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Mesenchymal stromal cell delivery of full-length tumor necrosis factor–related apoptosis-inducing ligand is superior to soluble type for cancer therapy

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Abstract

Background aims. Mesenchymal stromal cell (MSC) delivery of pro-apoptotic tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is an attractive strategy for anticancer therapy. MSCs expressing full-length human TRAIL (flT) or its soluble form (sT) have previously been shown to be effective for cancer killing. However, a comparison between the two forms has never been performed, leaving it unclear which approach is most effective. This study addresses the issue for the possible clinical application of TRAIL-expressing MSCs in the future. *Methods.* MSCs were transduced with lentiviruses expressing flT or an isoleucine zipper-fused sT. TRAIL expression was examined and cancer cell apoptosis was measured after treatment with transduced MSCs or with MSC-derived soluble TRAIL. *Results.* The transduction does not adversely affect cell phenotype. The sT-transduced MSCs (MSC-sT) secrete abundant levels of soluble TRAIL but do not present the protein on the cell surface. Interestingly, the flT-transduced MSCs (MSC-flT) not only express cell-surface TRAIL but also release flT into medium. These cells were examined for inducing apoptosis in 20 cancer cell lines. MSC-sT cells showed very limited effects. By contrast, MSC-flT cells demonstrated high cancer cell-killing efficiency. More importantly, MSC-flT cells can overcome some cancer cell resistance to recombinant TRAIL. In addition, both cell surface flT and secreted flT are functional for inducing apoptosis. The secreted flT was found to have higher cancer cell-killing capacity than either recombinant TRAIL or MSC-secreted sT. *Conclusions.* These observations demonstrate that MSC delivery of flT is superior to MSC delivery of sT for cancer therapy.

Key Words: *apoptosis, cancer, mesenchymal stromal cell, TRAIL, tumor*

Introduction

Cancers are one of the leading causes of human death in the world. Each year, more than 10 million new cases of cancer occur globally. Current treatments for metastatic cancers including chemotherapies or radiotherapies often provide limited benefits to patients and are frequently accompanied by undesired side effects. Novel therapies are needed.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a promising agent for cancer therapy. TRAIL is a type II transmembrane protein with homology to other members in the TNF family [1,2], and it selectively triggers apoptosis in tumor cells while sparing normal cells [1–3]. It is safe to deliver, with the ligand exhibiting no detectable cytotoxicity to normal tissues in murine and primate models [4,5] or in humans [6]. Agonist monoclonal antibodies to TRAIL receptors (TRAIL-Rs) have also been used in phase II clinical trials and have shown good safety and tolerability [5,7,8]. However, the enthusiasm in developing TRAIL as a novel cancer therapeutic has been tempered by the challenges of recombinant TRAIL's short half-life (~30 min), limited bioavailability and poor pharmacokinetics. The clinical trials of recombinant soluble TRAIL and agonistic TRAIL-R antibodies have thus far shown limited therapeutic benefit [9].

We and others have used mesenchymal stromal cells (MSCs) as a vector to target TRAIL therapy directly to tumor metastases [10–13]. MSCs preferentially migrate to and incorporate within tumors and their metastases-forming tumor stroma [10,14–18]. Several groups, including our own, have demonstrated that intravenously delivered MSCs preferentially

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localize in lung, breast and melanoma lung metastases [10,19,20], Kaposi's sarcoma [21], colorectal cancer [22] and glioma [23]. MSC tumor tropism has also been demonstrated after intraperitoneal delivery of MSCs for ovarian cancer [24] and intracerebral delivery of MSCs in a glioma model [25].

MSC-delivered targeted TRAIL overcomes the limited half-life of systemically delivered recombinant TRAIL. In murine models, we have shown that systemic injection of MSCs expressing full-length human TRAIL leads to a reduction in subcutaneous tumor growth and reduced, or indeed eliminated, lung metastases [10], and attenuates malignant pleural mesothelioma development [13]. Others have shown that MSCs engineered to express soluble TRAIL are able to kill cancer cells both *in vitro* and *in vivo* [26,27]. MSCs expressing soluble TRAIL may have an advantage *in vivo* in secreting TRAIL throughout the tumor rather than relying on the cell-cell contact that is required by the membrane-bound full-length TRAIL expressed on the MSC surface. In our preclinical development of MSC TRAIL therapy work, we wished to define the relative sensitivity of cancer cells to the different TRAIL forms expressed from a clinically approved lentiviral backbone. To elucidate which strategy is optimal, we created MSCs expressing full-length or soluble TRAIL and compared their activity in inducing cancer cell apoptosis.

Methods

Cell culture

Cell culture reagents were purchased from Invitrogen unless otherwise stated. Twenty cancer cell lines were used, including six lung cancer lines, A549, NCI-H460, NCI-H727, NCI-H23, H226 and PC9; seven malignant pleural mesothelioma lines, NCI-H2052, H2795, H2804, H2731, H2810, H2452 and H2869; three colon cancer lines, Colo205, HT29 and RKO; two renal cancer lines, RCC10 and HA7-RCC; one human oral squamous cell carcinoma line, H357; and one human breast adenocarcinoma line, MDAMB231 (M231). A549, H357 and M231 were obtained from Cancer Research United Kingdom. Other cell lines were kind gifts from Dr Ultan McDermott of the Wellcome Trust Sanger Institute, Cambridge, United Kingdom. NCI-H23, HT29 and Colo205 cells were cultured in Roswell Park Memorial Institute−1640 medium with 10% fetal bovine serum (FBS); RKO cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 with 10% FBS; H357 cells were cultured in DMEM/F-12 (3:1) supplemented with 0.5 μg/mL hydrocortisone and 10^{-10} mol/L cholera toxin (Sigma-Aldrich), 10 ng/mL epithelial growth factor (Cambridge Biosciences) and 5 μg/mL human insulin (MP Biomedicals); all other cell lines were grown in the DMEM containing 10% FBS. Well-characterized human adult MSCs (passage 1) were purchased from the Texas A&M Health Science Center and cultured in the α-minimum essential medium containing 17% FBS.

Construction of TRAIL vectors

The construction of the lentiviral vectors for the expression of flT and its soluble form (sT) was based on the lentiviral plasmid pCCL-c-Fes-Gfp [28]. The promoter of the backbone plasmid was replaced by the cytomegalovirus (CMV) promoter/enhancer [29] at XhoI and BamHI restriction sites. The CMV promoter/enhancer was amplified by means of polymerase chain reaction (PCR) with the use of the pCMV−dR8.74 plasmid as a template (a kind gift from Dr Thrasher, University College London). To create the flT vector, the flT-encoding complementary DNA (cDNA) was amplified by means of PCR with the use of our previously constructed inducible flT plasmid [10] as a template and inserted into the backbone in place of the green fluorescent protein (GFP) sequence through the use of BamHI and SalI sites; the resulting new plasmid is designated pCCL-CMV-flT. To create the sT vector, an open reading frame encoding an N-terminal−truncated extracellular portion of human TRAIL (amino acids 95−281) was amplified by means of PCR, which was then used as template for sequential PCRs to fuse the isoleucine zipper (IZ) (MKQIEDKIEEILSKIY HIENEIARIKKLIGERE) [30] in-frame and the murine immunoglobulin K-chain (IgK; 5′-ATGG AGACAGACACACTCCTGCTATGGGTACTG CTGCTCTGGGTTCCAGGTTCCACTGGTGA C-3′) leader sequence [31] to its N-terminal. The obtained sT sequence was inserted into the pCCL-CMV-flT in place of flT through the BamHI and SalI sites, creating the sT vector designated pCCL-CMV-sT.

Lentivirus preparation and transduction of MSCs

The lentivirus supernatants were produced by co-transfection of 293T cells with construct plasmids together with the packaging plasmids pCMV-dR8.74 and pMD2.G in the presence of a DNA transfection reagent jetPEI (Source Bioscience UK Ltd). The pMD2.G and pCMV-dR8.74 plasmids were kindly provided by Dr Thrasher, University College London (UCL). Lentiviruses in supernatants were concentrated by ultracentrifugation at 17,000 rpm

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