A novel four transfection protocol for deriving iPSC cell lines from human blood-derived endothelial progenitor cells (EPCs) and adult human dermal fibroblasts using a cocktail of non-modified reprogramming and immune evasion mRNAs

Sarah Eminli-Melissen1, Jung-Ill Moon1, Kevin Y1, Marco Polegaonar2, Tim Beilstein2, Ugur Sahin2, *Brad Hamilton3

1 Stemgent, 51 Moulton St, Cambridge, MA 02139, 2 TRON - Translational Oncology at University Medical Center Mainz, Mainz, Germany

*Corresponding Author: brad.hamilton@stemgent.com

Introduction

Peripheral blood provides easy access to adult human cell types for reprogramming purposes. In late 2012, two groups demonstrated the effective isolation, expansion, and subsequent generation of reprogrammed iPSC cell lines from endothelial progenitor cells (EPCs) derived from human peripheral blood. While circulating EPCs are a rare population of cells in blood, we have effectively isolated and established multiple adherent and expandable primary EPCs from fresh and frozen human peripheral and cord blood samples, some from as little as 1 x 10^3 mononuclear cells (MNCs) (Figure 1). The EPCs adhere to culture surfaces and exhibit the features of endothelial cell identity, including proliferation capacity when cultured in complete media. Here we present data demonstrating the unique combined application of non-modified reprogramming mRNA (Oct4, Sox2, Klf4, c-Myc, Nanog), and Lin28B and immune evasion mRNAs (E3, K3, and B16) derived from Vaccinia virus with reprogramming-associated mature double stranded miRNA (302/367 cluster) for the cellular reprogramming of human EPC lines derived from peripheral blood and cord blood into a stable, pluripotent and clinically relevant iPSC lines (Figure 4). Inclusion of the E3, K3, and B16 immune evasion mRNAs in the RNA transfection cocktail eliminates the need to supplement cell culture medium with recombinant B16 protein during the reprogramming process. Additionally, adjusting reprogramming factor mRNA stoichiometries to elevate Oct4 transcript levels in the RNA cocktail resulted in a four transfection protocol (Figure 3) that efficiently generated TRA-1-81 positive iPSC cell colonies from both human blood-derived EPCs (≤0.02%) and MNCs (≤0.04%) within 10 days. Moreover, by increasing the total number of RNA transfections from 4 to 6, the reprogramming efficiency for EPCs increased to ≤0.25% while MNCs increased to ≤0.60%. Equivalent application of the non-modified reprogramming RNA technology to human fibroblasts resulted in a four transfection protocol (Figure 5) where lower fibroblast seeding densities, exclusive use of NutriStem™ Culture Medium, and elevated Oct4 transcript levels proved beneficial for increasing the reprogramming efficiencies of both neonatal (0.5%) and adult dermal fibroblasts (1.6%) (Table 1). The effective application of this non-modified RNA platform for efficient cellular reprogramming of multiple human cell types with only 4-6 repeat transfections demonstrates both the flexibility and robustness of the system, potentially opening the door to the use of non-modified RNA in cell fate conversions to non-proliferative populations of cells which appear refractory to existing modified RNA technologies.

Efficient delivery of non-modified GFP mRNA to EPCs

Protocol timeline and morphology progression for reprogramming EPCs into iPSC cells using a cocktail of non-modified RNAs

Characterization of EPC-ips cell line derived using a cocktail of non-modified RNAs

Protocol timeline for reprogramming human fibroblasts with a cocktail of non-modified RNAs

Establishment and immunochemistry characterization of EPCs derived from peripheral blood

References

Summary

• Generation of stable, pluripotent EPC-ips cell lines using a non-modified RNA
  • Only 4-6 RNA cocktail transfections
  • Two weeks for iPSC colony establishment (No screening required)
  • Protocol does not require iPS8 protein
  • Generation of stable, pluripotent adult and neonatal fibroblast-ips cell lines using a non-modified RNA
  • Only 4 RNA cocktail transfections
  • Ten days for iPSC colony establishment (No screening required)
  • Protocol does not require iPS8 protein
  • Establishment of fibroblast-ips cells in Kao-free NutriStem XF/F Culture Medium
  • Simple, efficient primary EPC line establishment
  • Human peripheral and cord blood samples
  • Fresh or frozen samples
  • Two week primary culture establishment

TABLE 1. Reprogramming efficiencies for non-modified RNA reprogramming of human fibroblasts into iPSC cells. Adult human fibroblasts and neonatal foreskin fibroblasts (NutriStem) were plated at 25K, 50K, or 100K on CompuGene 6-well plates. Each reprogramming well was transfected for 4 consecutive days with non-modified reprogramming mRNA (302/367), non-modified interferon-alpha miRNA (E3), and microRNA (302/367) reprogramming cocktail. Daily RNA transfusions (1.5 μg total). Reprogramming culture as well as maintenance of iPSC were carried out in NutriStem XF/F Culture Medium or traditional from NutriStem to human foreskin fibroblast conditioned NutriStem on day 5.


Corning and Stemgent are registered trademarks of Corning Incorporated. NutriStem is a registered trademark of Stembook Industries. Unless otherwise noted, Sigma, DEStem, Inc., and RepliCELL, Inc., logo, RepliCell Biotechnologies Ltd, and RepliCell Biotechnologies Ltd. logo, and all other trademarks are the property of RepliCell, Inc. © 2015 RepliCell, Inc. All rights reserved.