High expression of TRAIL by osteoblastic differentiated dental pulp stem cells affects myeloma cell viability

GIACOMINA BRUNETTI[1*], ADRIANA DI BENEDETTO[2*], FRANCESCA POSA[2], GRAZIANA COLAIANNI[1], MARIA FELICIA FAIENZA[3], ANDREA BALLINI[4], SILVIA COLUCCI[1], GIOVANNI PASSERI[5], LORENZO LO MUZIO[2], MARIA GRANO[6] and GIORGIO MORI[2]

[1]Department of Basic Medical Sciences, Neurosciences and Sense Organs, Section of Human Anatomy and Histology, University of Bari Aldo Moro, I-70121 Bari; [2]Department of Clinical and Experimental Medicine, University of Foggia, I-71122 Foggia; Departments of [3]Biomedical Sciences and Human Oncology, Pediatric Section and [4]Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, I-70121 Bari; [5]Department of Medicine and Surgery, University of Parma, I-43121 Parma; [6]Department of Emergency and Organ Transplantation, Section of Human Anatomy and Histology, University of Bari Aldo Moro, I-70124, Italy

Received October 9, 2017; Accepted January 25, 2018

DOI: 10.3892/or.2018.6272

Abstract. Cells from dental tissues have a mesenchymal stem cell (MSC) phenotype, are multipotent and can differentiate into osteoblastic cells, as we have previously found. MSCs, due to their tumor-homing ability, are currently being used as cell-based delivery systems for cancer protein therapeutics, such as the TNF-related apoptosis-inducing ligand (TRAIL). In the present study we revealed that dental pulp stem cells (DPSCs) expressed TRAIL to a greater extent when they were differentiated into the osteoblastic lineage. TRAIL affected the viability of undifferentiated DPSCs, while osteoblastic differentiated DPSCs were not sensitive to TRAIL. The expression trend of TRAIL receptors underwent changes during the osteoblastic differentiation of DPSCs exhibiting low DcR2 and high DR5 levels in the undifferentiated DPSCs and an opposite scenario was presented in the differentiated cells. The sensitivity of the undifferentiated DPSCs to the TRAIL-apoptotic effect was also associated with low levels of intracellular anti-apoptotic proteins, such as c-FLIP, XIAP and the activation of caspase-8 and -3. DPSC-differentiated osteoblasts expressing high TRAIL levels were capable to affect the cell viability of the human myeloma cell line H929, thus representing an effective anticancer therapeutic method.

Correspondence to: Professor Giacomina Brunetti, Department of Basic Medical Sciences, Neurosciences and Sense Organs, Section of Human Anatomy and Histology, University of Bari Aldo Moro, Piazza Giulio Cesare 11, I-70124 Bari, Italy
E-mail: giacomina.brunetti@uniba.it

*Contributed equally

Key words: TRAIL, apoptosis, dental pulp stem cells, myeloma

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells that have attracted a great interest for their noteworthy multilineage differentiation potential (1,2) and hypoimmunogenic features (3,4). All these properties of MSCs have led to their application in regenerative medicine (5,6). Furthermore, due to their tumor-homing ability (7), MSCs are currently used as cell-based delivery systems of therapeutic proteins for cancer treatment (8,9). In particular, through the tumor-homing ability of MSCs, the localized production of a specific therapeutic protein is more helpful than the systemic use of a recombinant protein considering both the effective *in situ* concentration of the molecule and the reduction of unwanted systemic actions. To this end, pro-apoptotic molecules have been linked to MSCs to counteract tumor growth and a particular interest is evident toward TRAIL (10-13), a cytotoxic protein inducing apoptosis mostly in tumor cells, upon binding to the death domain-containing receptor 4 (DR4) and 5 (DR5). The activity of TRAIL can be modulated following binding with two membrane-bound decoy receptors, namely DcR1 and DcR2, which lacking functional death domains, confer TRAIL resistance to expressing cells (14).

However, clinical studies based on the use of a recombinant soluble form of TRAIL, consisting of a non-covalently assembled homotrimer, as a whole, did not demonstrate therapeutic efficacy (15,16). Over the past decades, many recombinant versions of TRAIL have been generated to enhance its pharmacokinetics and/or antitumor activity (17). To date, it is evident that at least a hexavalent organization of TRAIL molecules bypass the pharmacokinetic problems, however not the trimeric form (18). In contrast, in order to manage the insufficient pharmacokinetic properties, several studies have examined the practice of *in situ* production of a standard soluble TRAIL molecule by different adult stem cells (19-21). Furthermore, two studies have reported the antitumor activity of human genetically modified MSCs expressing antibodies in a diabody format (22,23). Recently,

a MSC line, stably producing TRAIL which is activated in a xenotransplantation tumor model, has been generated (13). Although the main known source of MSCs is the bone marrow, a wide variety of MSCs have been recognized in dental tissues such as pulp, periodontal ligament and apical papilla, exhibiting several multilineage potencies including osteogenic, adipogenic and neurogenic (24-30), besides the expected odontogenic (31). In our previous studies, we demonstrated the stem cell properties of dental tissues such as pulp, follicle and bud, as well as their ability to differentiate into osteoblasts (32-38). According to our findings, differentiated dental pulp stem cells (DPSCs) express high levels of TRAIL. Therefore, we hypothesized that DPSCs could provide, through the production of TRAIL, an effective anticancer therapeutic method. Based on these parameters and considering the pro-apoptotic TRAIL effect, we investigated whether DPSCs differentiated into osteoblasts, expressing high TRAIL levels were capable to affect tumor cell viability.

Materials and methods

Cell cultures. Third molar teeth were obtained from 20 healthy young donors, who gave their written informed consent. The study was approved by the Institutional Review Board of the Department of Dental Science and Surgery-Unit of Periodontology, University of Bari. The dental pulps were dissected, gently washed with phosphate-buffered saline (PBS), reduced to small pieces and digested enzymatically with 3 mg/ml type I collagenase and 4 mg/ml dispase (Gibco; Thermo Fischer Scientific, Uxbridge, UK) in agitation for 1 h at 37˚C. To obtain single cell suspensions, the digested solutions were filtered through a 70-μm BD Falcon strainer (Falcon; BD Biosciences, Sunnyvale, CA, USA). Single cell suspensions, centrifuged at 1,300 rpm, were seeded at $5x10^3$ cells/cm^2 in mesenchymal stem cell culture medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin-G, 100 μg/ml streptomycin (Gibco; Thermo Fischer Scientific) at 37˚C, in 5% CO_2, replacing the medium every three days until cells reached confluence. The cells were then trypsinized and seeded into appropriate common culture dishes for characterization and experiments, that provide an efficacious substrate for DPSC adhesion, proliferation and differentiation (39). For the induction of osteogenic differentiation, the cells were seeded at a density of $3x10^3$ cells/cm^2 in α-MEM supplemented with 2% FBS, 10^{-8} M dexamethasone and 50 μg/ml ascorbic acid (35). For some experiments, osteogenic differentiated DPSCs were co-cultured with $1x10^3$/cm^2 H929 cells (from the ATCC, Rockville, MD, USA) with or without anti-TRAIL neutralizing monoclonal antibody (mouse; cat. no. MAB375; 500 ng/ml; R&D Systems, Minneapolis, MN, USA).

Cell viability assay. Cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. DPSCs were cultured in 96-well tissue-culture plates and some of them were selected for the time-point 0 (t_0), while the others were differentiated with 50 μg/ml ascorbic acid and dexamethasone (10^{-8} M) for 20 days (t_{20}). Both t_0 and t_{20} cultures were treated with rh-TRAIL (10-500 ng/ml, TRAIL/TNFSF10; R&D Systems) for 48 h. The cell viability was assessed by adding 0.5 mg/ml MTT to the culture medium followed by a 4-h incubation at 37˚C in a humidified 5% CO_2 atmosphere. To stop the reaction, 150 μl of 0.04 N HCl in absolute isopropanol, was added and the optical density (OD) was read at 570 nm through an automatic plate reader (550 Microplate Reader; Bio-Rad Laboratories Inc., Hercules, CA, USA). The obtained values were normalized to cells in control conditions.

RNA isolation and real-time PCR analysis. DPSCs at t_0 and at t_{20} were subjected to mRNA extraction using spin columns (RNeasy; Qiagen, Hilden, Germany) according to the manufacturer's instructions. The mRNA (1 μg) was reverse transcribed using SuperScript First-Strand Synthesis System kit (Invitrogen Life Technologies, Carlsbad, CA, USA). The resulting cDNA was subjected to PCR amplification with the iTaq SYBR Green Supermix with ROX kit (Bio-Rad Laboratories), using the Chromo 4 real-time PCR detection system (Bio-Rad Laboratories). The following pairs of oligonucleotides were used for the PCR amplification: TRAIL sense, 5'-AGCAACACATTGTCTCC-3' and antisense, 5'-CCA GTTCACCATTCCTCAAG-3'; GAPDH sense 5'-TCATCC CTGCCTCTACTG-3' and antisense 5'-TGCTTCACCACC TTCTTG-3'. The fold change values were calculated using the Pfaffl method (40).

Western blot analysis. Total cell lysates were obtained from cultures ceased at different time-points. Briefly, at the indicated time-points, lysis buffer [50 mmol/l Tris-HCl (pH 8.0), 150 mmol/l NaCl, 5 mmol/l ethylenediaminetetraacetic acid, 1% NP40 and 1 mmol/l phenylmethyl sulfonyl fluoride] was added to the cell monolayer and the lysates were recovered after incubation on ice for 30 min. The proteins were separated by SDS-PAGE gel and transferred onto nitrocellulose membranes (Hybond; Amersham Pharmacia, London, UK) and the blots were probed with the appropriate antibodies (Abs): Mouse caspase-3 (1:500; cat. no. 9662; Cell Signaling Technology, Danvers, MA, USA) and mouse anti-β-actin monoclonal Abs (1:1,000; Chemicon International Inc.; EMD Millipore, Billerica, MA, USA), rabbit anti-DR5 (1:200; cat. no. ab47179; Abcam, Cambridge, UK), anti-DcR2 (1:200; cat. no. ab2019; Abcam), anti-caspase-8 (1:500; cat. no. 552038; BD Biosciences, San Diego, CA, USA), anti-cFLIP (1:500; cat. no. 8510; Cell Signaling Technology) and anti-XIAP (1:500; cat. no. 3B6; Cell Signaling Technology) polyclonal Abs. Specific reactions with the appropriate fluorescent-dye-conjugated secondary Ab (1:10,000; IRDye 800 CW goat anti rabbit IgG or IRDye 800 CW goat anti mouse IgG; LI-COR Biosciences GmbH, Bad Homburg, Germany), were revealed with the LI-COR Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

Statistical analysis. Statistical analysis was performed using Student's t-test with the SPSS 22 (SPSS X/PC) software (SPSS, Inc., Chicago, IL, USA). A value of $P<0.05$ was considered to indicate statistically significant differences.

Results

Expression of TRAIL increases in DPSCs differentiated towards osteoblasts. We previously demonstrated that DPSCs cultured in osteogenic medium displayed an osteoblastic

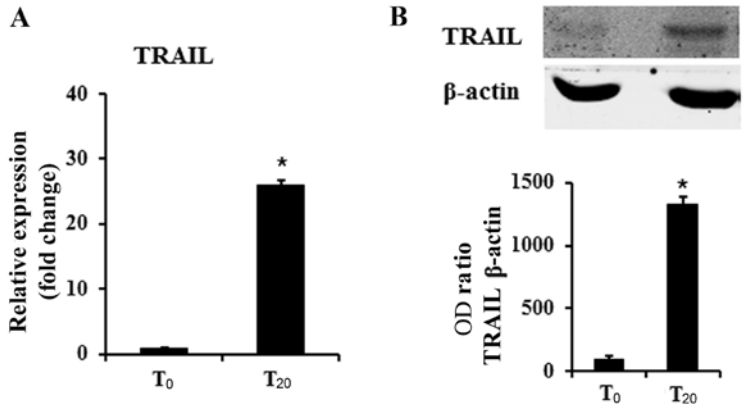

Figure 1. Expression of TRAIL in DPSCs. (A) qPCR of undifferentiated DPSCs (T_0) and DPSCs differentiated for 20 days in osteogenic conditions (T_{20}) revealed mRNA levels of TRAIL normalized to GAPDH. *P<0.01, compared to T_0. TRAIL expression reached a 25-fold increase in T_{20}-DPSCs. (B) The protein expression level of TRAIL was assessed by western blot analysis in same culture conditions (DPSCs T_0-T_{20}) and normalized to β-actin confirming mRNA level results. The graph represents means ± SE of three independent donors. *P<0.01 compared to T_0. Student's t-test was used for single comparison.

phenotype (32,33). It is also known from the literature that MSCs produced TRAIL. These findings prompted us to evaluate the expression of TRAIL in undifferentiated DPSCs (t_0) and in cultures differentiated for 20 days in osteogenic conditions (t_{20}). We found that undifferentiated DPSCs already expressed TRAIL, however, in the cells cultured for 20 days in osteogenic medium, TRAIL mRNA levels reached a 25-fold increase (Fig. 1A). These results were also supported by western blotting indicating a 15-fold increase of TRAIL in differentiated DPSCs in respect to undifferentiated cells (Fig. 1B).

Sensitivity of DPSCs to TRAIL-mediated apoptosis. DPSC sensitivity to TRAIL-apoptotic effect was investigated by analyzing cell viability through an MTT assay in undifferentiated (t_0) and differentiated (t_{20}) DPSCs in the presence of TRAIL. Undifferentiated and differentiated DPSCs were first characterized for their osteoblastic parameters (alkaline phosphatase, osteopontin and osteocalcin) exhibiting a weak expression at t_0 and a significant increase at t_{20} (data not shown). The cells in both conditions were treated with increasing concentrations of rh-TRAIL (ranging from 10 to 500 ng/ml) for 48 h and their viability was determined in both TRAIL-treated and untreated cells as a control. As observed in Fig. 2A, the viability of t_0-DPSCs was reduced by TRAIL in a dose-dependent manner. In detail, when undifferentiated cells were treated with 10 ng/ml rh-TRAIL for 48 h their viability was significantly reduced compared to untreated cultures. Treatment with TRAIL at 25 ng/m further decreased the viability of t_0-DPSCs, while the maximum decrease was observed at 50 ng/ml TRAIL and no additional reduction was observed in the presence of higher concentrations of the cytokine. Unexpectedly, we observed that TRAIL did not induce any effect on cell viability on differentiated DPSCs even at a dose of 50 ng/ml TRAIL (Fig. 2B), thus demonstrating that DPSCs T_{20}, were resistant to TRAIL-induced apoptosis.

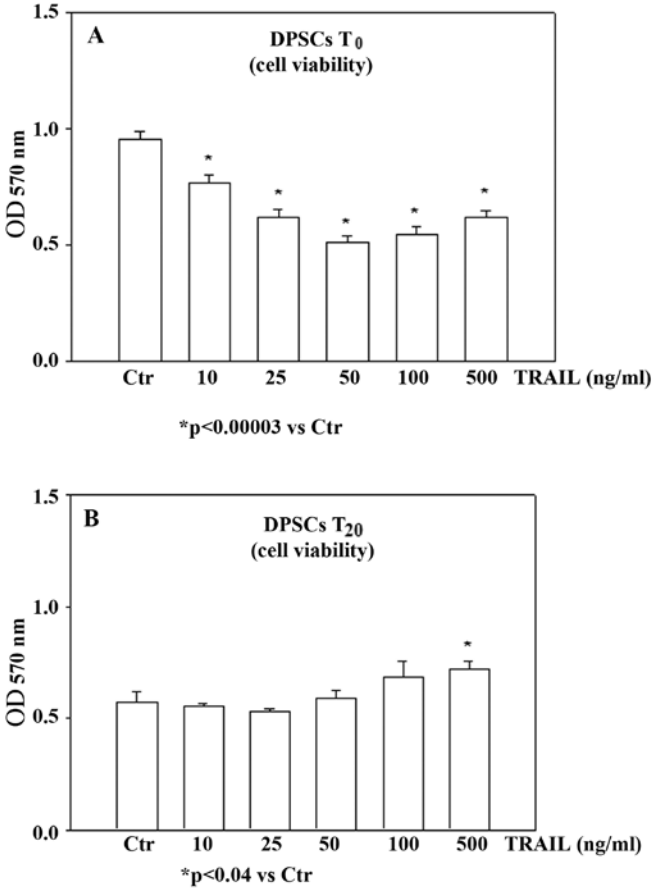

Figure 2. DPSC viability in the presence of exogenous TRAIL stimulation. An MTT assay performed on (A) DPSC-T_0 and (B) DPSC-T_{20} revealed the cell viability in the presence of an increasing dose of rh-TRAIL (10-500 ng/ml) for 48 h. The graphs are representative of the mean values ± SE of three independent experiments in which each treatment was performed in quadruplicate.

Expression of TRAIL receptors during DPSC differentiation. On the base of the aforementioned results, we explored the possibility that the progression of the osteoblastic differentiation could affect the expression of death and decoy TRAIL receptors in DPSCs. Using western blotting we found that DPSCs constitutively expressed all TRAIL receptors both in undifferentiated conditions and during their differentiation. In particular, the expression of DR5, was high in undifferentiated cells and progressively decreased during the

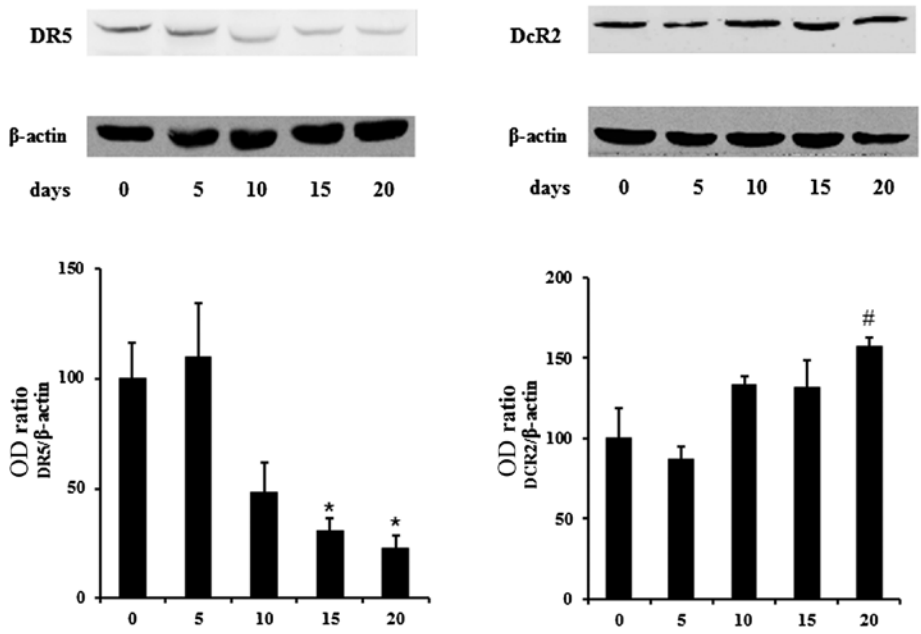

Figure 3. Expression of TRAIL receptors during DPSC osteogenic differentiation. Immunoblotting was performed to detect the expression of death (DR5) and decoy (DcR2) receptors during the osteogenic differentiation of DPSCs (0, 5, 10, 15 and 20 days). The graphs represent the quantified OD normalized to β-actin of each band. Data are reported as the means ± SE of three independent experiments. $^{*}P<0.01$ compared to T_0; $^{\#}P<0.05$ compared to T_0.

differentiation process, reaching the lowest level in t_{20}-DPSCs (Fig. 3). Additionally, the expression of the decoy receptor DcR2 was low in t_0-DPSCs and increased ~20% following 20 days of osteoblasic differentiation. The expression of DR4 and DcR1 was not modified (data not shown). Consequently, the ratio between decoy- and death-TRAIL receptors shifted in favor of the death-TRAIL receptors in the undifferentiated DPSCs thus, increasing their sensitivity to TRAIL apoptotic effect. By contrast, the ratio shifted in favor of the decoy receptors during the DPSC osteogenic differentiation. Collectively, these results indicated the absence of the TRAIL effect on the viability of osteogenic differentiated DPSCs (Fig. 2).

TRAIL activates caspase-8 and -3 in undifferentiated DPSCs. Based on the above described findings we evaluated the activation of caspase-8 and -3 in TRAIL-treated undifferentiated and differentiated DPSCs. It is known that caspase-8 is the early caspase activated during TRAIL-induced apoptosis in different cell types (41,42). Using western blot analysis, we demonstrated the activation of caspase-8 in undifferentiated DPSCs treated with 100 ng/ml TRAIL from 1 up to 8 h. In particular, caspase activation was evident after 6 h of TRAIL treatment (Fig. 4A). It is also well-known that activated caspase-8 cleaves caspase-3. Thus, we explored whether TRAIL treatment induced the cleavage of caspase-3. As depicted in Fig. 4B, the p17 cleaved form of caspase-3 was found in undifferentiated DPSCs following 6 and 8 h of TRAIL-treatment exposure. According to the MTT assay, we observed that TRAIL failed to induce caspase-8 and -3 activation in differentiated DPSCs (Fig. 4C and D).

Expression of cFLIP and XIAP during DPSC differentiation. The reported diverse TRAIL sensitivity of the undifferentiated and differentiated DPSCs prompted us to evaluate the different levels of the intracellular anti-apoptotic molecules cFLIP and XIAP during the DPSC differentiation. Using western blotting we observed that the expression of cFLIP and XIAP increased during the osteoblastic differentiation of DPSCs and the lowest levels of XIAP and cFLIP were demonstrated in undifferentiated DPSCs (Fig. 5). Thus, our findings indicated that differentiated DPSCs were further preserved from TRAIL pro-apoptotic effect through the increase of the intracellular inhibitors of caspases cFLIP and XIAP (43,44).

Differentiated DPSCs affect the viability of the H929 cells through TRAIL. The above-reported high expression of TRAIL in differentiated DPSCs led us to hypothesize that these cells could be a vehicle of the pro-apoptotic agent for cancer cells and we tested this hypothesis on the human myeloma cell line H929. Firstly, we verified the sensitivity of H929 cells to the TRAIL-apoptotic effect, stimulating the human myeloma cell line with 100 ng/ml TRAIL and assessing the cell viability with an MTT assay. The results indicated a significant decrease of H929 cell viability following treatment, demonstrating that the cells were sensitive to the TRAIL-apoptotic effect (Fig. 6A). Subsequently, in order to demonstrate that osteoblastic differentiated DPSCs producing TRAIL can induce apoptosis of tumor cells, we co-cultured osteoblastic differentiated DPSCs with H929, with or without a neutralizing anti-TRAIL antibody. Our results indicated that anti-TRAIL antibody treatment exhibited a significant increase of cell viability in the co-culture, which should be related to the neutralization of TRAIL produced by differentiated DPSCs (Fig. 6B). Anti-TRAIL antibody did not affect cell viability of H929 cells and differentiated DPSCs cultured alone (Fig. 6C and D). Notably, cell-cell contact was fundamental for this interaction; in fact the media from osteoblastic differentiated DPSCs did not affect the viability of H929 cells (data not shown).

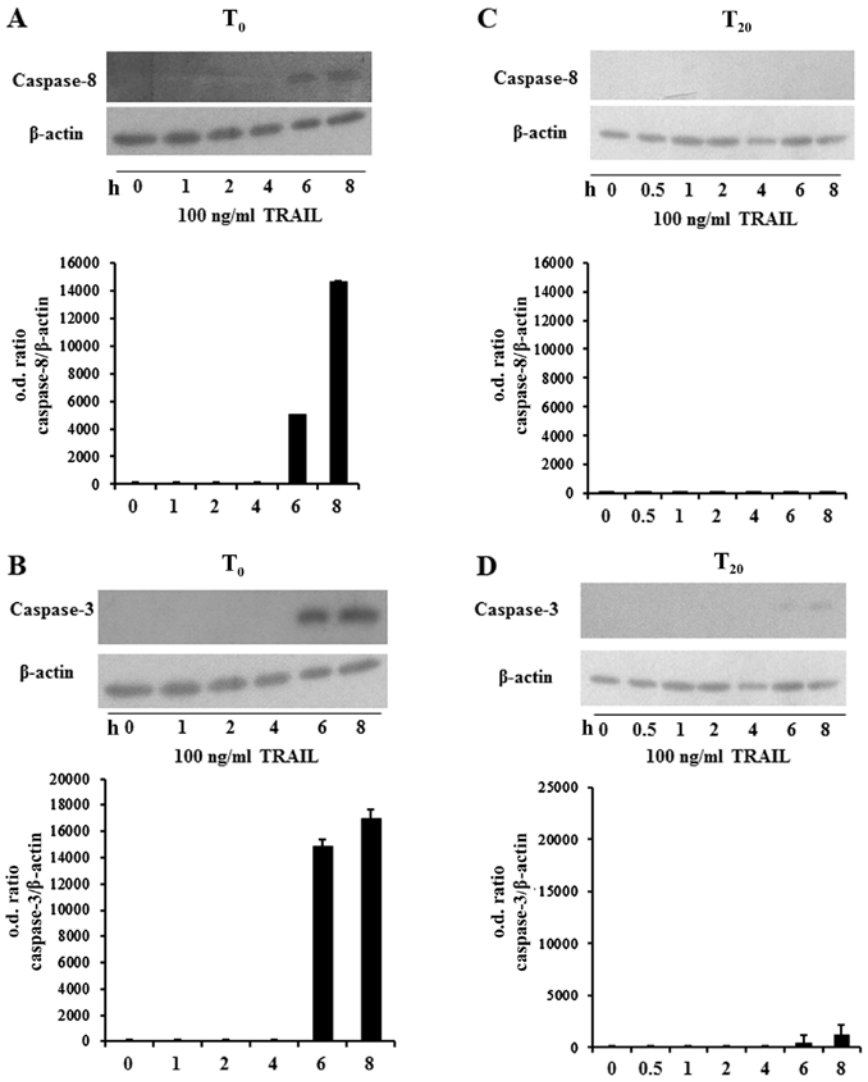

Figure 4. Activation of caspase-8 and caspase-3 in DPSC T_0 and T_{20}. (A and B) DPSCs at T_0 were stimulated with 100 ng/ml TRAIL for 1, 2, 4, 6 and 8 h and analyzed by western blotting to detect the activation of caspase-8 and -3. The blots revealed the bands corresponding to the active fragment of 23 kDa for caspase-8 and 17 kDa for caspase-3. (C and D) DPSCs at T_{20} stimulated with 100 ng/ml TRAIL for 1, 2, 4, 6 and 8 failed to demonstrate the activation of caspase-8 and caspase-3. Densitometric quantification of the results, normalized to β-actin, was reported in the graphs as the mean ± SE of three independent experiments.

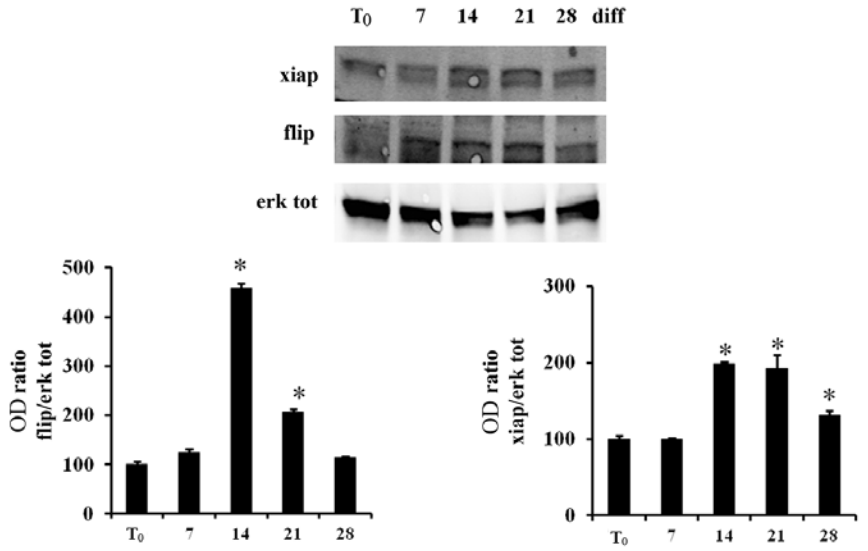

Figure 5. Expression of cFLIP and XIAP during the DPSC differentiation. The blots demonstrate the protein expression levels of XIAP and cFLIP in DPSCs differentiated in osteogenic conditions (T_0, 7, 14, 21 and 28 days). The graphs represent the mean ± SE values of three independent experiments. The results were normalized to total ERK (erk tot). *P<0.01 compared to T_0.

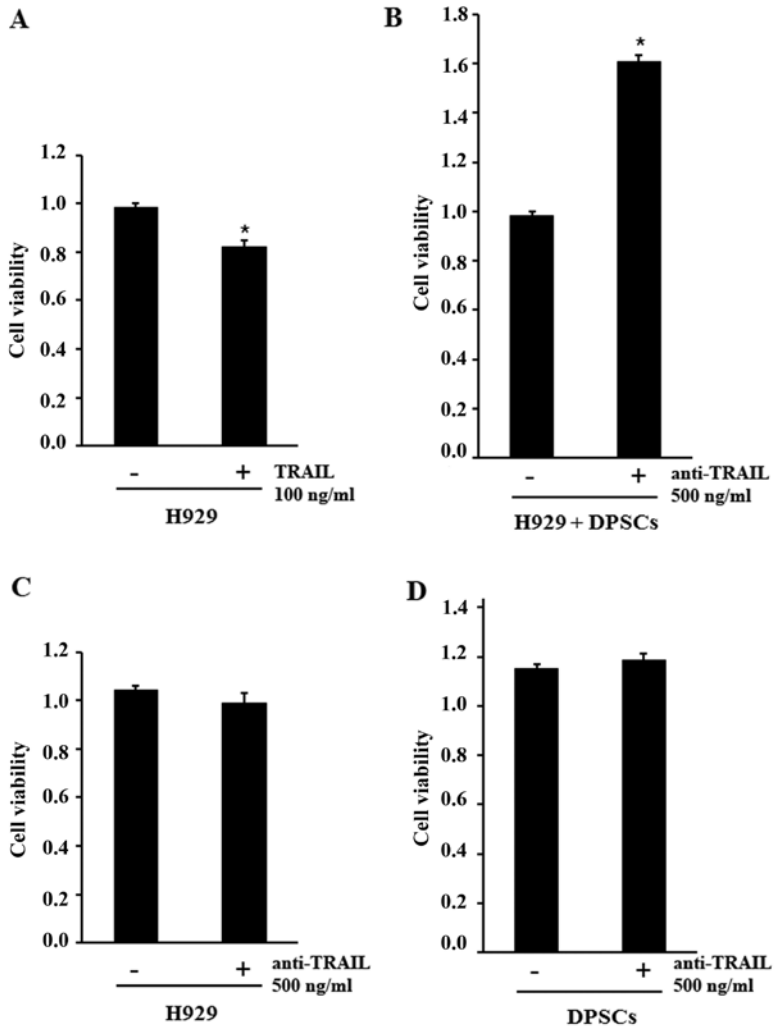

Figure 6. DPSCs T_{20} affect H929 cell viability. (A) H929 cells were cultured with or without TRAIL 100 ng/ml for 48 h and the cell viability was assessed by an MTT assay. (B) Differentiated DPSCs were co-cultured with H929 human myeloma cell line for 48 h in the presence or absence of a neutralizing anti-TRAIL antibody (500 ng/ml). The MTT assay performed on the H929 cells, growing in suspension, revealed that the treatment with the neutralizing anti-TRAIL significantly increased the cell viability of H929 cells, compared to the corresponding untreated co-cultures. (C) H929 cells and (D) differentiated DPSCs were cultured with or without 500 ng/ml anti-TRAIL antibody for 48 h and the cell viability was assessed by an MTT assay. The graphs are representative of the mean ± SE of three independent experiments in which each treatment was performed in quadruplicate. *P<0.01 compared to untreated samples.

Discussion

In the present study, we demonstrated that the expression of TRAIL increased during the osteoblastic differentiation of DPSCs and in parallel, that differentiated DPSCs lost their sensitivity to TRAIL-induced apoptosis. These two properties of DPSCs led us to use these cells as a TRAIL-vehicle to induce an apoptotic effect on H929 myeloma cell line *in vitro*.

Since its discovery (45,46), TRAIL has been broadly considered a potential therapeutic agent, due to its uniqueness of inducing apoptosis in cancer cells while not affecting normal cells (47,48). However, sensitivity to its pro-apoptotic effect has also emerged in normal cells, such as bone cells (49-54). TRAIL-based therapies performed in numerous clinical trials generated poor results for different reasons, including the defective delivery of the protein to cancer cells. These findings led to the use of DPSCs as a delivery system for cancer cells. We previously characterized DPSCs as an alternative source of MSCs (32,33,35) and demonstrated their ability to differentiate into osteoblastic cells. Notably, we observed that differentiated DPSCs expressed higher TRAIL levels and presented a different responsiveness to TRAIL pro-apoptotic effect with respect to undifferentiated DPSCs.

In detail, we found that only undifferentiated DPSCs were sensitive to TRAIL-induced apoptosis in a time- and dose-dependent manner, while DPSCs cultured in osteogenic conditions were resistant. We demonstrated that the diverse responses of undifferentiated and differentiated DPSCs to TRAIL were related to the different expression of TRAIL-receptors DR5 and DCR2 during osteoblastic differentiation. DR5 is a death domain-containing receptor, required for the induction of cell death (55-58), while DCR2 is a decoy factor able to counteract the pro-apoptotic action of TRAIL (59,60). Our results indicated that undifferentiated DPSCs expressed higher levels of DR5 and lower levels of DcR2 compared to differentiated DPSCs. Thus, in undifferentiated DPSCs the ratio of TRAIL-receptors moved towards the death receptors, leading these cells to be more sensitive to the pro-apoptotic action of TRAIL. Conversely, in differentiated DPSCs the receptor ratio shifted to the decoy

receptors, resulting in defense from TRAIL-mediated apoptosis. We demonstrated that cell viability in DPSCs at t_0 was negatively affected by TRAIL treatment, while DPSCs at t_{20} were protected from death. However, we hypothesized that cell-resistance or -sensitivity to TRAIL-mediated apoptosis was not only determined by the balance between the expression of its death and decoy receptors, but could be partially related to the levels of certain intracellular anti-apoptotic molecules, such as c-FLIP and XIAP (61-64). Supporting the observation of different TRAIL sensitivity between t_0- and t_{20}-DPSCs, we found low levels of the anti-apoptotic factors c-FLIP and XIAP in t_0-DPSCs, that increased during osteogenic differentiation.

Furthermore, according to the shifted ratio of receptors towards death receptors and low levels of cFLIP and XIAP in undifferentiated DPSCs, we demonstrated, in these cells, after the TRAIL administration, the immediate activation of caspase-8 that in turn resulted in the executioner caspase-3 cleavage starting at 6 h of incubation. These events represented the crucial intracellular steps in the initiation of the apoptotic pathway activated by TRAIL in other cells (65-67). Notably, the stimulation of TRAIL failed to activate this pathway in t_{20}-DPSCs, since caspase-8 and -3 were almost unexpressed. The latter finding was in agreement with the resistance to TRAIL-mediated cell death exhibited by these cells in osteogenic conditions and with the expression of receptors and anti-apoptotic molecules. The obtained results led us to hypothesize on the possible role of differentiated DPSCs as a vehicle of TRAIL to cancer cells and prompted us to test this hypothesis on cancer cells. We used the human myeloma cell line H929 to test our hypothesis due to its non-adherent behavior in culture. Previous studies have tested the use of MSCs as a vehicle of TRAIL demonstrating the ability to induce either *in vitro* apoptosis in several cancer cell lines (11), or *in vivo* remission of colon tumor and sarcomas established in nude mice (10,12). Furthermore, the localized action of TRAIL delivered by MSCs appeared to bypass the resistance of cancer cells, such as breast and colorectal, to soluble TRAIL (68,69). More recently, a stable MSC line expressing a highly bioactive form of TRAIL was generated and demonstrated a significant tumor regression in an *in vivo* Colo205 mouse xenograft tumor model (13). The advantage of our model is that DPSCs differentiated in osteogenic conditions, expressed high levels of endogenous TRAIL, thus this system did not require any transfection technology and the cells were resistant to TRAIL-mediated apoptosis. Our results indicated that the cell viability of human myeloma H929 cells was affected by the TRAIL-apoptotic effect (Fig. 6A). Notably when the H929 cells were co-cultured with DPSCs and treated with anti-TRAIL neutralizing antibody, they recovered high levels of cell viability, with a complete rescue of the apoptotic effect (Fig. 6A). In conclusion, our results revealed that differentiated DPSCs expressed high levels of TRAIL and were not sensitive to its pro-apoptotic effect, thus they may be an optimal carrier of this antitumor agent in cancer cells. Furthermore, the results demonstrated that this effect completely depended on TRAIL. When TRAIL, produced by DPSCs co-cultured with myeloma cells, was neutralized using blocking antibodies, an increase of H929 cell viability was obtained.

Notably, a recent well-designed study indicated that osteoblasts could counteract leukemia progression in mice, while osteoblast impairment promoted the disease. The authors concluded that osteoblasts could be a therapeutic target in akute leukemia, although the basic mechanism of this interesting finding has not yet been described (70). This study is aligned with our results and it is intriguing to hypothesize that TRAIL expressed by osteoblasts mediates this effect. Collectively, these emerging results suggested that osteoblasts and osteogenic differentiated MSCs could have a potential therapeutic role in hindering tumor progression.

Acknowledgements

The authors would like to thank Pasqua Bellocci for her technical support. The author A. Di Benedetto has received funding from the 'Fondo di Sviluppo e Coesione 2007-2013, APQ Ricerca Regione Puglia 'Programma regionale a sostegno della specializzazione intelligente e della sostenibilità sociale ed ambientale-Future In Research'.

Competing interests

The authors declare that they have no competing interests.

References

1. Aslan H, Zilberman Y, Kandel L, Liebergall M, Oskouian RJ, Gazit D and Gazit Z: Osteogenic differentiation of noncultured immunoisolated bone marrow-derived CD105+ cells. Stem Cells 24: 1728-1737, 2006.
2. Muraglia A, Cancedda R and Quarto R: Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 113: 1161-1166, 2000.
3. Davies LC, Heldring N, Kadri N and Le Blanc K: Mesenchymal stromal cell secretion of programmed death-1 ligands regulates T cell mediated immunosuppression. Stem Cells 35: 766-776, 2017.
4. Madec AM, Mallone R, Afonso G, Abou Mrad E, Mesnier A, Eljaafari A and Thivolet C: Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. Diabetologia 52: 1391-1399, 2009.
5. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE and Brenner MK: Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5: 309-313, 1999.
6. Scarano A, Crincoli V, Di Benedetto A, Cozzolino V, Lorusso F, Podaliri Vulpiani M, Grano M, Kalemaj Z, Mori G and Grassi FR: Bone regeneration induced by bone porcine block with bone marrow stromal stem cells in a minipig model of mandibular 'Critical Size'. Stem Cells Int 2017: 9082869, 2017.
7. Hagenhoff A, Bruns CJ, Zhao Y, von Luttichau I, Niess H, Spitzweg C and Nelson PJ: Harnessing mesenchymal stem cell homing as an anticancer therapy. Expert Opin Biol Ther 16: 1079-1092, 2016.
8. Niess H, von Einem JC, Thomas MN, Michl M, Angele MK, Huss R, Günther C, Nelson PJ, Bruns CJ and Heinemann V: Treatment of advanced gastrointestinal tumors with genetically modified autologous mesenchymal stromal cells (TREAT-ME1): Study protocol of a phase I/II clinical trial. BMC Cancer 15: 237, 2015.
9. Nowakowski A, Drela K, Rozycka J, Janowski M and Lukomska B: Engineered mesenchymal stem cells as an anti-cancer trojan horse. Stem Cells Dev: Sep 7, 2016 (Epub ahead of print).
10. Yu R, Deedigan L, Albarenque SM, Mohr A and Zwacka RM: Delivery of sTRAIL variants by MSCs in combination with cytotoxic drug treatment leads to p53-independent enhanced antitumor effects. Cell Death Dis 4: e503, 2013.

11. Yuan Z, Kolluri KK, Sage EK, Gowers KH and Janes SM: Mesenchymal stromal cell delivery of full-length tumor necrosis factor-related apoptosis-inducing ligand is superior to soluble type for cancer therapy. Cytotherapy 17: 885-896, 2015.
12. Grisendi G, Spano C, D'Souza N, Rasini V, Veronesi E, Prapa M, Petrachi T, Piccinno S, Rossignoli F, Burns JS, *et al*: Mesenchymal progenitors expressing TRAIL induce apoptosis in sarcomas. Stem Cells 33: 859-869, 2015.
13. Marini I, Siegemund M, Hutt M, Kontermann RE and Pfizenmaier K: Antitumor activity of a mesenchymal stem cell line stably secreting a tumor-targeted TNF-related apoptosis-inducing ligand fusion protein. Front Immunol 8: 536, 2017.
14. von Karstedt S, Montinaro A and Walczak H: Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. Nat Rev Cancer 17: 352-366, 2017.
15. Herbst RS, Eckhardt SG, Kurzrock R, Ebbinghaus S, O'Dwyer PJ, Gordon MS, Novotny W, Goldwasser MA, Tohnya TM, Lum BL, *et al*: Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. J Clin Oncol 28: 2839-2846, 2010.
16. Soria JC, Smit E, Khayat D, Besse B, Yang X, Hsu CP, Reese D, Wiezorek J and Blackhall F: Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. J Clin Oncol 28: 1527-1533, 2010.
17. Ganten TM, Koschny R, Sykora J, Schulze-Bergkamen H, Büchler P, Haas TL, Schader MB, Untergasser A, Stremmel W and Walczak H: Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs. Clin Cancer Res 12: 2640-2646, 2006.
18. Siegemund M, Seifert O, Zarani M, Džinić T, De Leo V, Göttsch D, Münkel S, Hutt M, Pfizenmaier K and Kontermann RE: An optimized antibody-single-chain TRAIL fusion protein for cancer therapy. MAbs 8: 879-891, 2016.
19. Guiho R, Biteau K, Grisendi G, Taurelle J, Chatelais M, Gantier M, Heymann D, Dominici M and Redini F: TRAIL delivered by mesenchymal stromal/stem cells counteracts tumor development in orthotopic Ewing sarcoma models. Int J Cancer 139: 2802-2811, 2016.
20. Yan C, Song X, Yu W, Wei F, Li H, Lv M, Zhang X and Ren X: Human umbilical cord mesenchymal stem cells delivering sTRAIL home to lung cancer mediated by MCP-1/CCR2 axis and exhibit antitumor effects. Tumour Biol 37: 8425-8435, 2016.
21. Lathrop MJ, Sage EK, Macura SL, Brooks EM, Cruz F, Bonenfant NR, Sokocevic D, MacPherson MB, Beuschel SL, Dunaway CW, *et al*: Antitumor effects of TRAIL-expressing mesenchymal stromal cells in a mouse xenograft model of human mesothelioma. Cancer Gene Ther 22: 44-54, 2015.
22. Compte M, Cuesta AM, Sánchez-Martin D, Alonso-Camino V, Vicario JL, Sanz L and Alvarez-Vallina L: Tumor immunotherapy using gene-modified human mesenchymal stem cells loaded into synthetic extracellular matrix scaffolds. Stem Cells 27: 753-760, 2009.
23. Zhang X, Yang Y, Zhang L, Lu Y, Zhang Q, Fan D, Zhang Y, Zhang Y, Ye Z and Xiong D: Mesenchymal stromal cells as vehicles of tetravalent bispecific Tandab (CD3/CD19) for the treatment of B cell lymphoma combined with IDO pathway inhibitor D-1-methyl-tryptophan. J Hematol Oncol 10: 56, 2017.
24. Gronthos S, Mankani M, Brahim J, Robey PG and Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97: 13625-13630, 2000.
25. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY and Shi S: Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364: 149-155, 2004.
26. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S and Shi S: Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1: e79, 2006.
27. Pisciotta A, Carnevale G, Meloni S, Riccio M, De Biasi S, Gibellini L, Ferrari A, Bruzzesi G and De Pol A: Human dental pulp stem cells (hDPSCs): Isolation, enrichment and comparative differentiation of two sub-populations. BMC Dev Biol 15: 14, 2015.
28. Nagatomo K, Komaki M, Sekiya I, Sakaguchi Y, Noguchi K, Oda S, Muneta T and Ishikawa I: Stem cell properties of human periodontal ligament cells. J Periodontal Res 41: 303-310, 2006.
29. Marrelli M, Paduano F and Tatullo M: Human periapical cyst-mesenchymal stem cells differentiate into neuronal cells. J Dent Res 94: 843-852, 2015.
30. Giorgini E, Conti C, Ferraris P, Sabbatini S, Tosi G, Centonze M, Grano M and Moric G: FT-IR microscopic analysis on human dental pulp stem cells. Vibrational Spectroscopy 57: 30-34, 2011.
31. Tatullo M, Falisi G, Amantea M, Rastelli C, Paduano F and Marrelli M: Dental pulp stem cells and human periapical cyst mesenchymal stem cells in bone tissue regeneration: Comparison of basal and osteogenic differentiated gene expression of a newly discovered mesenchymal stem cell lineage. J Biol Regul Homeost Agents 29: 713-718, 2015.
32. Mori G, Brunetti G, Oranger A, Carbone C, Ballini A, Lo Muzio L, Colucci S, Mori C, Grassi FR and Grano M: Dental pulp stem cells: Osteogenic differentiation and gene expression. Ann N Y Acad Sci 1237: 47-52, 2011.
33. Mori G, Centonze M, Brunetti G, Ballini A, Oranger A, Mori C, Lo Muzio L, Tetè S, Ciccolella F, Colucci S, *et al*: Osteogenic properties of human dental pulp stem cells. J Biol Regul Homeost Agents 24: 167-175, 2010.
34. Mori G, Ballini A, Carbone C, Oranger A, Brunetti G, Di Benedetto A, Rapone B, Cantore S, Di Comite M, Colucci S, *et al*: Osteogenic differentiation of dental follicle stem cells. Int J Med Sci 9: 480-487, 2012.
35. Di Benedetto A, Carbone C and Mori G: Dental pulp stem cells isolation and osteogenic differentiation: A good promise for tissue engineering. Methods Mol Biol 1210: 117-130, 2014.
36. Di Benedetto A, Brunetti G, Posa F, Ballini A, Grassi FR, Colaianni G, Colucci S, Rossi E, Cavalcanti-Adam EA, Lo Muzio L, *et al*: Osteogenic differentiation of mesenchymal stem cells from dental bud: Role of integrins and cadherins. Stem Cell Res 15: 618-628, 2015.
37. Posa F, Di Benedetto A, Colaianni G, Cavalcanti-Adam EA, Brunetti G, Porro C, Trotta T, Grano M and Mori G: Vitamin D effects on osteoblastic differentiation of mesenchymal stem cells from dental tissues. Stem Cells Int 2016: 9150819, 2016.
38. Di Benedetto A, Posa F, Carbone C, Cantore S, Brunetti G, Centonze M, Grano M, Lo Muzio L, Cavalcanti-Adam EA and Mori G: NURR1 downregulation favors osteoblastic differentiation of MSCs. Stem Cells Int 2017: 7617048, 2017.
39. Tatullo M, Marrelli M, Falisi G, Rastelli C, Palmieri F, Gargari M, Zavan B, Paduano F and Benagiano V: Mechanical influence of tissue culture plates and extracellular matrix on mesenchymal stem cell behavior: A topical review. Int J Immunopathol Pharmacol 29: 3-8, 2016.
40. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45, 2001.
41. Peter ME, Scaffidi C, Medema JP, Kischkel F and Krammer PH: The death receptors. Results Probl Cell Differ 23: 25-63, 1999.
42. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH and Peter ME: FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 16: 2794-2804, 1997.
43. Golks A, Brenner D, Fritsch C, Krammer PH and Lavrik IN: c-FLIPR, a new regulator of death receptor-induced apoptosis. J Biol Chem 280: 14507-14513, 2005.
44. Deveraux QL, Takahashi R, Salvesen GS and Reed JC: X-linked IAP is a direct inhibitor of cell-death proteases. Nature 388: 300-304, 1997.
45. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, *et al*: Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3: 673-682, 1995.
46. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A and Ashkenazi A: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 271: 12687-12690, 1996.
47. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, *et al*: Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 104: 155-162, 1999.
48. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, *et al*: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 5: 157-163, 1999.
49. Tinhofer I, Biedermann R, Krismer M, Crazzolara R and Greil R: A role of TRAIL in killing osteoblasts by myeloma cells. FASEB J 20: 759-761, 2006.

50. Brunetti G, Oranger A, Carbone C, Mori G, Sardone FR, Mori C, Celi M, Faienza MF, Tarantino U, Zallone A, *et al*: Osteoblasts display different responsiveness to TRAIL-induced apoptosis during their differentiation process. Cell Biochem Biophys 67: 1127-1136, 2013.
51. Roux S, Lambert-Comeau P, Saint-Pierre C, Lépine M, Sawan B and Parent JL: Death receptors, Fas and TRAIL receptors, are involved in human osteoclast apoptosis. Biochem Biophys Res Commun 333: 42-50, 2005.
52. Colucci S, Brunetti G, Cantatore FP, Oranger A, Mori G, Pignataro P, Tamma R, Grassi FR, Zallone A and Grano M: The death receptor DR5 is involved in TRAIL-mediated human osteoclast apoptosis. Apoptosis 12: 1623-1632, 2007.
53. Mori G, Brunetti G, Colucci S, Oranger A, Ciccolella F, Sardone F, Pignataro P, Mori C, Karapanou V, Ballini A, *et al*: Osteoblast apoptosis in periodontal disease: Role of TNF-related apoptosis-inducing ligand. Int J Immunopathol Pharmacol 22: 95-103, 2009.
54. Mori G, Brunetti G, Colucci S, Ciccolella F, Coricciati M, Pignataro P, Oranger A, Ballini A, Farronato D, Mastrangelo F, *et al*: Alteration of activity and survival of osteoblasts obtained from human periodontitis patients: Role of TRAIL. J Biol Regul Homeost Agents 21: 105-114, 2007.
55. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J and Dixit VM: The receptor for the cytotoxic ligand TRAIL. Science 276: 111-113, 1997.
56. Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, Timour MS, Gerhart MJ, Schooley KA, Smith CA, *et al*: TRAIL-R2: A novel apoptosis-mediating receptor for TRAIL. EMBO J 16: 5386-5397, 1997.
57. Wu GS, Burns TF, McDonald ER III, Jiang W, Meng R, Krantz ID, Kao G, Gan DD, Zhou JY, Muschel R, *et al*: KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 17: 141-143, 1997.
58. Schneider P, Thome M, Burns K, Bodmer JL, Hofmann K, Kataoka T, Holler N and Tschopp J: TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-kappaB. Immunity 7: 831-836, 1997.
59. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, Yuan J, Gurney A, Goddard AD, Godowski P and Ashkenazi A: A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol 7: 1003-1006, 1997.
60. Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA and Goodwin RG: The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. Immunity 7: 813-820, 1997.
61. Safa AR: c-FLIP, a master anti-apoptotic regulator. Exp Oncol 34: 176-184, 2012.
62. Chawla-Sarkar M, Bae SI, Reu FJ, Jacobs BS, Lindner DJ and Borden EC: Downregulation of Bcl-2, FLIP or IAPs (XIAP and survivin) by siRNAs sensitizes resistant melanoma cells to Apo2L/TRAIL-induced apoptosis. Cell Death Differ 11: 915-923, 2004.
63. Lee TJ, Lee JT, Park JW and Kwon TK: Acquired TRAIL resistance in human breast cancer cells are caused by the sustained cFLIP(L) and XIAP protein levels and ERK activation. Biochem Biophys Res Commun 351: 1024-1030, 2006.
64. Deveraux QL, Leo E, Stennicke HR, Welsh K, Salvesen GS and Reed JC: Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. EMBO J 18: 5242-5251, 1999.
65. Sprick MR, Rieser E, Stahl H, Grosse-Wilde A, Weigand MA and Walczak H: Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signalling complexes in a FADD-dependent manner but can not functionally substitute caspase-8. EMBO J 21: 4520-4530, 2002.
66. Nagata S: Apoptosis by death factor. Cell 88: 355-365, 1997.
67. Ashkenazi A and Dixit VM: Death receptors: Signaling and modulation. Science 281: 1305-1308, 1998.
68. Mueller LP, Luetzkendorf J, Widder M, Nerger K, Caysa H and Mueller T: TRAIL-transduced multipotent mesenchymal stromal cells (TRAIL-MSC) overcome TRAIL resistance in selected CRC cell lines in vitro and in vivo. Cancer Gene Ther 18: 229-239, 2011.
69. Lin T, Huang X, Gu J, Zhang L, Roth JA, Xiong M, Curley SA, Yu Y, Hunt KK and Fang B: Long-term tumor-free survival from treatment with the GFP-TRAIL fusion gene expressed from the hTERT promoter in breast cancer cells. Oncogene 21: 8020-8028, 2002.
70. Krevvata M, Silva BC, Manavalan JS, Galan-Diez M, Kode A, Matthews BG, Park D, Zhang CA, Galili N, Nickolas TL, *et al*: Inhibition of leukemia cell engraftment and disease progression in mice by osteoblasts. Blood 124: 2834-2846, 2014.