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Intercellular Communication Via Gap Junctions Influences Stem Cell Therapy

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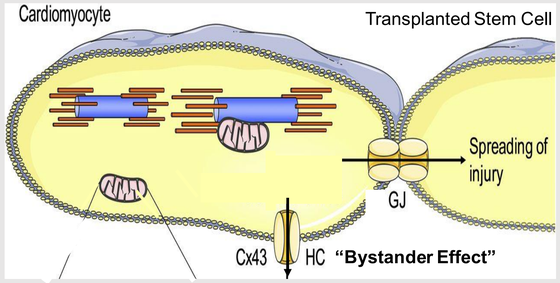
Introduction

Adult somatic stem cell therapy can be beneficial for cardiac muscle recovery following injury to the heart. However, a common problem with various approaches to this therapy has been the early (< 24 hrs) loss of a large percentage of the implanted stem cells, which decreases their therapeutic potential. We have demonstrated that cell coupling occurs between stem and cardiac cells through Cx43 gap junctions.Thus, we hypothesize that gap junctions may act as conduits for molecular apoptotic signals from injured cardiac muscle cells that impair the viability of implanted stem cells ('Bystander Effect'). In our initial experiments, we will use a fluorescently-tagged RNAi control oligo to transfect mouse bone marrow-derived mesenchymal stem cells (mBM-MSCs) and measure transfection efficiency with fluorescence-activated cell sorting (FACS). Additional studies will determine mBM-MSC Cx43 protein expression and the level of Cx43 protein down-regulation in the mBM-MSCs.



Adapted and revised from Leybaert et al. Pharmacol Rev 2017;69:396-478

Figure 1. Proposed role of Cx43 in cell-to-cell coupling (bystander effect) between cardiac myocytes and adult bone marrow-derived mesenchymal stem cells

Previously Published Data from our Laboratory

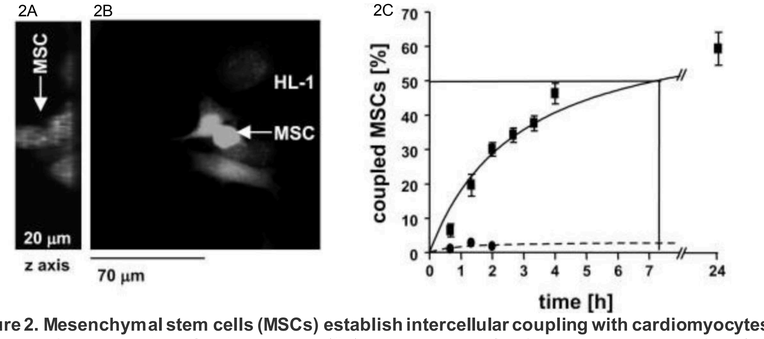


Figure 2. Mesenchymal stem cells (MSCs) establish intercellular coupling with cardiomyocytes. Three-dimensional reconstruction of z-stack images (*2A*) obtained by confocal microscopy shows calcein/AM-loaded MSCs on top of an HL-1 monolayer (*2B*). Dye transfer through gap junction channels was determined between the 2 cell types. Heterocellular coupling between MSCs and HL-1 cells occurs rapidly over the first hours of coculture (*2C;* solid line) and is suppressed by the gap junction inhibitor, carbenoxolone (*2C*; dotted line).
Am J Physiol Heart Circ Physiol. 2013 Feb 15; 304(4): H600–H609.. doi: 10.1152/ajpheart.00533.2012

Objectives

- The goal of this project is to transiently knock-down Cx43 within cultured mBM-MSCs
- Use an indicator of lipid-mediated transfection efficiency for RNAi experiments (BLOCK-IT ™ Red Fluorescent Control Oligo)
- Optimize conditions for maximum transfection efficiency (cell density, reagent concentrations)
- Western Blot analysis of mBM-MSCs for Cx43 protein expression in non-transfected (without siRNA) and transfected (with siRNA) cells

Cell Culture and Transfection Methods

Bone Marrow-Derived Mouse Mesenchymal Stem Cells (mBM-MSC)
mBM-MSCs were obtained from the Phinney laboratory (UF, Scripps). Cells were derived from femurs and tibias of adult FVB/n mice. The mBM-MSCs were negative for CD11b, CD34, CD45, SSEA1, and CD106. The cells were positive for SCA1, CD29, CD44, CD73, and CD105. Once thawed, cells were dispersed into 6-well plates at a density of 50,000 cells/well and cultured in 5% O_2, 5% CO_2, and 90% N_2 to achieve 80% confluency.

Lipofectamine RNAiMax Transfection Protocol
The BLOCK-iT™ Alexa Fluor™ Red Fluorescent Control is an indicator of lipid-mediated transfection efficiency for RNAi experiments. The sequence of the BLOCK-iT™ Alexa Fluor™ Red Fluorescent Control is not homologous to any known gene, preventing induction of nonspecific cellular events caused by introduction of the duplex into cells.

Methods (Continued)

Figure 3. Lipofectamine^R + RNAiMax Transfection Protocol with mBM-MSCs

Figure 3A.

Steps
Seed cells to be 60-80% confluent at transfection
Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium
Dilute siRNA in Opti-MEM® Medium
Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)
Incubate
Add siRNA-lipid complex to cells

Figure 3B.

Component	6-well
Adherent cells	0.25–1 × 10^6
Opti-MEM® Medium	150 µL
Lipofectamine® RNAiMAX Reagent	9 µL
Opti-MEM® Medium	150 µL
siRNA (10 µM)	3 µL (30 pmol)
Diluted siRNA	150 µL
Diluted Lipofectamine® RNAiMAX Reagent	150 µL

Figure 3C.

Component	6-well
siRNA-lipid complex per well	250 µL
Final siRNA used per well	25 pmol
Final Lipofectamine® RNAiMAX used per well	7.5 µL

Results

Figure 4. Lipofectamine^R + RNAiMax Transfection Experiment. The graphs below depict FACS analysis for one lipofectamine + RNAiMax (Alexafluor) and two control cell cultures. The right-most portion of the figures represent the percentage of cells that expressed the highest levels of a red fluorescent signal indicating successful transfection with the RNA. Approximately 14% of the Lipofectamine^R + RNAiMax cells displayed high levels of fluorescent activity while the control cultures expressed only baseline levels of red fluorescence.

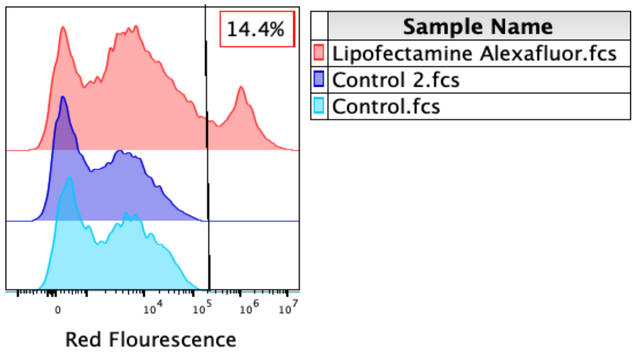
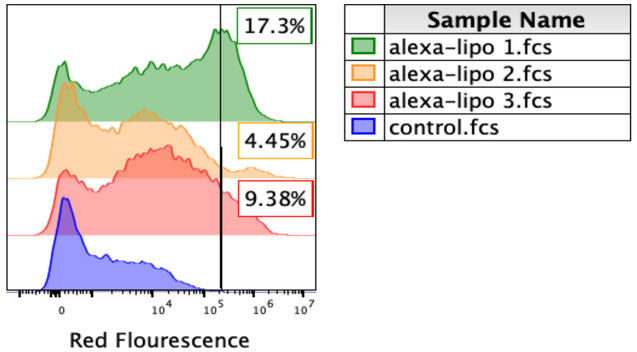
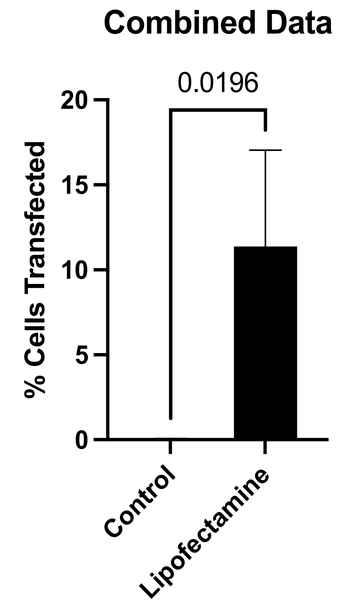


Figure 5. Lipofectamine^R + RNAiMax Transfection Experiment. The graphs below depict fluorescent activated cell sorting (FACS) analysis for three lipofectamine + RNAiMax and one control cell culture. The right-most portion of the figures represent the percentage of cells that expressed the highest levels of a red fluorescent signal indicating successful transfection with the RNA. In the three separate experiments, 17.3%, 4.45%, and 9.38% of cells exhibit high fluorescence activity compared to baseline activity in the control.



Results (continued)

Figure 6. Combined Lipofectamine^R + RNAiMax Transfection Results. Aggregate data of the four lipofectamine + RNA and the three control transfections (11.38%; p<0.0196).



Conclusions

- The lipid-mediated methodology used in our mBM-MSC cell culture experiments was successful in transfecting cells with the fluorescently-tagged RNA, but the transfection efficiency was low.
- Additional experiments with varying concentrations of the lipofectamine and RNA reagents will be conducted to determine whether this method will be effective in transfecting mBM-MSCs. Other non-lipid-based methods (e.g. electroporation) have also been used to transfect adult somatic stem cells.
- Once we have achieved an efficiency of > 50%, we will transiently transfect the stem cells with a commercially available siRNA to knock-down expression of Cx43. Reduced protein expression will be confirmed by immunoblot analysis. These studies will allow us to test our hypothesis that gap junctions act as a conduit for apoptotic signals between ischemic cardiocytes and implanted stem cells.

Acknowledgements

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