Bone marrow derived mesenchymal stem/stromal cells transduced with full length human TRAIL repress the growth of rhabdomyosarcoma cells *in vitro*

Rhabdomyosarcoma (RMS) is the most frequent juvenal cancer originating from skeletal muscle and patient survival is poor in case of metastatic disease. New targeted therapeutics are critically needed. In a recent Hematologica paper Kuci *et al.*[1] reported that *in vitro* cultured cytokine-induced killer (CIK) cells effectively lysed the targeted rhabdomyosarcoma (RMS) cell lines and proposed that CIK cells may be used as a novel adoptive immunotherapy for the treatment of patients with RMS after allogeneic stem cell transplantation.

The authors showed that TRAIL expression contributes to the antitumor effect of CIK cells by inducing caspase activation in rhabdomyosarcoma cell lines. Furthermore, they provided evidence that not every RMS cell line is equally sensitive for TRAIL-mediated CIK-induced cytotoxicity.

We have previously shown for the first time on a panel of RMS cell lines that around half of them were highly sensitive for the hr-TRAIL-induced caspase-mediated apoptosis.[2] RMS cell line type did not determine their response to TRAIL as several alveolar RMS cell lines were sensitive to TRAIL while an embrional RMS cell line was resistant.[2] Further research revealed that resistance of RMS cells to TRAIL can be the result of dysregulation of various molecular components such as FLIP overexpression,[2] casein kinase II (CK2) overactivity[3] or Bcl-2 overexpression.[3,4]

TRAIL receptor agonist therapies (hr-TRAIL or anti-TRAIL-receptor antibodies) are under clinical trials for various cancer types.[5] Phase I results with sarcoma patients indicate high frequency of adverse effects as well as enduring partial responses.[6] Recently mesenchymal stem/stromal cell (MSC)-mediated targeted delivery of TRAIL to tumor sites was proposed as a new approach to enhance the relative TRAIL concentration in tumors and in their metastatic sites.[7,8,9] Application of MSCs for cancer treatment is a promising cell based therapeutic strategy.[10] MSC provide an alternative experimental approach to CIK cell mediated growth inhibition of metastatic RMS. We have recently shown that MSC derived from adipose tissue (AD-MSC) is an appropriate vehicle for TRAIL production for inducing apoptosis in various cancer types (cell lines and primary tumor cells) and for targeted delivery of TRAIL to tumor sites *in vivo*.[11] Here we provide evidences that bone marrow derived MSC-s (BM-MSC) transduced with a TRAIL vector can effectively target an RMS cell line *in vitro*.

We have generated MSC cultures from the tumor free bone marrow of 2 patients. Samples were obtained after informed consent by the patients and research was approved by the Ethical Committee of Healthcare Scientific Committee (ETT). Samples were used in passages 6-8 in this study. BM-MSC were isolated by adherence on plastic, and characterized with immunofluorescence markers and with differentiation potential to adipose cells or to osteoblast cells according to Muller *et al.*[12] MSC-s were nucleoporated and transduced with pORF-TRAIL vector (Invivogen) that resulted in TRAIL protein overexpression in MSC-s (Figure 1). TRAIL produced by MSC-s was secreted to the supernatant determined by ELISA test (*data not shown*). We examined the growth inhibitory effect of the TRAIL-transduced MSC (MSC-TRAIL) for RD rhabdomyosarcoma cells *in vitro* by counting the cultured cell number. GFP expressing control MSC-s did not significantly reduce the growth of co-cultured RD cells while MSC-TRAIL cells inhibited the growth of RD-cells with almost 85% at a low effector to target ratio (1:4) (Figure 2).

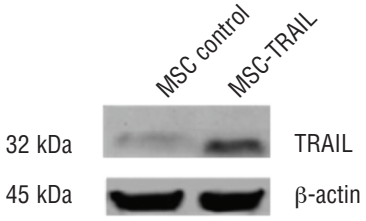

Figure 1. TRAIL expression in MSC-s. MSC ($1x10^5$) were transduced with pORF-h-TRAIL vector (Invivogen) or control vector with Amaxa nucleofector (Lonza) and cultured for 48 h in DMEM (PAA) supplemented with 10% FBS (Hyclone) before being lysed for Western blot and labeled with antibodies against TRAIL (MBL) or β-actin (Sigma) and developed by chemiluminescence.

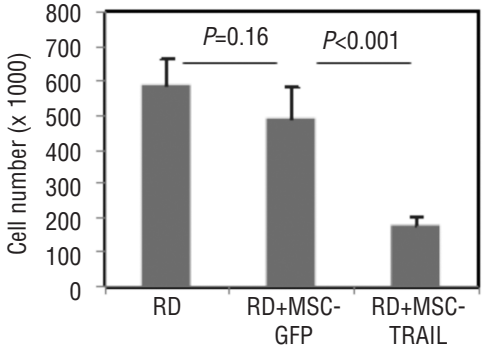

Figure 2. MSC-TRAIL suppressed growth of RD rhabdomyosarcoma cells. Transduced MSC-s ($0.25x10^5$) were plated on 6-well plates for one day then RD cells (ATCC CCl-136) ($1x10^5$) were seeded, co-cultured in low glucose DMEM and RPMI 1640 1:1 (PAA), supplemented with 10% FBS (HyClone) and cell number was determined with Bürker chamber after five days. Mean ±SD of 4 determinations are plotted. Two-tailed, non-homocedastic Student's t-test (Excel) was applied as statistical probe.

Our results with BM-MSCs together with the results of Kuci *et al.* with CIK cells[1] emphasize that cell mediated delivery of TRAIL to metastatic RMS tumor sites can be a useful approach in RMS therapy. Targeted delivery of TRAIL to tumors may allow systemic exposure of patients to drugs (e.g. the proteasome inhibitor bortezomib) that may overcome resistance for TRAIL-induced apoptosis in RMS cells[10] (and Barti-Juhasz H *et al.*, manuscript in preparation, 2011).

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