MESENCHYMAL STEM CELLS WITH OVEREXPRESSION OF TRAIL, IL2 OR IFNA17 CAN KILL HUMAN MELANOMA CELLS

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1. Introduction

Currently, immunotherapy (in particular, cytokine-based therapy) of cancer is considered as a promising method to treat various types of tumors. Cytokines and chemokines play an important role in the processes of selective destruction of tumor cells, while not affecting healthy cells of the body. Mesenchymal stem cells (MSCs) can become an ideal vector for delivering cytokines to the tumor microenvironment since they exhibit a homing behavior toward tumor sites.

The aim of the study: production of mesenchymal stem cells overexpressing TRAIL, IL2, IFNA17 or GM-CSF and analysis of its antitumor properties.

2. Materials and methods

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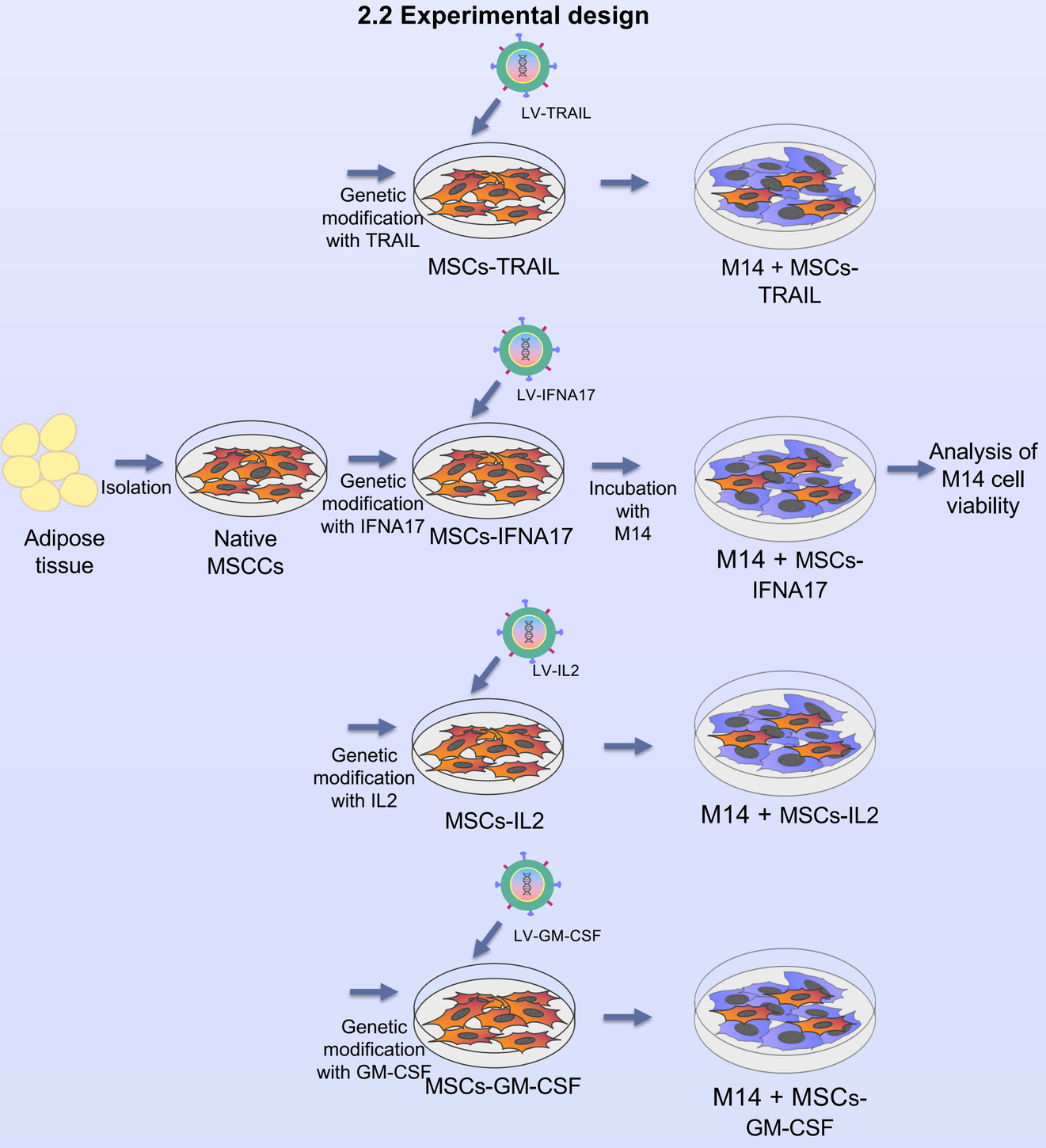
Human MSCs were isolated from human adipose tissue and transduced with recombinant lentiviral vectors encoding tumor necrosis factor ligand superfamily member 10 (TRAIL), interferon alpha-17 (IFNA17), interleukin-2 (IL2), granulocyte macrophage colony-stimulating factor (GM-CSF) or reporter red fluorescent protein (RFP). The gene and protein expression was confirmed by qPCR and Western blot analysis. To evaluate the influence of MSCs with overexpression of various cytokines on the melanoma M-14 cell viability M-14 cells were co-cultured with native and genetically modified MSCs in 1:1 ratio (5×10^4 cells of each type) for 72 h. Viability of M-14 cells were determined using Annexin V assay.

2.2 Experimental design



3. Results and discussion

3.1 Genetic modification of MSCs

Human MSCs were isolated from human adipose tissue. The cells were largely positive for MSC surface markers including CD29, CD44, CD73, CD90 and CD105 and negative for haematopoietic stem cell surface markers. MSCs expressed tumor necrosis factor ligand superfamily member 10 (TRAIL), interferon alpha-17 (IFNA17), interleukin-2 (IL2), granulocyte macrophage colony-stimulating factor (GM-CSF) as confirmed by qPCR and Western blot analysis (Fig. 1-2).

Co-culture of M-14 cells with MSCs-TRAIL (79.1±0.42%), MSCs-IFNA17 (78.0 ± 5.3%) and MSCs-IL2 (84.45±7.5%) resulted in significant decrease in cell viability as compared to that when tumor cells were co-cultured with native MSCs (93.3±0.42%) or MSCs-RFP (91.75±0.49%). At the same time co-culture of M-14 cells with MSCs-GM-CSF increased the cell viability compared to control co-cultures (Fig. 3).

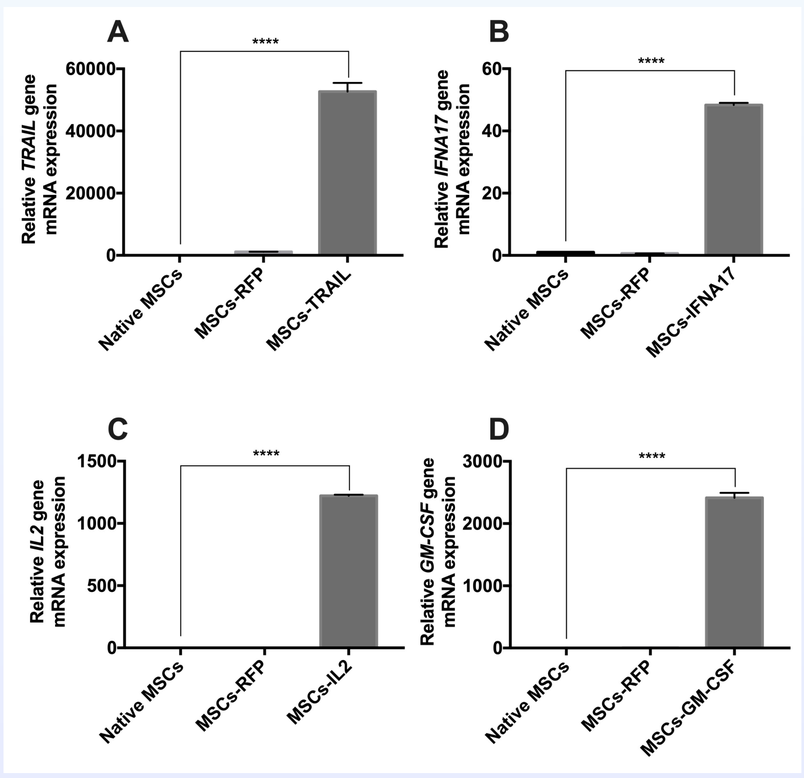


Fig. 1. Relative mRNA expression of TRAIL, IFNA17, IL2 and GM-CSF genes in native and genetically modified MSCs. A – relative TRAIL gene mRNA expression; B – relative IFNA17 gene mRNA expression; C – relative IL2 gene mRNA expression; D – relative GM-CSF gene mRNA expression. ** – p <0,0001.**

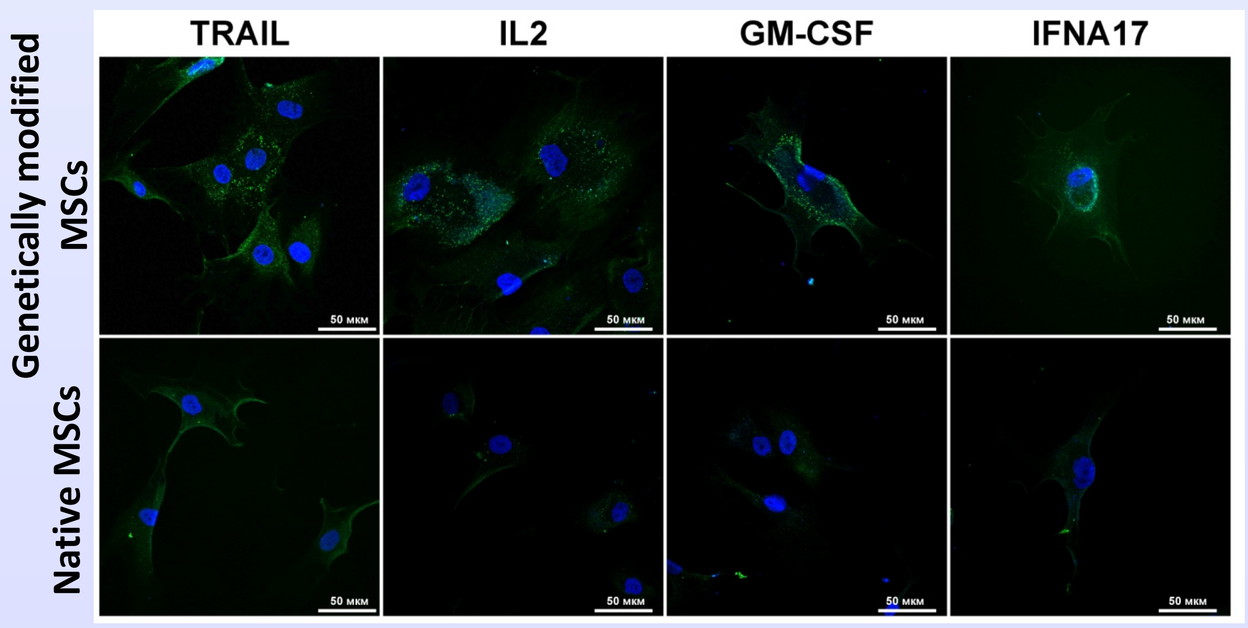


Fig. 2. Analysis of the secretion of recombinant proteins IFNA17 (A), TRAIL (B), GM-CSF (C), PTEN (D) and IL2 (E) in native and genetically modified MSCs by Western blot analysis.

3.2 Analysis of apoptosis/necrosis and proliferation of M14 cells after co-culture with MSCs

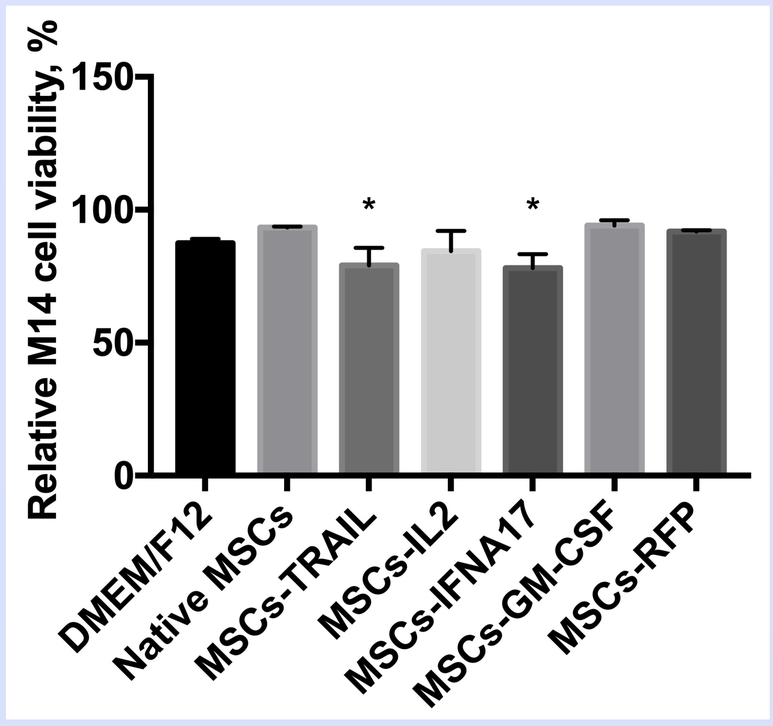


Fig. 3. Analysis of M14 cell viability after cultivation with native and genetically modified MSCs.

4. Conclusion

The use of MSCs-TRAIL, MSCs-IFNA17 or MSCs-IL2 can be effective in the treatment of melanoma. However, further studies of MSC efficiency in animal tumor models are required.

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This study was supported by the Russian Science Foundation grant 18-74-10044 and Kazan Federal University Strategic Academic Leadership Program