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Pre-activated human mesenchymal stromal cells in combination with doxorubicin synergistically enhance tumor-suppressive activity in mice

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Abstract

Background aims. Previously, we showed that human mesenchymal stromal cells (hMSCs) were activated to express tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) upon TNF-α stimulation, induced cell death in triple-negative breast cancer (TNBC) MDA-MB-231 cells (MDA), and RNA released from apoptotic MDA further increased TRAIL expression in hMSCs. This feed-forward stimulation increased apoptosis in MDA cells. Here, we tested whether TRAIL-expressing hMSCs, in combination with a sub-toxic-dose of a chemotherapy drug doxorubicin, would overcome TRAIL resistance and create synergistic effects on targeting metastatic TNBC. *Methods.* To optimize conditions for the combination treatment, we (i) selected an optimal condition to activate hMSCs for TRAIL expression, (ii) selected an optimal dose of doxorubicin treatment, (iii) examined underlying mechanisms *in vitro* and (iv) tested the efficacy of the optimized conditions in a xenograft mouse model of human breast cancer lung metastasis. *Results.* The results showed that DNA fragments from apoptotic MDA triggered hMSCs to increase further TRAIL expression in an absent in melanoma 2 (AIM2)–dependent manner, and thus higher TRAIL-expressing hMSCs stimulated with synthetic DNA, poly(deoxyadenylic-deoxythymidylic) acid [poly(dA:dT)], more effectively suppressed tumor progression *in vivo*. Furthermore, activated hMSCs increased apoptosis in MDA cells when combined with a sub-toxic dose of doxorubicin, which was mediated by up-regulating TRAIL and Fas-related pathways. When we combined the optimized conditions, pre-activated hMSCs with poly (dA:dT) synergistically reduced tumor burden even with minimal doxorubicin treatment in a xenograft mouse model of human breast cancer lung metastasis. *Conclusions.* These results suggest that the treatment of hMSCs with a sub-toxic dose of doxorubicin can overcome TRAIL resistance and be a potential novel therapy for TNBC metastasis treatment.

Key Words: *bone marrow, IFIH1, multipotent stromal cells, Poly (I:C)*

Introduction

Triple-negative breast cancer (TNBC) has a poor prognosis due to a lack of specifically targeted therapeutic options. The only currently available therapy for TNBC is chemotherapy [1–6]. Chemotherapy agents are relatively effective in patients with early-stage cancer. However, chemotherapy regimens show poor outcomes in patients with advanced TNBC metastasis due to chemoresistance against the drug [7–9].

Giving these agents as monotherapy frequently entails significant toxicity that is exacerbated by multiple drug combinations, and chemotherapy-induced side effects often lead to a compromised quality of life for patients [10,11].

Despite the lack of a target for hormone receptors and poor outcome, TNBC cell lines are sensitive to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced cell death [12]. However, TNBC cell lines show variations in sensitivity to TRAIL-induced cell death [12,13], which could be a major challenge of TRAIL therapy for TNBC treatment. Indeed, many studies demonstrated clinically poor anti-cancer responses toward recombinant human TRAIL [14], TRAIL-R1 [14] or TRAIL-R2 agonist [15–17]. Such outcomes suggest that therapeutic approaches with a single agent are not suitable for effective treatment of TNBC.

Previously, we showed that human mesenchymal stromal cells (hMSCs) expressed membrane-bound TRAIL protein on stimulation of TNF-α, and the expression of TRAIL in hMSCs was further increased

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by RNA released from apoptotic MDA cells in a TLR3-dependent manner after co-culture with MDA cells [18]. In this study, we found that DNA released from apoptotic MDA cells also increased TRAIL expression in hMSCs in absent in melanoma−2 (AIM2), a cytosolic protein that detects dsDNA fragments originated in a foreign cell−dependent manner [19]. Additionally, stimulated hMSCs with synthetic DNA induced more cell death in MDA cells compared with synthetic RNA or TNF-α stimulated hMSCs. In addition, such anti-tumor effect of hMSCs was enhanced when combined with a low-dose chemotherapy drug, and thus this combination treatment significantly reduced the size of tumor burden in mice.

Methods

Cell preparations

hMSCs were prepared as previously described [20]. MDA-MB-231 cells (ATCC) were cultured as described previously [18].

Preparation of apoptotic MDA cells

MDA cells were thawed and plated in α-MEM with 100 ng/mL rhTRAIL (R&D Systems). After 24 h at 37°C, floating cells were collected and washed by centrifugation at 500 *g* for 5 min. Apoptotic cells were stained with 300 ng/mL annexin-V (AnnexinV-FITC Apoptosis Detection Kit; Sigma-Aldrich) and 4 μg/mL 7-aminoactinomycin D (7AAD; Sigma-Aldrich), and analyzed by flow cytometry (Cytomics FC500; Beckman Coulter) to show that 99% of these cells were apoptotic, as shown previously [18]. The pellet was resuspended in 2% culture medium [CM; 2% CM; alpha-minimum essential medium containing 2% fetal bovine serum; lot-selected for rapid growth of hMSCs; (Atlanta Biologicals), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine; (Life Technologies)] and plated on hMSC-containing wells (10^4/cm^2) with 20 ng/mL TNF-α. For RNase and DNase treatment, apoptotic MDA cells were washed by centrifugation, resuspended in 0.2 mL phosphate-buffered saline (PBS) containing either 20 μg of RNase or 30 units of DNase (both from Qiagen), and incubated for 2 h at 37°C. The apoptotic cells were washed by centrifugation and resuspended in 2% CM added to hMSC-containing wells (Apoptotic MDA:hMSC = 1:2).

Animals

Six-week old female NOD/SCID mice (NOD.CB17-Prkdcscid/J) from the Jackson Laboratory were used under a protocol approved by the Institutional Animal Care and Use Committee of Texas A&M University, Health Science Center, College of Medicine.

Lung xenograft model, hMSC infusion and tissue collection

Animals were injected through a tail vein with 2×10^6 MDA cells to mimic lung metastases. At the time of treatment, mice were injected intravenously with Hank's Balanced Salt Solution (HBSS) or pre-activated 2×10^6 hMSCs as described previously [18]. The number of injections for hMSCs varies among experiments. Pre-activated hMSCs were prepared by treating cells for 24 h before injection with recombinant human TNF-α protein (20 ng/mL; R&D Systems) or poly(deoxyadenylic-deoxythymidylic) [poly (dA:dT); 0.5 μg/mL; InvivoGen] in 2% CM. For injection, hMSCs were harvested with 0.25% trypsin/1 mmol/L EDTA and resuspended at 2×10^6 cells in 200 μL of HBSS.

For combination treatment of doxorubicin/hMSCs, the animals were infused weekly with doxorubicin (DOXOrubicin HCl injection, USP; Pfizer Injectables) intraperitoneally once or twice, 4 weeks after intravenous infusion of 2×10^6 MDA cells. Twenty-four hours after doxorubicin infusion, either HBSS or pre-activated hMSCs with TNF-α or poly (dA:dT)/TNF-α were infused intravenously once a week for 2 weeks.

One week after the last injection, lungs were collected for genomic DNA isolation to detect human Alu using quantitative real-time polymerase chain reaction (PCR).

Genomic DNA extraction/real-time PCR assays for human Alu

Genomic DNA from lung samples was extracted and real-time PCR was performed to detect human Alu signals, as previously described [18].

Coculture of hMSCs and MDA cells and flow cytometry

MDA cells were treated with 100 ng/mL doxorubicin (Sigma-Aldrich) in 2% CM for 24 h at 37°C, before co-culture with hMSCs. For co-culture, 10^5 hMSCs were plated in six-well plates containing doxorubicin-treated MDA cells with or without TNF-α (20 ng/mL) and incubated for 24 h. After 24 h, supernatants and cells were collected, suspended in PBS and incubated with anti-CD90-PE (Clone Thy1/310; Beckman Coulter) for 45 min on ice. The suspension was then washed with PBS by centrifugation, incubated at room temperature for 20 min with 300 ng/mL annexin-V (AnnexinV-FITC Apoptosis Detection Kit)