Osteoclast apoptosis: Role of TRAIL and its receptors

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Abstract

Control of osteoclast (OC) apoptosis has been recognized as a critical regulatory factor in bone remodelling, and alteration in OC death and/or survival has been shown to contribute to the pathogenesis of osteoporosis or malignant osteolysis. TNF-related apoptosis-inducing ligand (TRAIL) has received considerable attention as a molecule showing the unique property to induce apoptosis in a variety of neoplastic cells, however, in many normal cells TRAIL can activate the apoptotic process. In this paper, we demonstrate for the first time a different TRAIL receptor expression pattern during OC differentiation. Moreover, we show that TRAIL treatment causes an increased apoptotic OC number, caspase 8 and 3 activation and the formation of the DNA ladder. The apoptotic process is mediated by death receptor 5 (DR5) as demonstrated by the increase in its expression following TRAIL treatment and by the rescue of the cell viability in OCs treated simultaneously with TRAIL and anti-DR5 neutralizing antibody. Elucidation of the mechanisms controlling OC apoptosis may reveal novel strategies for drug development.

Key Words: TRAIL, TRAIL receptors, osteoclasts, apoptosis, caspase-8, Bid

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Bone is a metabolically active tissue that undergoes continuous remodelling by two counteracting processes, bone formation and bone resorption. These events are strongly linked and tightly regulated to maintain skeletal homeostasis. The bone cells responsible for the dual processes include the bone-resorbing cells, i.e. the osteoclasts (OCs), which are differentiated cells derived from hematopoietic cells of the monocyte-macrophage lineage, and bone forming cells, i.e. the osteoblasts, which are of mesenchymal origin [24]. The life span of OCs is relatively short both in vitro and in vivo, and once differentiated, they rapidly die in the absence of supporting cells such as osteoblasts or bone marrow stromal cells, or of growth factors [11]. Although the factors regulating OC formation and activity are well known, those involved in OC apoptosis are much less well clarified. Considerable progress has been made in understanding the mechanisms of OC apoptosis in rodents, but little is known about the human mechanism. Apoptosis is a tightly regulated process that is characterized by specific morphological and biochemical properties [12, 21, 32, 37]. It is known that the induction of apoptosis by extracellular signals involves ligands, related to the TNF family proteins, and death receptors, belonging to the TNF receptor superfamily. Some death receptors have been described in detail, including CD95 (Fas) that binds the CD95 ligand (FasL), and TRAIL receptors that bind the TNF-related apoptosis-inducing ligand (TRAIL). TRAIL, also known as the Apo2 ligand (Apo2L), induces cell death in a wide variety of tumor cell lines [19]. Moreover, increasing experimental evidence shows that TRAIL exhibits regulatory role in various normal tissues as well [39]. In vitro studies have reported that thymocytes [31], neurons [20], hepatocytes [13] and OCs [4, 25] may be sensitive to TRAIL-induced apoptosis. Consequently, the role of TRAIL can not only be considered that of an apoptotic agent for tumor cells. TRAIL is a type II transmembrane protein that induces apoptosis by binding to its death domain-containing receptors, DR4 and DR5 [3, 22, 23, 30, 34]. Furthermore, TRAIL interacts with antagonistic "decoy" receptors that somehow prevent TRAIL-induced apoptosis in resistant cells. To date, at least three homologous human decoy receptors for TRAIL have been identified, DcR1, DcR2, and osteoprotegerin [3, 5, 6, 8]. Although the selective cytotoxicity of TRAIL is not entirely clear it may be related to the balance between death and "decoy" receptors expressed by the cells. TRAIL binding to DR4 and DR5 induces recruitment of the adapter molecules Fas-associated death domain (FADD) that leads to direct activation of the caspase cascade. This activation is accomplished by recruit-

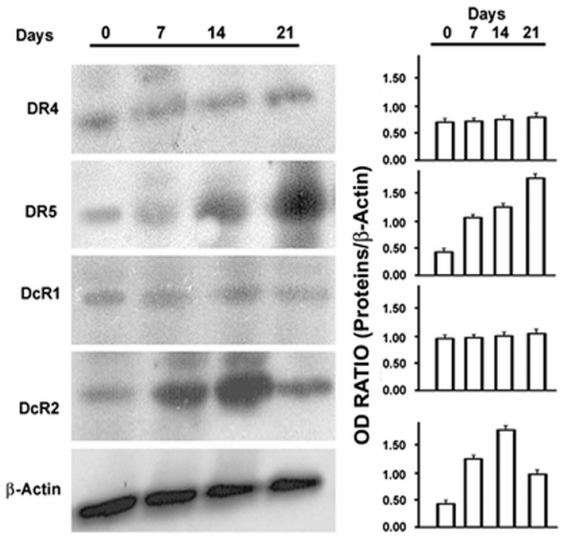

Fig. 1 *Expression of TRAIL receptors during osteoclast differentiation. Human PBMCs were differentiated by the addition of MCSF and RANKL for different times (0–21 days). On day 0, precursors were collected after Ficoll-Hypaque gradient centrifugation; on days 7, 14 and 21 cells were collected after incubation with MCSF and RANKL. Different expression of TRAIL receptors was demonstrated by western blotting. The intensity of the bands obtained by Western blot analysis was quantified by densitometry (histograms) and normalized to β-actin.*

ment of caspase-8, followed by its proteolytic activation. Once activated, caspase-8 can proteolytically cleave Bid, a pro-apoptotic member of the Bcl-2 family proteins, leading to the formation of a truncated Bid form (tBid) that in turn activates the mitochondrial apoptotic pathway [9, 16, 17].

Although in vitro studies reported by us and other investigators [4, 25] have demonstrated that human OCs generated by two different systems, peripheral blood mononuclear cells (PBMCs) and cord blood monocytes, express TRAIL receptors, the TRAIL-induced apoptotic pathway has not yet been elucidated in human OCs. In the present study we reported new data demonstrating that TRAIL induces apoptosis in human differentiated OCs by means of DR5, and activates an intracellular pathway involving caspase-8 and Bid cleavage in the active forms.

Materials and Methods

Cell cultures

OCs were obtained from heparinized human peripheral blood mononuclear cells (PBMCs) collected from healthy donors. PBMCs were isolated by centrifugation over a Histopaque 1077 density gradient

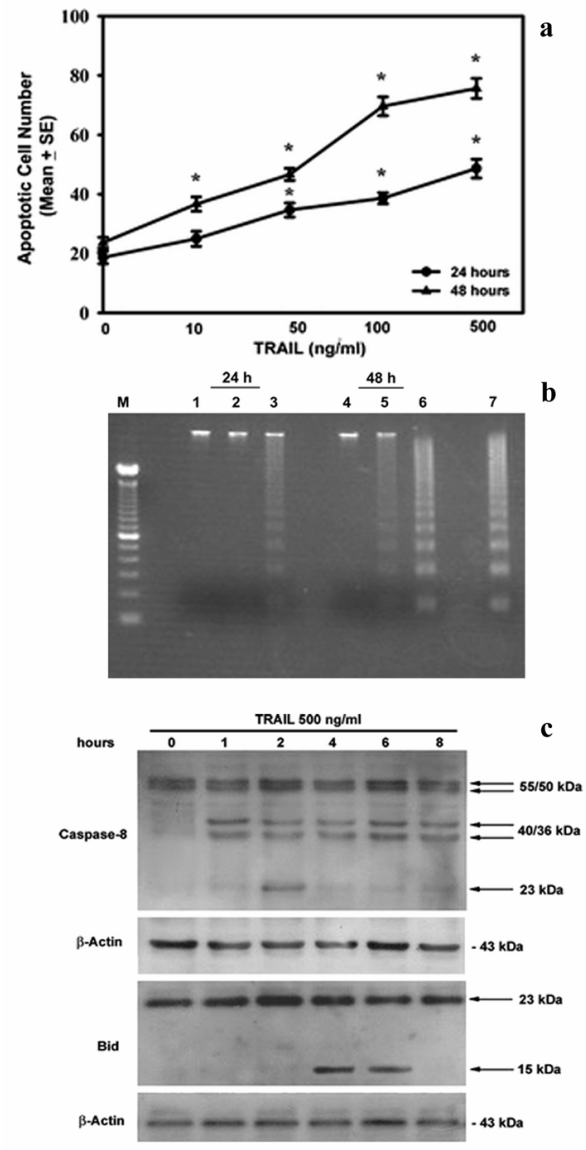

Fig. 2 *TRAIL sensitivity in fully differentiated osteoclasts. (a) Osteoclasts were treated for 24 and 48 h with TRAIL at the indicated concentrations and the number of apoptotic cells was determined by counting the osteoclasts after DAPI staining, *P < 0.001. (b) Lines: M, DNA molecular weight markers; 1–3: DNA extracts from osteoclasts treated with 0 (line 1), 100 (line 2) and 500 ng/ml TRAIL (line 3) for 24 h; 4–6: DNA extracts from osteoclasts treated with 0 (line 4), 100 (line 5) and 500 ng/ml TRAIL (line 6) for 48 h; 7: apoptotic U937 cells utilized as positive control. (c) Human mature osteoclasts, treated with 500 ng/ml TRAIL, were lysed at the indicated times and analysed by western blot analysis to detect the protein levels of inactive and cleaved forms of caspase-8 and Bid. The fragment at 23 kDa and the fragment at 15 kDa represent the active cleaved form for caspase-8 and Bid, respectively. β-actin was utilized as control.*

(Sigma Chemical Co., St. Louis, MO, USA), plated at 1x10^{6}cells/cm2 in α-Minimal Essential Medium (α-MEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco Limited, Uxbridge, UK). PBMCs were cultured in the presence of 25 ng/ml recombinant human Macrophage Colony Stimulating Factor (rh-MCSF) and 30 ng/ml receptor activator of the nuclear factor-kappa B ligand (rh-RANKL - R&D Systems Inc MN, USA) for 18-21 days. The medium was replaced every 2-3 days with fresh medium supplemented with the above-described agents. After 21 days, tartrate-resistant acid phosphatase positive (TRAP+) and multinucleated cells, containing 3 or more nuclei, identified as mature OCs, had formed in culture. Cell coltures, containing mature OCs, used to study protein expression by Western Blot analysis, were subjected to trypsin treatment to remove from the cultures any residual mononuclear cells weakly attached to the substrate, and thus more easily detachable as compared to the mature OCs. This treatment was followed by a recovery period of 48 hours (hrs) in which mature OCs were cultured in medium supplemented with serum and then utilized for the experiments.

Cell viability assay

Cell viability experiments were performed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, as previously described (12). PBMCs were cultured in 96-well tissue-culture plates with 25 ng/ml of M-CSF and 30 ng/ml of RANKL. Mature OCs, after removal of M-CSF and RANKL, were treated with 500 ng/ml rh-TRAIL for 48 hrs to evaluate cell viability in mature OCs. In parallel, the effect of 500 ng/ml TRAIL on OC viability was evaluated in cells pretreated for 30 minutes with 5 µg/ml of antagonist anti-DR5 neutralizing monoclonal antibody (mAb) (Alexis, San Diego, CA) or an irrelevant anti-IgG.

Detection of apoptosis by DAPI staining

Cells were cultured on coverslips in the presence of M-CSF and RANKL. Once they had differentiated into mature OCs, after removal of M-CSF and RANKL, they were treated with rh-TRAIL (10-500 ng/ml) for 24 and 48 hrs. OCs were gently washed three times with PBS and fixed in 3% paraformaldehyde and 2% sucrose in PBS, pH 7.6, for 10 min at RT. After rinsing in PBS, coverslips were incubated with 0.25 µg/ml 4,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich) for 45 min at 37 °C. Stained coverslips were then mounted in 20% Mowiol 4-88 (Hoechst AG, Frankfurten on Main, Germany). Cells with nuclei containing clearly condensed chromatin or cells with fragmented nuclei were scored as apoptotic. Results are expressed as number of apoptotic cells/coverslip.

DNA fragmentation

PBMCs were seeded in 6-well plates and cultured with 25 ng/ml of M-CSF and 30 ng/ml of RANKL for 21 days. After removal of M-CSF and RANKL, DNA extracts were prepared from mature OCs treated with rh-TRAIL at different concentration (10-500 ng/ml) for 24 and 48 hrs. DNA was purified using the Apoptotic DNA Ladder Kit (Roche Applied Science, Mannheim, Germany), to detect the typical apoptotic DNA fragments (ladder), according to the manufacturer's instructions. The kit provides a lyophilized apoptotic U937 cell sample as positive control. Samples were then electrophoresed on 2% agarose gel containing 0.01% ethidium bromide, and the resulting bands were detected by a light sensitive CCD video system (BioDocAnalyze, Whatman Biometra, Göttingen, Germany).

Western blot analysis

PBMCs were cultured in 6-well tissue-culture plates with 25 ng/ml of M-CSF and 30 ng/ml of RANKL. After removal of M-CSF and RANKL, the cells were treated with 500 ng/ml rh-TRAIL for 0-8 hrs to evaluate DcR1, DcR2, DR4, DR5, caspase-8 and Bid expression on preosteoclasts, on days 7 and 14, and mature OCs on day 21. Cells were lysed by incubation on ice for 30 min in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 150 mM NaCl, 10% glycerol, 1 mM Na3VO4, 50 mM NaF, 100 mM phenylmethylsulfonyl fluoride, and a commercial protease inhibitor mixture (Sigma Aldrich). After insoluble debris had been pelleted by centrifugation at 14,000 × g for 15 min at 4 °C, the supernatants were collected. Protein determination was performed with the BCA Protein assay Reagent Kit (Pierce Biotecnology, Rockford, MN USA). 50 µg of cell proteins were subjected to 12 % SDS-PAGE gel and subsequently transferred to nitrocellulose membranes (Hybond, Amersham Pharmacia, London, UK). The blots were probed overnight at 4 °C with: mouse anti-βactin (Chemicon International Inc.), rabbit anti-DR4, anti-DR5, anti-DcR1, anti-DcR2 (Oncogene Research Product Inc), anti-caspase-8 and Bid polyclonal Abs (BD Pharmingen, San Diego, CA, USA). After incubation with the appropriate peroxidase-conjugated secondary Ab, specific reactions were revealed with the ECL detection kit and visualized on HyperfilmTM (Amersham Pharmacia, UK).

Statistical analyses

Statistical analyses were performed by Student's t-test with the Statistical Package for the Social Sciences (spssx/pc) software (SPSS, Chicago, IL, USA). A value of $p<0.05$ was taken to be statistically significant.

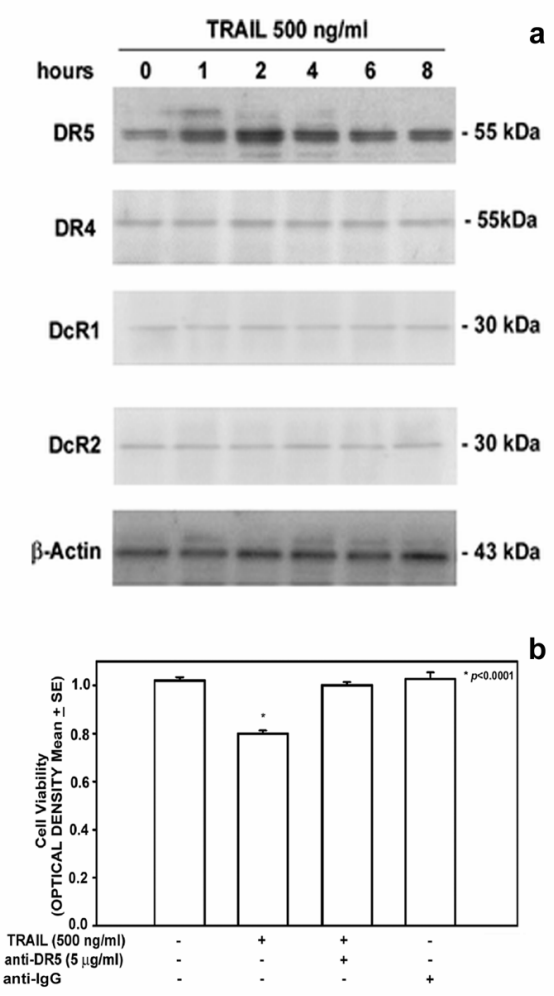

Fig. 3 *TRAIL-mediated human OCs apoptosis involves the death receptor 5 (a) Human mature osteoclasts, treated with 500 ng/ml TRAIL at the indicated times, were lysed and analyzed by western blot analysis to detect the protein levels of all TRAIL receptors. (b) Osteoclasts were cultured in control conditions (−), in the presence of TRAIL (+) with (+) or without (−) anti-DR5 neutralizing antibodies and cell viability was assessed by MTT assay. Osteoclasts were pre-treated for 30 min with the indicated antibodies before the addition of TRAIL. The anti-IgG was utilized as negative control. The graph represents the mean values ± SE of optical density at 570 nm..*

Results

TRAIL receptor expression during osteoclast differentiation

The expression of TRAIL receptors has been detected in preosteoclasts and fully differentiated OCs. By western blot analysis we demonstrated that a weak expression of both death and decoy TRAIL receptors was found in freshly isolated human PBMC lysates (Figure 1: day 0). Among the death TRAIL receptors only the expression of DR5 was increased during the OC differentiation process, and reached an increase of about three-fold on days 7 and 14 while a five-fold increase was observed on day 21. Among the decoy TRAIL receptors a modulation of DcR2 expression was found, in particular a three-fold increase was found on day 7, a five-fold increase on day 14, while a significant reduction was demonstrated in completely differentiated OCs on day 21 (Figure 1).

TRAIL sensitivity in fully differentiated osteoclasts

Sensitivity to TRAIL in human OCs, cultured with or without serum, was demonstrated by counting the number of apoptotic cells after DAPI staining. Mature OCs were generated from PBMCs in the presence of appropriate osteoclastogenic cytokines, M-CSF and RANKL. On day 21, when most of the cells had acquired the characteristics of mature OCs, M-CSF and RANKL were removed from the media and different concentrations of rh-TRAIL (10-500ng/ml) were added for 24 and 48hrs. The nuclei of apoptotic cells were condensed and fragmented, while the nuclei of non-apoptotic cells were round and smooth. Only multinucleated cells showing condensed chromatin were counted as apoptotic. As reported in panel a of Figure 2 the number of apoptotic cells after 24 hrs was 18±1 in control cultures, 25±1.2 in the presence of TRAIL at the concentration of 10ng/ml, 35±1.3 at 50ng/ml TRAIL, 39±0.9 at 100ng/ml, 49±2.4 at 500ng/ml. After 48 hrs the number of apoptotic cells was 24±0.9 in control cultures, 37±0.9 in the presence of TRAIL at the concentration of 10ng/ml, 47±1.2 at 50ng/ml TRAIL, 70±0.9 at 100ng/ml, 76±1.5 at 500ng/ml (Figure 2a).

Furthermore, the loss of nuclear integrity detected by the morphological study was biochemically confirmed by DNA fragmentation. Following the addition of TRAIL to the culture media, the formation of the DNA ladder was clearly evident in the OCs treated for 24 hrs with 500 ng/ml, and for 48 hrs with both 100 and 500 ng/ml TRAIL (Figure 2b lines 3, 5 and 6 respectively). DNA ladder formation was compared to the positive control, represented by U937 apoptotic cells provided in the apoptotic DNA ladder kit (Figure 2b line 7).

On the basis of the described results we investigated signaling molecules involved in TRAIL-mediated OC apoptosis. Caspase-8 has been reported to be the initial caspase activated during TRAIL-induced apoptosis in other cell types [7, 10, 14, 15, 29]. Procaspase-8 exists as two spliced-forms, p55 and p50, which are activated by a process involving removal of small fragments generating p40 and p36 intermediate forms. A second cleavage then results in the formation of the p23 subunit. By Western blot analysis, we demonstrated that processing of procaspase-8 occurred in OCs

treated with TRAIL for 1 to 8 hrs. In particular, the generation of intermediate forms (p40, p36) could be observed already after 1 hour of TRAIL treatment, and after 2 hours a second cleavage took place, inducing formation of the p23 active caspase fragment (Figure 2c). It is also known that activated caspase-8 cleaves Bid (p23), one of the proapoptotic members of the Bcl2 family proteins. Thus, we examined whether cleavage of Bid could be induced by TRAIL treatment. As shown in Figure 2C, the tBid form was detected in OCs after 4 and 6 hrs TRAIL treatment.

TRAIL-mediated human OCs apoptosis involves the death receptor 5

The expression of TRAIL receptors in differentiated OCs after 500ng/ml TRAIL treatment was investigated by western blot. Results showed that the expression of DR4, DcR1 and DcR2 was not affected by TRAIL treatment. Conversely, the expression of DR5 was significantly increased by TRAIL, reaching the maximum level within 2 hrs and remaining higher than untreated OCs up to 8 hrs treatment (Figure 3a). Furthermore, to assess the specific involvement of DR5 in TRAIL-induced OC apoptosis we investigated OC viability in the presence of TRAIL using an anti-DR5 neutralizing antibody. We demonstrated that the reduction of OCs viability induced by TRAIL after 48 hrs was completely abolished only in the presence of anti-DR5 neutralizing antibody. Anti-IgG antibody was utilized as negative control (Figure 3b).

Discussion

Control of OC apoptosis has been recognized as a critical regulatory factor in bone remodelling [18], and alterations in OC death and/or survival have been shown to contribute to the pathogenesis of osteoporosis or malignant osteolysis [18, 35]. Although it has been demonstrated that OC apoptosis is mainly regulated by the Fas/FasL system [36], other apoptotic molecules such as TRAIL cannot be excluded in the regulation of this process. In the present paper we demonstrated that fully differentiated human OCs, derived from PBMCs collected from healthy subjects, are sensitive to TRAIL-induced apoptosis, mainly through the involvement of DR5.

Initially, TRAIL received considerable attention as a molecule showing the ability to induce apoptosis in a variety of neoplastic cells [1]. However, many normal cells, such as thymocytes [1], neural cells [20] and hepatocytes [13], expressing TRAIL and TRAIL-receptors, were sensitive to TRAIL-induced apoptosis, implying that the physiological role of TRAIL is more complex than just induction of apoptosis in cancer cells. With particular regard to the bone cells, the expression of TRAIL receptors has been described in normal and transformed osteoblasts [2] as well as in OCs in pathological and physiological conditions [4, 25], suggesting that TRAIL could play a role in bone cell apoptosis [25,33]. Further studies demonstrated that TRAIL exhibits other roles in different normal and pathological tissues [39]. In particular, an anti-differentiating activity of TRAIL on both the erythroid [27] and osteoclastic [38] lineages has been shown. In one of the few studies available in TRAIL –/– deficient mice no overt differences in gross radio-density of bones were described [28]. These investigators demonstrated in vitro that OC differentiation was normal in these animals, suggesting that TRAIL cannot play an essential role in mouse bone development. However, it is interesting to note that this study was performed in relatively young mice, and the authors did not study OC survival in vitro. It is also important to stress that TRAIL receptors differ in humans and mice, and the two systems may function according to different mechanisms [26]. Thus, experiments in mice cannot be directly related to the human situation, and these studies do not conclusively rule out the possibility that the TRAIL/TRAIL-receptor pathway may be involved in human OC apoptosis.

In the present paper, we have demonstrated that human OCs, once differentiated, were sensitive to TRAIL-induced apoptosis. This evidence lies in the increased number of apoptotic OCs induced by TRAIL and degradation of chromatin. None of these effects were induced by TRAIL in OC precursors during the differentiation process, in line with the study by Zauli et al. [38]. The lack of TRAIL effect we found on preosteoclasts can be explained by our results showing that in the early stages of OC differentiation there was overexpression of the decoy receptor DcR2 as compared to the other TRAIL receptors. Consequently, in preosteoclasts the ratio between death and decoy TRAIL receptors is in favour of decoy receptors, thereby inhibiting TRAIL mediated-cell death. Thus, we can speculate that overexpression of DcR2 could be a protective strategy of preosteoclasts against TRAIL-induced apoptosis. Instead, once differentiated OCs showed a different scenario in the expression of TRAIL receptors. The death receptor DR5 was highly expressed as compared to the other receptors, while expression of the decoy receptor DcR2, that was up-regulated in OC precursors, was down-regulated and so the OCs became susceptible to TRAIL-induced apoptosis.

Additionally, in fully differentiated human OCs we have herein documented for the first time that TRAIL caused upregulation of the levels of DR5 but no modulation of either DR4 or the two decoy receptor expression. These findings suggest that DR5 could be the major receptor involved in TRAIL-mediated apoptosis of human mature OCs. This hypothesis is in agreement with the demonstration that only anti-DR5 neutralizing antibody completely restored the TRAIL-induced reduction of OC viability. Moreover, following the upregulation of DR5, the proteolytic

activation of caspase-8 and consequent activation of Bid suggested that both the extracellular and the mitochondrial pathways were involved in TRAIL-mediated OC apoptosis. These findings provide evidence of a fully functional TRAIL receptor in human mature OCs in which a sequential apoptotic pathway is activated. Our results are consistent with literature data showing that caspase-8 activation and the subsequent Bid cleavage are a prerequisite for the TRAIL apoptotic effect in other cell types [7, 10, 14, 15, 17, 29].

In conclusion, this study provides evidence that TRAIL-mediated apoptosis may represent an additional and/or alternative mechanism for apoptosis of human OCs. It is essential to bear in mind that large amounts of TRAIL are produced in several pathological conditions, including diseases with bone involvement [4]. Thus, we can speculate that when OCs are in the presence of high concentrations of TRAIL, the expression of TRAIL death domain-containing receptors could be up-regulated, and the apoptotic pathway could be activated. On the other hand, it is of interest to note that regulation of the ratio of TRAIL death and decoy receptors, that could depend on both the stage of OC differentiation and other cells present in the bone microenvironment, becomes critical in determining cell sensitivity or resistance. For instance, we previously demonstrated that OCs from multiple myeloma bone disease patients were protected from TRAIL-induced apoptosis via the upregulation of decoy receptors and downregulation of death TRAIL receptors through a T cell-dependent mechanism [4]. Thus on the basis of our results and literature data, we can conclude that TRAIL-induced OC apoptosis, mainly mediated by DR5, could be an additional and/or alternative mechanism inducing apoptosis of bone resorbing cells. This is a complex mechanism that needs to be further studied. Elucidation of the mechanisms controlling OC apoptosis will not only help to improve the efficacy of available drugs, but, more importantly, may also reveal novel strategies for drug development.

This study shows that TRAIL-induced OC apoptosis, mainly mediated by DR5, could be an additional and/or alternative mechanism inducing apoptosis of bone resorbing cells.

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References

[1] Almasan A, Ashkenazi A: Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. Cytokine Growth Factor Rev. 2003; 14:337-348.

[2] Bu R, Borysenko CW, Li Y, Cao L, Sabokbar A, Blair HC: Expression and function of TNF-family proteins and receptors in human osteoblasts. Bone 2003;33(5):760-770.

[3] Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L: Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. Immunity 1997; 7:821-830.

[4] Colucci S, Brunetti G, Rizzi R, Zonno A, Mori G, Colaianni G, Del Prete D, Faccio R, Liso A, Capalbo S, Liso V, Zallone A, Grano M: T cells support osteoclastogenesis in an in vitro model derived from human multiple myeloma bone disease: the role of the OPG/TRAIL interaction. Blood 2004; 104: 3722-3730.

[5] Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA, Goodwin RG: The novel receptor TRAIL-R4 induces NF-κB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. Immunity 1997; 7:813-820.

[6] Degli-Esposti MA, Smolak PJ, Walczak H, Waugh J, Huang CP, DuBose RF, Goodwin RG, Smith CA: Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. J Exp Med 1997; 186:1165-1170.

[7] Eggert A, Grotzer MA, Zuzak TJ, Wiewrodt BR, Ho R, Ikegaki N, Brodeur GM: Resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in neuroblastoma cells correlates with a loss of caspase-8 expression. Cancer Res 2001; 61:1314-1319.

[8] Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR: Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 1998;273:14363-14367.

[9] Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P, Korsmeyer SJ: Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. J Biol Chem 1999; 274:1156-1163.

[10] Hao C, Beguinot F, Condorelli G, Trencia A, Van Meir EG, Yong VW, Parney IF, Roa WH, Petruk KC: Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis in human malignant glioma cells. Cancer Res 2001; 61:1162–1170.

[11] Hughes DE, Boyce BF: Apoptosis in bone physiology and disease. Mol. Pathol 1997; 50: 132-137.

[12] Janmey PA: The cytoskeleton and cell signaling: component localization and mechanical coupling. Physiol Rev 1998; 78: 763-781.

[13] Jo M, Kim TH, Seol DW, Esplen JE, Dorko K, Billiar TR, Strom SC: Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nat Med 2000; 6:564-567.

[14] Lacour S, Hammann A, Wotawa A, Corcos L, Solary E, Dimanch-Boitrel MT: Anticancer agents sensitize tumor cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated caspase-8 activation and apoptosis. Cancer Res 2001; 61:1645 -1651.

[15] Leverkus M, Neumann M, Mengling T, Rauch CT, Bröcker EB, Krammer PH, Walczak H: Regulation of tumor necrosis factor-related apoptosis-inducing ligand sensitivity in primary and transformed human keratinocytes. Cancer Res 2000; 60: 553–559.

[16] Li H, Zhu H, Xu CJ, Yuan J: Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998; 94:491-501.

[17] Luo X, Budihardjo I, Zou H, Slaughter C, Wang X: Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 1998; 94:481-490.

[18] Manolagas SC: Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000; 21:115–137.

[19] Marsters SA, Pitti RA, Sheridan JP, Ashkenazi A: Control of apoptosis signaling by Apo2 ligand. Recent Progr Horm Res 1999; 54:225-234.

[20] Martin-Villalba A, Herr I, Jeremias I, Hahne M, Brandt R, Vogel J, Schenkel J, Herdegen T, Debatin KM: CD95 ligand (Fas-L/APO-1L) and tumor necrosis factor-related apoptosis-inducing ligand mediate ischemia-induced apoptosis in neurons. J Neurosci 1999; 19:3809-3817.

[21] Oberhammer F, Wilson JW, Dive C, Morris ID, Hickman JA, Wakeling AE, Walker PR, Sikorska M: Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. EMBO J 1993; 12:3679-3684.

[22] Pan G, Ni J, Yu G, Wei YF, Dixit VM: TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signaling. Fed Eur Biol Lett 1998; 424:41-45.

[23] Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM: The receptor for the cytotoxic ligand TRAIL. Science 1997; 276:111-113.

[24] Parfitt AM: The cellular basis of bone remodeling: the quantum concept reexamined in light of recent advances in the cell biology of bone. Calcif. Tissue Int 1984; 36: S37-S45.

[25] Roux S, Lambert-Comeau P, Saint-Pierre C, Lepine M, Sawan B, Parent JL: Death receptors, Fas and TRAIL receptors, are involved in human osteoclast apoptosis. Biochem Biophys Res Commun. 2005 22;333(1):42-50.

[26] Schneider P, Olson D, Tardivel A, Browning B, Lugovskoy A, Gong D, Dobles M, Hertig S, Hofmann K, Van Vlijmen H, Hsu YM, Burkly LC, Tschopp J, Zheng TS: Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). J Biol Chem 2003;278:5444-5454.

[27] Secchiero P, Melloni E, Heikinheimo M, Mannisto S, Di Pietro R, Zauli G: TRAIL regulates normal erythroid maturation through an ERK-dependent pathway. Blood 2004; 103: 517-522.

[28] Sedger LM, Glaccum MB, Schuh JC, Kanaly ST, Williamson E, Kayagaki N, Yun T, Smolak P, Le T, Goodwin R, Gliniak B: Characterization of the in vivo function of TNF-alpha-related apoptosis-inducing ligand, TRAIL/Apo2L, using TRAIL/Apo2L gene-deficient mice. Eur J Immunol 2002; 32: 2246–2254.

[29] Seol DW, Li J, Seol MH, Park SY, Talanian RV, Billiar TR: Signaling events triggered by tumor necrosis factor-related apoptosis-inducing lignat (TRAIL): caspase-8 is required for TRAIL-induced apoptosis. Cancer Res 2001; 61:1138 - 1143.

[30] Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD,

Godowski P, Ashkenazi A: Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997; 277:818-821.

[31] Simon AK, Williams O, Mongkolsapaya J, Jin B, Xu XN, Walczak H, Screaton GR: Tumor necrosis factor-related apoptosis-inducing ligand in T cell development: sensitivity of human thymocytes. Proc Natl Acad Sci USA 2001; 98: 5158-5163.

[32] Steller H: Mechanisms and genes of cellular suicide. Science 1995; 267:1445-1449.

[33] Tinhofer I, Biedermann R, Krismer M, Crazzolara R, Greil R: A role of TRAIL in killing osteoblasts by myeloma cells. FASEB J 2006;20(6):759-761.

[34] Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, Timour MS, Gerhart MJ, Schooley KA, Smith CA, Goodwin RG, Rauch CT: TRAIL-R2: A novel apoptosis-mediating receptor for TRAIL. EMBO J 1997; 16: 5386–5397.

[35] Weinstein RS, Manolagas SC: Apoptosis and osteoporosis. Am J Med 2000; 108:153–164.

[36] Wu X, McKenna MA, Feng X, Nagy TR, McDonald JM: Osteoclast apoptosis: the role of Fas in vivo and in vitro. Endocrinology 2003;144(12):5545-55.

[37] Wyllie AH, Kerr JF, Currie AR: Cell death: the significance of apoptosis. Int Rev Cytol 1980; 68:251-306.

[38] Zauli G, Rimondi E, Nicolin V, Melloni E, Celeghini C, Secchiero P: TNF-related apoptosis-inducing ligand (TRAIL) blocks osteoclastic differentiation induced by RANKL plus M-CSF. Blood 2004; 104:2044–2050.

[39] Zauli G, Secchiero P: The role of the TRAIL/TRAIL receptors system in hematopoiesis and endothelial cell biology. Cytokine Growth Factor Rev 2006; 17:245-257.