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In vitro and *in vivo* discrepancy in inducing apoptosis by mesenchymal stromal cells delivering membrane-bound tumor necrosis factor–related apoptosis inducing ligand in osteosarcoma pre-clinical models

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Abstract

Background: Osteosarcoma (OS) is the most frequent pediatric malignant bone tumor. OS patients have not seen any major therapeutic progress in the last 30 years, in particular in the case of metastatic disease, which requires new therapeutic strategies. The pro-apoptotic cytokine Tumor necrosis factor (TNF)–Related Apoptosis Inducing Ligand (TRAIL) can selectively kill tumor cells while sparing normal cells, making it a promising therapeutic tool in several types of cancer. However, many OS cell lines appear resistant to recombinant human (rh)TRAIL-induced apoptosis. We, therefore, hypothesized that TRAIL presentation at the membrane level of carrier cells might overcome this resistance and trigger apoptosis. *Methods:* To address this, human adipose mesenchymal stromal cells (MSCs) transfected in a stable manner to express membrane-bound full-length human TRAIL (mbTRAIL) were co-cultured with several human OS cell lines. *Results:* This induced apoptosis by cell-to-cell contact even in cell lines initially resistant to rhTRAIL. In contrast, mbTRAIL delivered by MSCs was not able to counteract tumor progression in this OS orthotopic model. *Discussion:* This was partly due to the fact that MSCs showed a potential to support tumor development. Moreover, the expression of mbTRAIL did not show caspase activation in adjacent tumor cells.

Key Words: *mesenchymal stromal cells, osteosarcoma, Tumor necrosis factor (TNF)–Related Apoptosis Inducing Ligand*

Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor in children. OS is characterized by the direct formation of osteoid matrix by tumor cells derived from osteoblasts [1]. Its incidence peaks during the second decade of life coinciding with adolescent growth but its pathogenesis is not clearly determined. Most OS tumors are of high grade and tend to develop pulmonary metastases. Despite clinical improvements, patients with metastatic or relapsed disease still have a poor prognosis of approximately 20% at 5 years [2]. New areas of research have been opened using Tumor necrosis factor (TNF)–Related Apoptosis Inducing Ligand (TRAIL), a pro-apoptotic cytokine from the TNF superfamily. TRAIL can bind to five receptors: two death receptors, TRAIL-R1 (Death Receptor 4 [DR4]) and TRAIL-R2 (DR5), and three decoy receptors that do not transmit the death signal but that can confer resistance toward TRAIL-induced apoptosis, TRAIL-R3 (Decoy Receptor 1 [DcR1]), TRAIL-R4 (DcR2) and the soluble osteoprotegerin

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(OPG). TRAIL, by binding to DR4 and DR5, is able to induce apoptosis of tumor cells by activating the recruitment and cleavage of pro-caspase-8 in a complex called Death-Inducing Signaling Complex (DISC). Caspases activation cascade leads to tumor cell death by the extrinsic apoptosis pathway. This mechanism can be amplified in certain cell types by activating the intrinsic apoptosis pathway, depending on the mitochondria and apoptosome. Both pathways lead to the final cleavage of pro-caspase-3 into active caspase-3 [3].

In the early 2000s, two publications reported a significant inhibitory effect of TRAIL overexpression in an allograft mouse model [4] and in a xenograft model [5] of OS. However, most OS models remain resistant to TRAIL-induced apoptosis. Of the various strategies for increasing the therapeutic effect of TRAIL, using cells carrying TRAIL looks promising [6−9]. This method overcomes limitations resulting from the short half-life of recombinant TRAIL in plasma and allows presentation of trimeric TRAIL. These transporter cells can be adipose-derived mesenchymal stromal cells (AD-MSCs), selected for their natural affinity for tumors, modified to encode human TRAIL [10−12]. Here, we further challenge the promising therapeutic potential of AD-MSCs as mbTRAIL cellular vectors in a variety of OS cell lines *in vitro*, additionally assessing this anti-cancer gene delivery approach in an OS orthotopic *in vivo* model.

Materials and Methods

Cell cultures

Tumor cell lines

Six human OS cell lines were studied: K-HOS (ATCC CRL-1544), U-2 OS, MG-63, G-292, SJSA-1 and Saos2. All cell lines were purchased from the American Type Culture Collection and were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 2 mmol/L L-glutamine.

MSCs

AD-MSCs expressing green fluorescent protein (GFP) only or TRAIL with GFP (named MSC-GFP and MSC-TRAIL, respectively) were obtained as previously described [7,11,12]. In this setting, AD-MSC were cultured in Minimum Essential Medium (αMEM, Lonza) supplemented with 10% FBS (Thermo Scientific), 2 mmol/L L-glutamine and 2 ng/mL basic Fibroblast Growth Factor (bFGF; R&D Systems). As previously described, MSC-TRAIL exhibits a large and homogeneous expression of TRAIL, both intracellular and at the membrane level (Supplementary Figure 1A).

In vitro *experiments*

Cell growth and viability

Two thousand tumor cells per well were seeded into 96-well plates and cultured for 72 h in the presence of increasing concentrations (0−1000 ng) of recombinant human TRAIL (rhTRAIL; R&D Systems). Cell growth and viability were determined using a stable tetrazolium salt WST-1 cell proliferation reagent assay kit (Roche). After the culture period and addition of the WST-1 reagent, the absorbance was then determined at 490 nm. The half maximal inhibitory concentration (IC50) were calculated using Graph Pad Prism v6.01 software.

Annexin V assay using flow cytometry

Ten thousand MSC-TRAIL or MSC-GFP cells were seeded into 6-well plates. The next day, tumor cells were added and co-cultured at a tumor cell/AD-MSC ratio of 3:1. After 48 h, the cells were detached and stained for the Annexin V assay (BD Bioscience). The percentage of tumor cell apoptosis was quantified using cytometry gating on GFP-negative cells with the FC500 flow cytometer (Beckman Coulter).

Effect of MSC supernatant on tumor cell proliferation

Five thousand MSC-TRAIL or MSC-GFP cells were seeded into 24-well plates. The next day, 2000 tumor cells were added to Boyden chamber inserts of 3-μm porosity in 24-well plates (BD Bioscience). After 48 h, the cells in the inserts were fixed, stained and photographed as previously described [12]. The cell surfaces were analyzed with imageJ software (National Institutes of Health). The results are reported as a percentage of tumor cell proliferation in the presence of MSC-GFP as control.

Caspase 3/7 enzymatic activity

Caspase 3/7 activity was measured using an Apo-ONE Homogeneous assay kit (Promega) as previously described [12]. Briefly, cell protein extracts were incubated at room temperature for 16 h with reagent. The fluorescence of each sample was measured and the concentration of total protein was determined with a Bicinchoninic acid assay (BCA) kit.

In vivo *experiments*

OS animal models

Four-week-old Rj:NMRI-nude mice (Janvier Labs) were housed under pathogen-free conditions at the Experimental Therapy Unit (Faculty of Medicine, Nantes, France) in accordance with the institutional guidelines of the French Ethical Committee (CEEA

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