow–derived OCs and RAW264.7–derived OCs), a potential role of OSCAR is likely to regulate OC function/differentiation. Therefore, we cloned and characterized the full-length OSCAR cDNAs from murine and human OC cDNA libraries. The full-length OSCAR cDNA encodes a type I transmembrane protein (Fig. 2). The extracellular domain of OSCAR contains two Ig-like domains with significant homology to murine PIR family proteins (26, 27) and to members of the human LRC–encoded proteins (28–37). The predicted transmembrane region contains a charged Arg residue, suggesting that OSCAR may associate with another transmembrane

protein. Mouse OSCAR (mOSCAR) is localized near the proximal end of mouse chromosome 7, the region where PIR family proteins reside (26), while human OSCAR (hOSCAR) gene was found to reside in the human chromosome 19q13.4, which contains the LRC (28, 37). These results thus indicate that OSCAR is a bona fide novel member of the PIR/LRC family proteins. The amino acid identity between mouse and human OSCAR is ~73%, which distinguishes it from other PIR–related and LRC family proteins, most of which lack orthologs in mouse and man. To date, in addition to OSCAR, the only PIR/LRC family member with orthologs in humans, mice, and rats is the p46 NK cell receptor, NKp46 (28, 37–39).

Northern blot analysis of OSCAR indicated that OSCAR expression was predominant in mature OCs but was also detected during OC differentiation before mature OCs were visibly detected in the culture, suggesting that some OC differentiation intermediates might express OSCAR

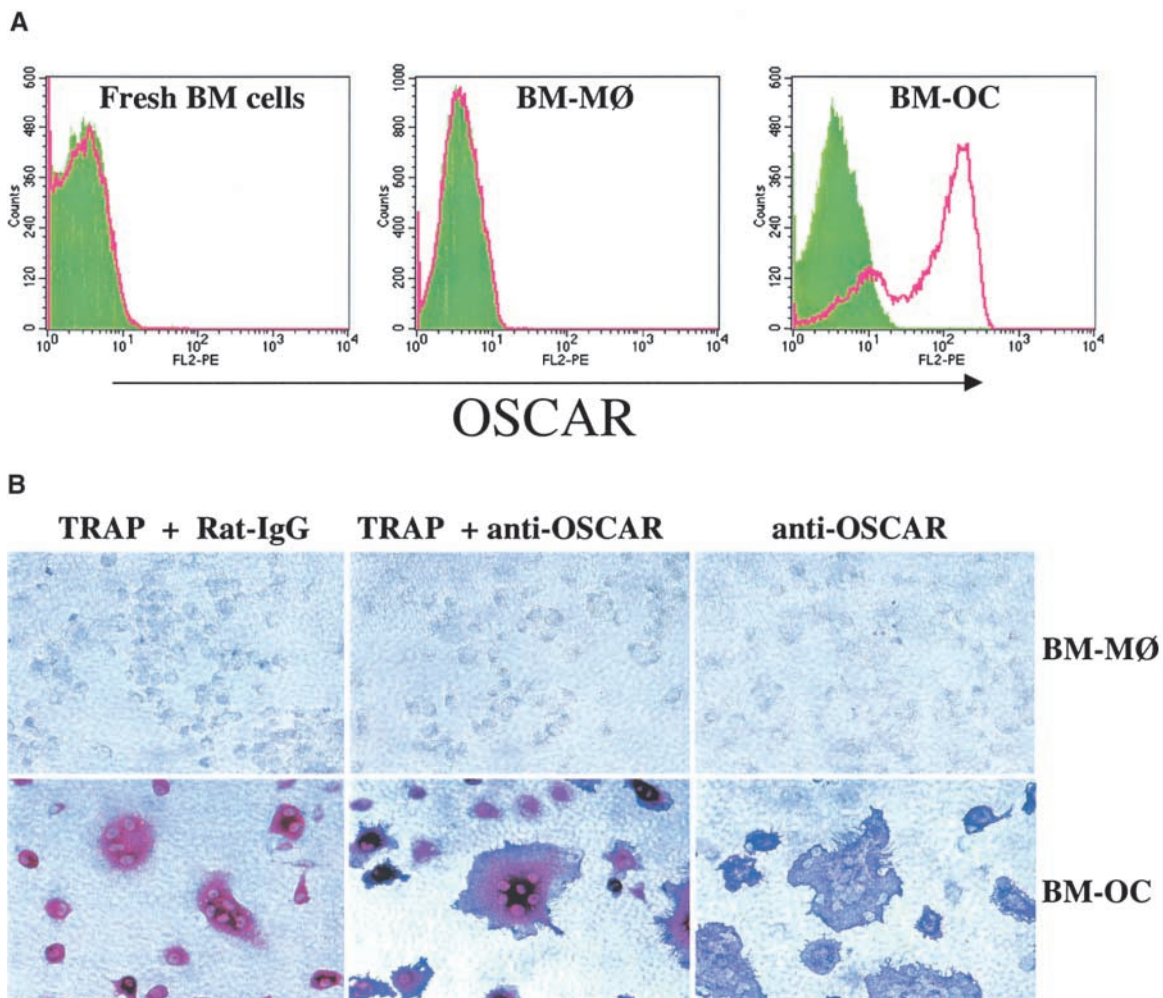


Figure 3. Expression and regulation of OSCAR protein. (A) FACS[®] analysis of cell surface OSCAR expression on freshly isolated bone marrow cells (BM), bone marrow–derived MØ (BM-MØ), and OCs (BM-OC). After differentiation, cells were stained with anti-OSCAR mAb (open histogram) or isotype control mAb (shaded histogram). (B) Immunohistochemistry of OSCAR on *in vitro* bone marrow–derived macrophage (BM-MØ) and bone marrow–derived OC (BM-OC). For immunohistochemistry, cells were fixed with 4% paraformaldehyde, and followed by TRAP assay (left and middle), staining with rat anti-OSCAR mAb (middle and right) or rat IgG as a control (left). TRAP–positive cells are stained red and OSCAR–expressing cells are stained blue.

(Fig. 1). To further determine when OSCAR is expressed during osteoclastogenesis, a rat anti-mOSCAR mAb was generated and used for FACS[®] and immunohistochemical analysis. Surface OSCAR was detected predominantly on mature OCs but not on other cells including macrophages, DCs, NK cells, and lymphocytes (Fig. 3 A), consistent with mRNA expression profiles. In addition, we showed by immunohistochemical analyses that a fraction of mononuclear, TRAP-positive preosteoclasts expressed surface OSCAR, albeit at low levels (Fig. 3 B). None of the TRAP-negative cells expressed measurable levels of OSCAR. These results indicate that OSCAR protein is predominantly expressed on the surface of mature OCs as well as some cells of OC lineage. OSCAR expression is likely to begin at an intermediate stage of differentiation from TRAP-positive OC precursors to fully mature multinucleated OCs.

Since OSCAR expression is detected in pre-OCs and LRC family members have been shown to regulate various functions of cells in the immune system (28, 37), we hy-

pothesized that OSCAR may be involved in the differentiation of OC-precursors to mature, multinucleated OCs. To test this hypothesis, we made a soluble form of OSCAR by fusing the extracellular domain of OSCAR to the Fc portion of human IgG1 (OSCAR-Fc). To determine the role of OSCAR in osteoclastogenesis, OSCAR-Fc was added to a coculture of bone marrow precursor cells and osteoblast/stromal cells in the presence of an osteotropic factor, 1,25(OH)₂D₃ (Fig. 4 A–C). The addition of OSCAR-Fc significantly inhibited the formation of multinucleated TRAP⁺ OCs in the coculture stimulated by 1,25(OH)₂D₃. Furthermore, when cultures were made on dentine slices, OSCAR-Fc inhibited bone resorption as measured by pit formation (Fig. 4 A and D). As a control, we also used TRANCE-R-Fc to inhibit the interaction of the TNF family member TRANCE and its receptor (TRANCE-R/RANK), which has been previously shown to be essential for osteoclastogenesis in the coculture system (Fig. 4 A–D; references 1, 2, 5, 6, 18, 22, and 40). These

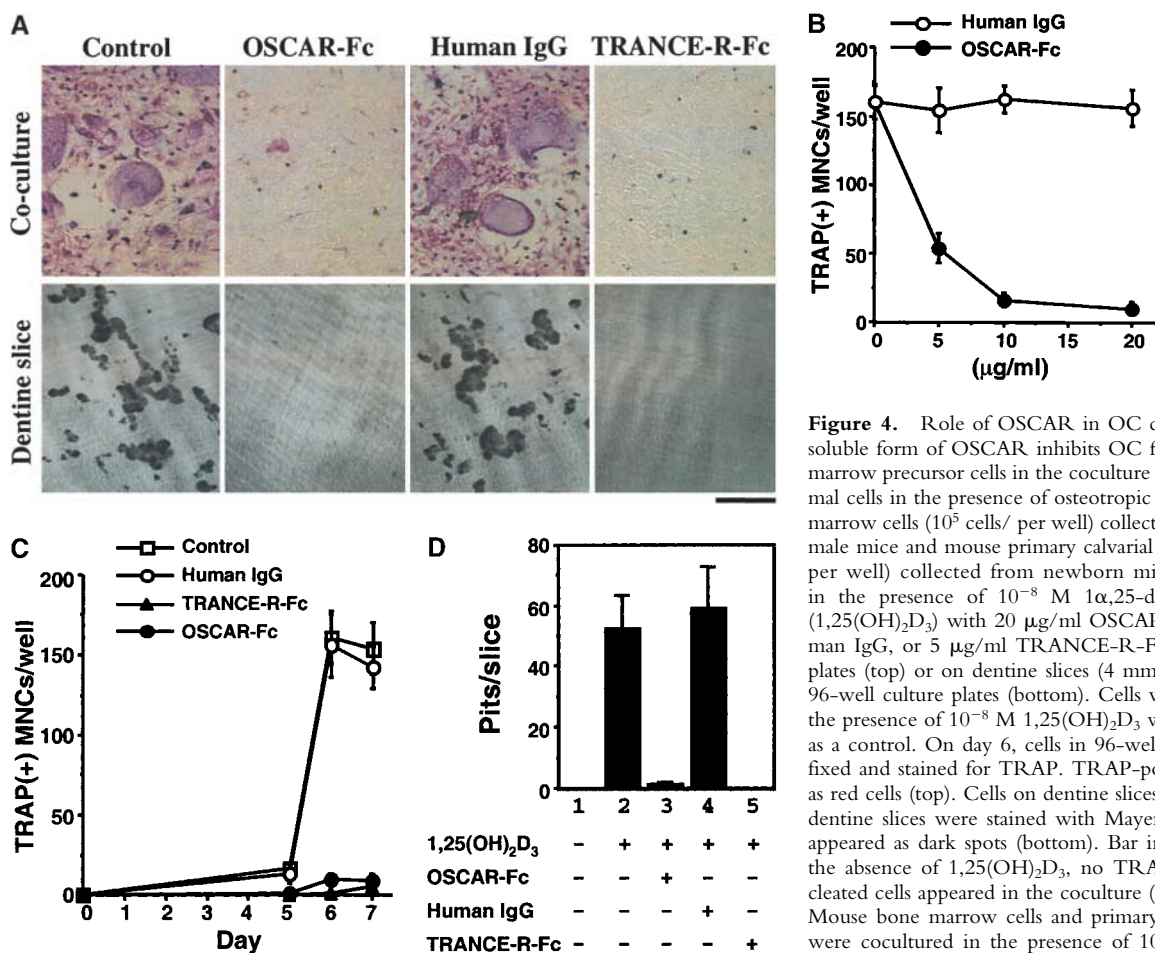


Figure 4. Role of OSCAR in OC differentiation. (A) A soluble form of OSCAR inhibits OC formation from bone marrow precursor cells in the coculture with osteoblast/stromal cells in the presence of osteotropic factors. Mouse bone marrow cells (10^5 cells/ per well) collected from 5–7-wk-old male mice and mouse primary calvarial osteoblasts (10^4 cells per well) collected from newborn mice were cocultured in the presence of 10^{-8} M $1\alpha,25$ -dihydroxyvitamin D₃ ($1,25(\text{OH})_2\text{D}_3$) with 20 $\mu\text{g}/\text{ml}$ OSCAR-Fc, 20 $\mu\text{g}/\text{ml}$ human IgG, or 5 $\mu\text{g}/\text{ml}$ TRANCE-R-Fc in 96-well culture plates (top) or on dentine slices (4 mm diameter) placed in 96-well culture plates (bottom). Cells were also cultured in the presence of 10^{-8} M $1,25(\text{OH})_2\text{D}_3$ without any additives as a control. On day 6, cells in 96-well culture plates were fixed and stained for TRAP. TRAP-positive cells appeared as red cells (top). Cells on dentine slices were removed, and dentine slices were stained with Mayer's hematoxylin. Pits appeared as dark spots (bottom). Bar indicates 250 μm . In the absence of $1,25(\text{OH})_2\text{D}_3$, no TRAP-positive multinucleated cells appeared in the coculture (data not shown). (B) Mouse bone marrow cells and primary calvarial osteoblasts were cocultured in the presence of 10^{-8} M $1,25(\text{OH})_2\text{D}_3$ with increasing concentrations (0, 5, 10, and 20 $\mu\text{g}/\text{ml}$) of

OSCAR-Fc (black circles) or human IgG (white circles) in 96-well culture plates for 6 d. After fixing and staining cells for TRAP, numbers of TRAP-positive multinucleated cells (MNCs) were counted as OCs. (C) Mouse bone marrow cells and mouse primary calvarial osteoblasts were cocultured in the presence of 10^{-8} M $1,25(\text{OH})_2\text{D}_3$ with 20 $\mu\text{g}/\text{ml}$ OSCAR-Fc (black circles), 20 $\mu\text{g}/\text{ml}$ human IgG (white circles), or 5 $\mu\text{g}/\text{ml}$ TRANCE-R-Fc (black triangles) in 96-well culture plates. Cells were also cultured in the presence of 10^{-8} M $1,25(\text{OH})_2\text{D}_3$ without any additives as control (white squares). On days 0, 5, 6, and 7, cells were fixed and stained for TRAP. TRAP-positive multinucleated cells were counted as OCs. (D) Number of pits formed on dentine slices in A was counted. Media of each culture were replaced with fresh media on day 3 (A–D). Results are representative of at least three independent sets of similar experiments (A–D). All data are expressed as mean \pm SD of quadruplicate cultures.

results thus indicate that OSCAR is required for formation of mature OCs from bone marrow precursor cells stimulated by osteoblasts.

Although OSCAR-Fc inhibited osteoclastogenesis mediated by osteoblasts, it did not inhibit the formation of mature OCs from the RAW264.7 cell line stimulated with soluble TRANCE (data not shown). These results suggest that when there is a supraphysiological amount of TRANCE available and/or cells are CSF-1 independent as in the RAW264.7 myeloid tumor cell line, OSCAR stimulation may be bypassed in osteoclastogenesis. Moreover, when OCs were derived from bone marrow cells by the combination of recombinant M-CSF and TRANCE in the absence of any osteoblasts/stromal cells, OSCAR-Fc did not inhibit osteoclastogenesis (data not shown). Taken together, OSCAR-Fc appears to affect osteoclastogenesis only when it is induced by physiological levels of TRANCE and/or M-CSF produced by osteoblasts in response to bone resorbing hormones. Although future experiments are necessary to understand how OSCAR/OSCAR-L interaction regulates osteoclastogenesis, the level of TRANCE expression in osteoblasts appears not to be regulated by OSCAR/OSCAR-L interaction (data not shown).

It is currently thought that the formation and activation of OCs are tightly regulated by osteoblasts, which provide at least two known essential factors for osteoclastogenesis, TRANCE, and M-CSF. In addition, the ultimate osteoblast-induced osteoclastogenesis from bone marrow precursors are further influenced by various stromal cells that produce different osteotropic factors (1, 2). These factors can be divided into two groups: one influencing the activity of osteoblasts, e.g., TNF regulating the expression of TRANCE in osteoblasts (1, 2, 9), and the other affecting the OC precursors or OCs per se. The factors in the latter group include TGF- β , which has been recently shown to be a powerful costimulatory molecule for osteoclastogenesis induced by M-CSF and TRANCE (14–17, 41).

As reported previously, M-CSF and TRANCE together appear to be sufficient to induce the differentiation of bone marrow precursors, spleen cells, or blood monocytes to mature OCs in vitro (2). However, the expression of M-CSF, TRANCE, and their receptors are not limited to bone cells. For example, M-CSF and TRANCE are important cytokines for the activity/viability of macrophages and DCs (18). Despite this pleiotropy, OCs are not found in most soft tissues. This raises question of why OCs are not observed in various soft tissues. One possibility is that there may be costimulatory molecules present only in the bone. Alternatively, there is a powerful inhibitor of osteoclastogenesis in most soft tissues but not in bone.

Although most of the costimulatory molecules for osteoclastogenesis described to date (e.g., TGF- β , TNF, or IL-1) are not specific to the bone system (1, 2), it is still possible that there may be costimulatory receptors expressed specifically on OCs and/or costimulatory factors specifically expressed on osteoblasts. As we have described in this study, we have indeed identified a novel cell surface receptor OSCAR specifically expressed in OCs, which appears

to regulate osteoclastogenesis. Moreover, a putative ligand for OSCAR (OSCAR-L) is detected solely on osteoblasts (Fig. 5). Therefore, this new receptor/ligand pair (OSCAR and OSCAR-L) may provide an evidence for the role of various bone-specific costimulation pathways for osteoclastogenesis to control the differentiation of OCs in conjunction with the two essential factors M-CSF and TRANCE, which are not bone specific. Finally, it is interesting that the novel OC-specific costimulatory receptor OSCAR is a member of the PIR/LRC family proteins that have been shown to provide various costimulatory/inhibitory functions during the regulation of both innate and adaptive immune responses (28, 37). OSCAR is unique among the PIR/LRC family members in that it is not detected in cells of the immune system such as macrophages or DCs. It suggests that certain PIR/LRC family proteins may have evolved to influence cells far beyond the immune system, as it certainly controls bone metabolism as shown in this study.

Under normal circumstances, the activity of OCs is integrated with the requirements of calcium homeostasis and skeletal morphogenesis and restructuring, while excessive OC activity causes bone destruction in diseases such as rheumatoid arthritis, Paget's disease, periodontal disease, chronic infections, and metastases of cancers (1, 2). Most importantly, excessive osteoclastic activity is responsible for the development of osteoporosis, a very common cause of fracture, with consequent morbidity and mortality, in elderly people. Since OCs are the principal, if not the only, cells which can resorb bone, the understanding of the molecular pathways leading to the differentiation and activation of OCs will help to make strides in the treatment and prevention of osteoporosis as well as other diseases involving bone destruction. That OCs differentiate from monocyte/macrophage lineage precursors which also give rise to macrophages and DCs, key components of the immune system, and that M-CSF and TRANCE, two essential fac-

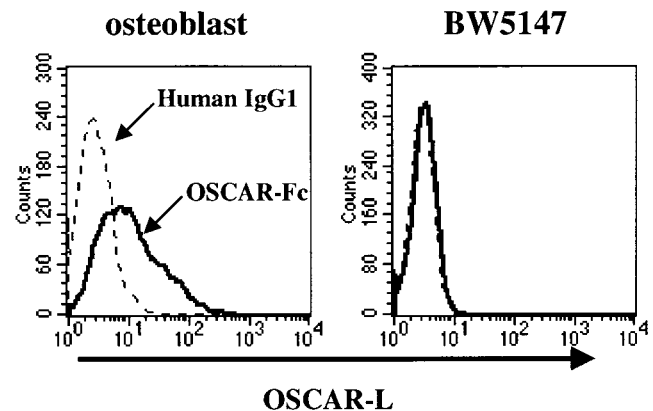


Figure 5. Expression of OSCAR-L in osteoblasts. Primary calvarial osteoblasts are derived as described in the Materials and Methods. Surface OSCAR-L expression on various cells was determined by FACS[®] analysis using control human IgG1 (dotted lines) or OSCAR-Fc (solid lines) and FITC-labeled anti-human IgG1. A thymoma cell line, BW5147, which does not express OSCAR-L, was also shown here.

tors for osteoclastogenesis, also regulate cells of the immune system, clearly indicate that many aspects of bone and the immune system are shared at the cellular and molecular levels. Such similarities in the molecular regulation of bone and the immune system would make it rather difficult to control pathological conditions of one or the other system specifically. Therefore, an elucidation of various costimulation pathways specific to bone but not to the immune system, as shown in this study, will ultimately aid the design of therapeutic approaches to bone diseases that do not compromise immune function.

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