mRNA-based Methods for Cell Fate Manipulation

Chenmei Luo1, Alice Chen2, Kevin Yi1, Shuya Zhai1, Dirk Hockemeyer3, Johanna Goldmann3, Brad Hamilton1, Kerry Mahon1

1Stemgent, One Kendall Square, Cambridge, MA 02139
2Stemgent, 10575 Roselle St., San Diego, CA 92121
3Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142

*Corresponding Author: kerry.mahon@stemgent.com

www.stemgent.com

INTRODUCTION

Several recent publications have demonstrated the potential of mRNA to reprogram somatic cells to induced pluripotent stem (iPS) cells, albeit with varying levels of efficiency, reproducibility and colony stability.2,3,16,20, Myogenin and eomesoderm specific markers MF-20, Myogenein and Desmin.
The ability to manipulate cell fate has significant potential to provide useful tools in regenerative medicine and drug screening applications. Current methods primarily rely on media additives or viral induction to induce the transformation of the cell type of interest. However, these methods are limited by a number of factors, most notably low efficiency and genomic insertion. More robust cell fate manipulation methods would lead to a higher yield of target cell types; non-viral methods would lead to more clinically relevant cells. Here, we present results demonstrating the utility of mRNA-based methods for the generation of induced pluripotent stem (iPS) cells and the transdifferentiation of fibroblasts to myoblasts. We also introduce two new tools that enable these mRNA methods to be successful: 1) Stemgent Pluriton™ Reprogramming Medium, which allows for efficient cellular reprogramming of human fibroblasts to iPS cells, and 2) Stemfect RNA Transfection kit, a new lipid-based formulation for highly efficient mRNA delivery with translatable control over protein expression. These methodologies allow for the reproducible and efficient alteration of cell fate without insertional mutagenesis.

OBJECTIVES

The objective of the study was to evaluate the performance of the mRNA-based reprogramming methods, including the use of the Stemgent Pluriton™ Reprogramming Medium and Stemfect RNA Transfection kit, and to compare these methods to previously published results. The study also aimed to evaluate the potential of the mRNA-based methods for transdifferentiation of fibroblasts to myoblasts.

METHODS

Mammalian fibroblasts were obtained from human foreskin (BJ) and mouse tail (C3H) tissues. The BJ fibroblasts were used for all reprogramming experiments, while the C3H fibroblasts were used for transdifferentiation experiments. The BJ fibroblasts were seeded in a 24-well format and transduced with 125, 250 or 500 ng of eGFP mRNA. The cells were cultured at 37°C / 5% CO2 and analyzed at 18-24 hrs. post transfection. DAPI staining was used to visualize the nucleus, and the mean fluorescence intensity and transfection efficiency as determined by flow cytometry. 

RESULTS

Figure 1. Expression of reprogramming factors using mRNA

BJ fibroblasts were transduced with mRNA encoding the transcription factors OCT4, SOX2, KLF4, c-MYC, LIN-28 or nGFP and fixed with 4% paraformaldehyde after incubation for 18 hours. The cells were then stained with the appropriate antibody and DAPI for visualization. Merged images are shown.

Figure 2. Modified protocol for mRNA reprogramming of human fibroblasts.

A) Timeline for the reprogramming of human fibroblasts by repeated mRNA transfection using Stemgent’s optimized no-slip mRNA Reprogramming protocol. B) Primary culture morphology progression (Phase - 10x) during the emergence of a primary iPS cell colony resulting from the reprogramming of Parkinson’s disease patient dermal fibroblasts with Stemgent mRNA Reprogramming Factor Set in combination with Pluriton™ mRNA Reprogramming Medium. Last panel: Day 20 primary iPS colony identification using the Stemgent StainAlive™ Tra-1-81 antibody. C) Key protocol adjustments, optimizations, by Stemgent, to the mRNA reprogramming protocol as published in Cell Stem Cell. For the full protocol, please visit www.stemgent.com.

Figure 3. Characterization of BJ mRNA iPS cell lines.

A) Pluripotency immunocytochemistry for mRNA iPS cell line (c) derived from BJ fibroblasts. Nuclear pluripotency for  Nanog, Oct4, and Rex1. B) Relative endogenous mRNA expression level comparison for Nanog, Rex1, and Oct4, qRT-PCR calculations and data normalization against HuES9 p62 human ES positive control cell line. C) Bisulphite sequencing analysis of Oct promoter for BJ mRNA iPS cell lines c3 p11 and c8 p14. Lines were also found to have a normal karyotype (data not shown). D) Hematoxylin and eosin staining and analysis of a teratoma resulting from the injection of the BJ mRNA iPS cell line into SCID mice. E) Immunocytochemical analysis of neural progenitor cells (NPCs) derived from BJ mRNA iPS cell line stained for Pax6 (green) and Nestin (red) expression.

Figure 4. Comparison of Pluriton™ mRNA Reprogramming Medium and other common human ES culture media for iPS cell colony generation during mRNA based reprogramming.

Different target cell densities (50k, 25k, or 10k per well) of BJ fibroblasts were plated on human fibroblast feeder (250K/well) in a single well of 6-well plate. Each condition was incubated at 5% O2 and transfected with 1.2 ug of mRNA reprogramming cocktail for 16 consecutive days without enzymatic passaging. Primary cultures were assayed with the Stemgent StainAlive™ Tra-1-81 antibody (1:100) on Day 19 and Tra-1-81 positive colonies were counted using a fluorescent microscope. Each bar in the graph is individually labeled with the number of iPSC colonies generated.

REFERENCES


SUMMARY

1. Developed functionally validated mRNA reprogramming protocol
   - Integration-free
   - Increased reprogramming efficiency
   - Faster iPS cell colony generation – colony isolation within 3 weeks
   - Demonstrated on diseased patient fibroblasts
   - Validated at 5% O2 and 21% O2

2. Developed novel, xeno-free media essential for mRNA reprogramming success

3. Demonstrated the utility of mRNA for the transdifferentiation of human and mouse fibroblasts to myoblasts

4. Developed highly efficient RNA transfection reagent for broadened cell application