Paracrine engineering of cardiac stem cells using non-viral somatic gene transfer

Tatsiana Yeuchyk, Darryl R. Davis

Cardiac Translational Research Lab, University of Ottawa Heart Institute, Ottawa, Ontario

Introduction

Heart failure afflicts over 500,000 Canadians and, despite innovations in other realms of cardiovascular care, the 1-year mortality attributable to heart failure has only decreased from 27 to 25% over the last 10 years. These findings rationalize searching for novel means of repairing damaged hearts. As such, treatments using a patient's own resident cardiac stem cells (CSCs) are being actively investigated and have shown promising abilities to rescue, repair and re-vascularize damaged cardiac tissue.

Given that the majority of CSC-mediated repair is mediated through indirect cytokine/exosome stimulation of endogenous repair, our lab has shown that overexpression of pro-healing cytokines (i.e., insulin-like growth factor 1 (IGF-1) or stromal cell-derived factor (SDF-1α)) by human CSCs increases transplanted cell survival and therapeutic regeneration. Genetic alterations of CSCs have been traditionally accomplished using lentivirus but the risk of random insertion mutagenesis obviates straightforward clinical translation.

Therefore, we explored the ability of DNA minicircle and direct mRNA transfection to promote the expression of foreign transcripts to enable genetic engineering.

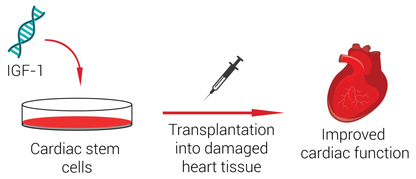

Figure 1. Flowchart of CSC-mediated therapy for heart failure with overexpressed IGF-1.

Study Aim: To compare the ability and durability of DNA minicircle and mRNA transfection of CSCs.

Hypothesis: Although both DNA minicircles and mRNA provide robust expression of foreign transgenes, DNA minicircles will provide sustained expression after transfection.

Methods

Minicircle vectors were prepared containing either green fluorescent protein (GFP) or human IGF-1. Minicircle preparation followed the protocol of the Systems Biosciences MC-Easy minicircle DNA production kit. Plasmids were grown in *E.coli* ZYCY10P3S2T strain bacteria and isolated using a Maxiprep kit.

Mouse CSCs were grown from mouse cardiac tissue biopsies in cell culture for up to 4 weeks. CSCs were transfected with minicircles using lipofectamine or electroporation (Invitrogen Neon Transfection System). A dosage of 5 µg of minicircles per 100,000 cells was used. For conditioned media experiments, cells were cultured for 48 hours in hypoxic (1% oxygen) low-serum (1% serum) media to replicate the harsh infarct environment.

GFP expression was quantified using flow cytometry. IGF-1 expression was analyzed using an enzyme-linked immunosorbent assay (ELISA) specific to human IGF-1.

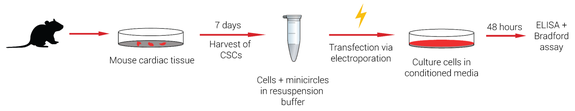

Figure 2. Flowchart of minicircle experimental methodology.

TriLink Biotechnologies GFP mRNA was transfected into CSCs at varying doses using Lipofectamine MessengerMax vesicles with subsequent GFP expression evaluated using flow cytometry.

Figure 3. Flowchart of mRNA experimental methodology.

Results

Minicircle Transfection Method: Results

Figure 4. Results of agarose gel electrophoresis comparing newly-made IGF-1 minicircles (N) to a known sample of IGF-1 minicircles (K). Aligning bands (arrows) confirm the presence of IGF-1.

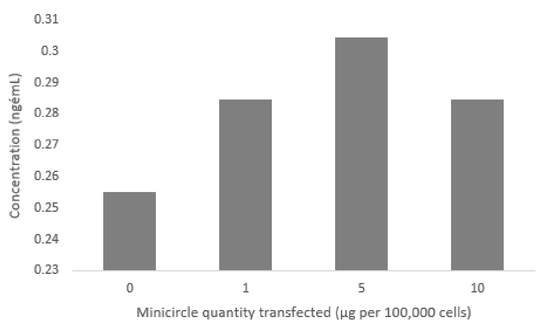

Figure 5. Results of IGF-1 ELISA following Human Embryonic Kidney 298 cell transfection with IGF-1 minicircles using Lipofectamine 2000. These results show that a minicircle dosage of 5 µg per 100,000 cells promotes the highest rate of IGF-1 overexpression, compared with control.

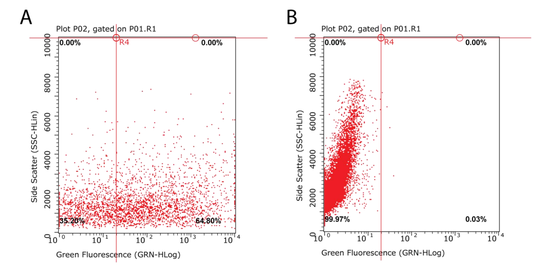

Figure 6. DNA minicircle transfection promotes GFP expression in CSCs. (A) Flow cytometry demonstrating GFP expression 3 days after GFP minicircle transfection of mouse CSCs (n=1), compared with control (B). The results showed a 64.8% expression of GFP in transfected cells. However, the electroporation process also resulted in 80% cell death.

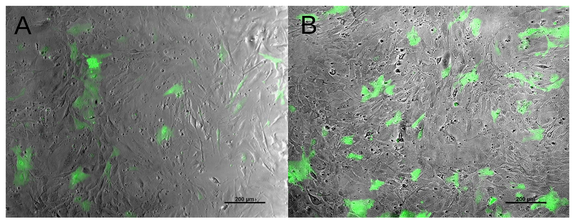

Figure 7. Representative images of GFP-transfected mouse CSCs taken 3 days after transfection. These images correspond to the results of Figure 4, demonstrating a 64.8% transfection efficiency using minicircles.

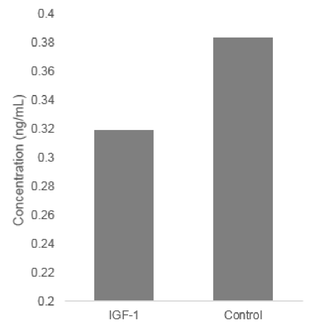

Figure 8. DNA IGF-1 ELISA results 3 days following IGF-1 minicircle transfection of mouse CSCs, compared with control. A dosage of 5 µg of minicircles per 100,000 cells was used. The control sample exhibits a greater IGF-1 expression than the sample with overexpressed IGF-1.

mRNA Transfection Method: Results

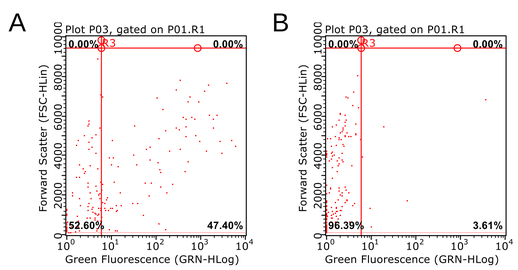

Figure 9. mRNA Lipofectamine MessengerMax-mediated transfection promotes GFP expression. (A) Flow cytometry results show 47% GFP expression in mouse CSCs transfected with GFP mRNA (n=1), 3 days after transfection, compared with control (B).

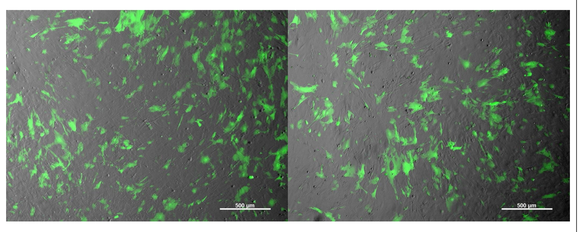

Figure 10. Representative images of mouse CSCs transfected with GFP mRNA using Lipofectamine MessengerMax vesicles. Images were taken 3 days after transfection. These cells correspond to the results of Figure 7, showing a 47% rate of transfection efficiency using GFP mRNA.

Conclusion

Compared to established stable immortalized cell lines (i.e., HEK cells), CSCs are remarkably resistant to transfection- thus rationalizing the use of electroporation. Electroporation promoted GFP minicircle uptake but at a very high rate of cell death (~80%).

Surprisingly, IGF-1 DNA minicircles did not promote the IGF-1 expression by CSCs. Using minicircles did not prove to be an effective CSC transfection strategy for IGF-1; refuting our initial hypothesis.

mRNA transfection provided robust GFP expression with little cell death. Future work will focus on designing IGF-1 mRNA expression cassettes. If this method proves to be successful, it will enable straightforward clinical translation of genetic engineering to boost the regenerative potential of CSCs.

References

1. Jackson et al., *J Am Heart Assoc*. 2015;4:e002104.
2. Tilokee et al., *Stem Cells*. 2016;34(7):1826-35.
3. Davis et al. *Expert Opin. Biol. Ther.* 2011;11(4):489-508.
4. Bergmann et al, *Science*. 2009;101(15):98-102.

Acknowledgements

Contact: Tatsiana Yeuchyk
TYeuchyk@ottawaheart.ca

Many thanks to Dr. Darryl Davis, Ghazaleh Rafatian, Bin Ye, and the rest of the Davis lab.

Support for this project was provided by the Canadian Institutes of Heath Research, the Ontario Heart and Stroke Foundation, and the Undergraduate Research Opportunity Program.