MECHANISMS OF ENHANCED NON-VIRAL GENE DELIVERY TO HUMAN MESENCHYMAL STEM CELLS INDUCED BY GLUCOCORTICOID PRIMING

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Background: Because of unique roles in wound healing, trophic tissue support, immunomodulation, differentiation ability, and immune privileged status, human mesenchymal stem cells (hMSCs), which can be easily derived from many adult tissues (e.g. bone marrow (BMSCs) and adipose tissue (AMSCs)), are under intense study for the applications of cell and gene therapeutics, as well as tissue engineering and regenerative medicine[1]. Genetic modification of hMSCs could allow for targeted delivery of transgenic therapeutic factors or genetically-guided differentiation. Non-viral gene delivery (i.e. cationic polymer- and lipid-mediated) is safer and more flexible than immunogenic and mutagenic viral vectors[2], but it is less effective, especially in hMSCs (i.e. maximum 10-30% transfection)[3]. As part of an approach to understand molecular mechanisms of non-viral gene delivery[4] and 'prime' cells to be more receptive to transfection[5], our lab recently demonstrated that transgene expression from lipofected hMSCs can be increased about 10-fold by priming cells, 30 mins before plasmid DNA (pDNA) transfection, with 100 nM dexamethasone (DEX), a glucocorticoid (Gc) drug, relative to EtOH vehicle control (VC)[6]. This work investigates the mechanisms by which Gc priming enhances non-viral gene delivery, which are currently unknown. Studies provide insights into the biological processes of Gc priming and transfection to inform future gene delivery technologies, and characterize a simple protocol to significantly enhance non-viral gene delivery of therapeutic transgenes for future clinical applications.

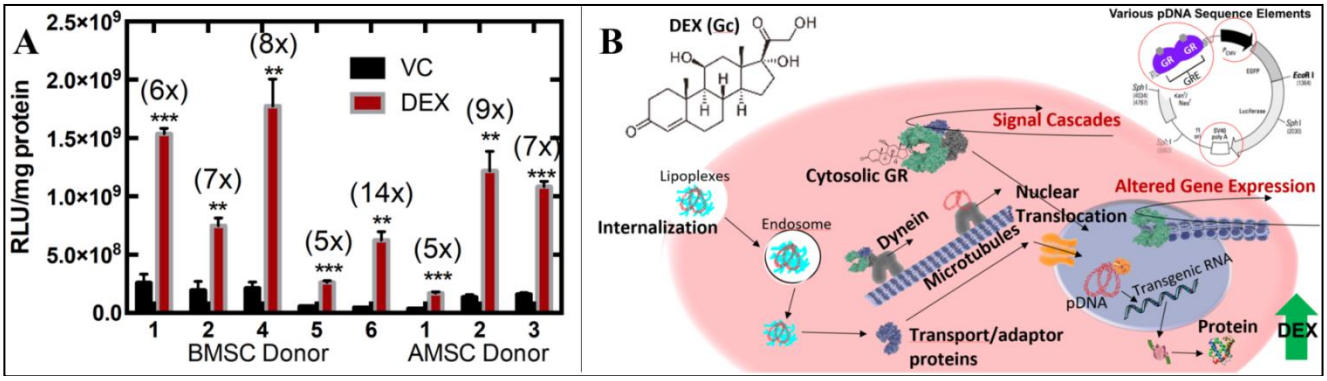

Figure 1-- A) BMSC and AMSC luciferase transgene expression treated with EtOH vehicle control (VC) or 150 nM DEX B) Summary of investigations into molecular mechanisms of hMSC transfection and DEX priming (e.g. pDNA internalization and intracellular transport, endogenous signaling and gene expression, pDNA sequence interactions, transgene transcription and translation).

Results & Discussion: DEX significantly increased transgene expression over VC in all BMSCs and AMSCs, derived from multiple human donors (i.e. 5- to 14-fold) (Fig. 1A). Enhanced non-viral gene delivery by Gc treatment was mediated by binding of the cytosolic glucocorticoid receptor (GR) (Fig. 1B), as treatment with other steroids resulted in no enhancement. Inhibiting nuclear import pathways dramatically decreased transgene expression in the absence of DEX, but did not decrease the fold-change enhancement by DEX priming. Cytoplasmic transport inhibition modestly decreased fold-change enhancement by DEX priming, possibly by inhibiting GR or pDNA trafficking. Enhancement by DEX treatment occurs regardless of any pDNA sequence element changes. DEX priming did not increase cellular or nuclear internalization of pDNA, or increase transgene RNA expression. DEX treatment modulated endogenous gene expression to increase hMSC total protein synthesis after transfection, contributing to increased transgenic protein production. Studying mechanisms of priming cells for transfection provides greater understanding of molecular parameters important to gene delivery and characterizes practical protocols to improve delivery of clinically-relevant transgenes.

References: [1] D'Souza et al, BMC Med, 2015, 13, 186. [2] Nayerossadat et al, Adv Biomed Res, 2012, 1, 27. [3] Hoare et al, J Gene Med, 2010, 12, 207. [4] Plautz et al, Mol Ther, 19, 2144. [5] Nguyen et al, Bioeng Transl Med, 2016, 1, 123. [6] Kelly et al, Mol Ther, 2016, 24, 331.