Bone marrow mesenchymal stem cells: improving transgene expression level, transfection efficiency and cell viability.


Abstract

PURPOSE:
Advanced cancer is a catastrophic medical condition that is generally treated with surgery and conventional anticancer drugs, which are very toxic and often fail. A promising alternative is using genetically engineered mesenchymal stem cells. A popular method for genetically engineering mesenchymal stem cells (MSCs) is by employing transfection reagents. Nevertheless, a serious limitation of this procedure is its consistently low transfection efficiency. Therefore, the utility of transfection reagents in regenerative medicine - including cancer treatment - might increase strikingly by increasing their transfection efficiency and maintaining, to the greatest extent possible, cell viability and transgene expression levels. The purpose of this study was to analyze various effects on gene expression level, transfection efficiency, and cell viability by increasing the volume of transfection reagents and the plasmid DNA mass.

METHODS:
Mouse bone marrow MSCs were transfected with trademarked Xfect®, Turbofect® or Lipofectamine 3000® and the plasmid pTracer-EF-His-A® expressing the green fluorescent protein (GFP). Additionally, we tested a protocol modification recommended by the Xfect manufacturer. The GFP expression level, transfection efficiency, and cell viability were evaluated together using a performance index.

RESULTS:
By doubling the quantities recommended by the manufacturers (reagent volume), plasmid DNA mass or both variables and by following a modified Xfect method, the transfection efficiency improved to 70%, the cell viability did not diminish, and the performance index increased to 47.7% with respect to the values determined using the original Xfect protocol.

CONCLUSION:
Transgene expression levels, transfection efficiency, and cell viability may be strikingly improved, by increasing the volume of the transfectant agent, the plasmid DNA mass or both, beyond those recommended by transfection kit manufacturers.

In Vitro Induction of Tendon-Specific Markers in Tendon Cells, Adipose- and Bone Marrow-Derived Stem Cells is Dependent on TGFβ3, BMP-12 and Ascorbic Acid Stimulation.

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Author information

Abstract

Mesenchymal Stem Cells (MSCs) and tissue-specific progenitors have been proposed as useful tools for regenerative medicine approaches in bone, cartilage and tendon-related pathologies. The differentiation of cells towards the desired, target tissue-specific lineage has demonstrated advantages in the application of cell therapies and tissue engineering. Unlike osteogenic and chondrogenic differentiation, there is no consensus on the best tenogenic induction protocol. Many growth factors have been proposed for this purpose, including BMP-12, b-FGF, TGF-β3, CTGF, IGF-1 and ascorbic acid (AA). In this study, different combinations of these growth factors have been tested in the context of a two-step differentiation protocol, in order to define their contribution to the induction and maintenance of tendon marker expression in adipose tissue and bone marrow derived MSCs and tendon cells (TCs), respectively. Our results demonstrate that TGF-β3 is the main inducer of scleraxis, an early expressed tendon marker, while at the same time inhibiting tendon markers normally expressed later, such as decorin. In contrast, we find that decorin is induced by BMP-12, b-FGF and AA. Our results provide new insights into the effect of different factors on the tenogenic induction of MSCs and TCs, highlighting the importance of differential timing in TGF-β3 stimulation.


Role of mesenchymal stromal cell-derived extracellular vesicles in tumour microenvironment.

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Author information

Abstract

Stromal cells, deriving from mesenchymal stromal cells (MSCs), are crucial component of tumour microenvironment and represent key regulators of tumour processes. MSCs can be recruited to the tumour environment and interact with many cellular elements, thus influencing tumour biology. Cell-to-cell communication is in part mediated by the release of extracellular vesicle (EVs). EVs can induce significant molecular changes in recipient cells, delivering bioactive molecules. In this review, we describe the MSC-derived EVs content and discuss their role in different processes related to cancer biology. Furthermore, we summarize chemical or biological EVs modifications aiming to develop more efficient antitumor therapies.