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Effect of Replacement of C-Myc with L-Myc on Sendai-based Reprogramming Method

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ABSTRACT

As induced pluripotent stem cells (iPSCs) move toward clinical applications, it is necessary to minimize oncogenic risk in order to ensure safe transplantation of the cells. Previously used reprogramming protocols utilize the transcription factor c-Myc that is a known proto-oncogene associated with different cancers. Another transcription factor in the Myc family, L-Myc has been found to have low transformation activity and is less frequently observed in human cancers.
The aim of this study is to compare the reprogramming efficiencies of c-Myc and L-Myc in a variety of different blood cell types. Peripheral blood mononuclear cells (PBMCs), CD34+ cells, and T-cells were cultured using a xeno-free workflow and reprogrammed using both a Sendai virus containing c-Myc and L-Myc. The number of AP+ colonies and relative reprogramming efficiencies were determined by terminal alkaline phosphatase staining. Statistical data analysis was carried out using JMP. Results indicate that although for some cell types L-Myc yielded lower reprogramming efficiencies, the colonies obtained had the same morphology as c-Myc colonies. The ability of L-Myc to reprogram blood cells into iPSCs while reducing the oncogenic risk makes it a good candidate for the generation of clinical-grade iPSCs.

INTRODUCTION

Since iPSC generation first emerged in 2006, iPSCs have been acknowledged for their potential use in regenerative medicine (1). The ability to make patient-specific pluripotent stem cells that can be differentiated into any cell type gives researchers the ability to treat disease in a new way. In 2014, the first clinical trials using iPSC-derived cells began in Japan where iPSC-derived retinal pigment epithelial cells were used to treat macular degeneration (2). Clinical trials using iPSCs have not yet begun in the United States, but many are in the works and will be moving toward clinical trials in the coming years. As iPSCs move closer toward clinical applications, steps must be taken to ensure they are safe for use in patients. Typical reprogramming protocols utilize the 4 transcription factors Oct4, Sox2, Klf4, and c-Myc. Of these factors, c-Myc is the most concerning as it is a known proto-oncogene and has been found to be abnormally expressed in over 50% of human cancers (3). The goal of this study was to determine if L-Myc could serve as a suitable replacement for c-Myc. L-Myc was chosen as it is from the same family and has similar pluripotency-inducing properties as well as having lower transformation activity (4). This study utilizes blood cells for reprogramming as they are more easily and less invasively obtained than fibroblasts from skin biopsies.

MATERIALS AND METHODS

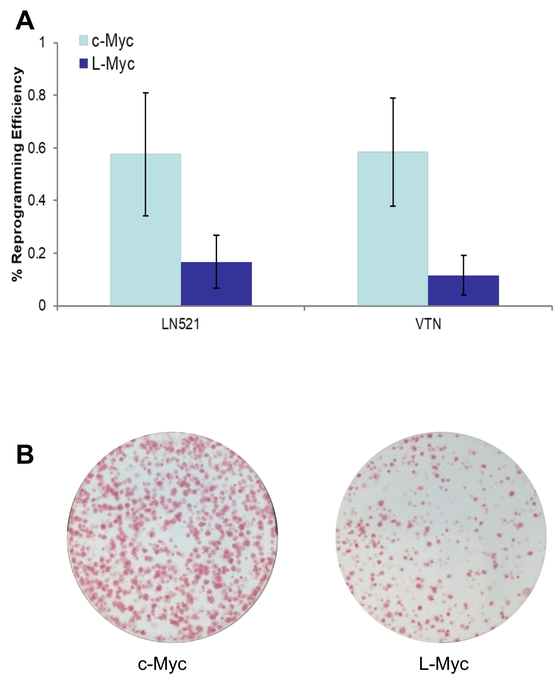
PBMC Reprogramming
PBMCs from two different donors were cultured in StemPro™34 SFM plus SCF (100ng/ml), FLT3 (100ng/ml), IL-3 (20ng/ml), IL-6 (20ng/ml) for 4 days post thaw. Cells were then either transduced with the CytoTune™-iPS 2.0 Sendai Reprogramming Kit containing KOS (Klf4, Oct4, Sox2), Klf4, and c-Myc reprogramming factors, or the CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming kit containing KOS (Klf4, Oct4, Sox2), Klf4, and L-Myc reprogramming factors. Virus was removed after 24 hours and cells were plated on rh-Vitronectin (VTN) or rh-Laminin521 (LN521) 3 days after transduction. At 7 days after transduction medium was changed to Essential 8™ Medium. At 14 days after transduction, cells were terminally stained using the Vector® Red Alkaline Phosphatase (AP) stain.

CD34+ Reprogramming
StemPro™ CD34+ cells were cultured in StemPro™34 SFM plus SCF (100ng/ml), IL-3 (50ng/ml), GM-CSF (25ng/ml) for 3 days post thaw. Cells were transduced, plated, and stained as described above for PBMCs.

T-Cell Reprogramming
T-Cells were isolated from PBMCs from two donors using Dynabeads™ FlowComp™ Human CD3 Kit according to manufacturer protocol. Cells were cultured in OpTmizer™ SFM plus 100U/ml IL-2 and CD3/CD28 Dynabeads™ for 7 days after isolation. Cells were then removed from beads and plated onto CD3-antibody coated plates. Cells were transduced as described above for PBMCs. At 48 hours after transduction, cells were plated on either VTN or LN521 in OpTmizer plus 100U/ml IL-2, and medium was changed to Essential 8™ Medium 24 hours later. At 14 days after transduction, cells were terminally stained using the Vector® Red Alkaline Phosphatase stain.

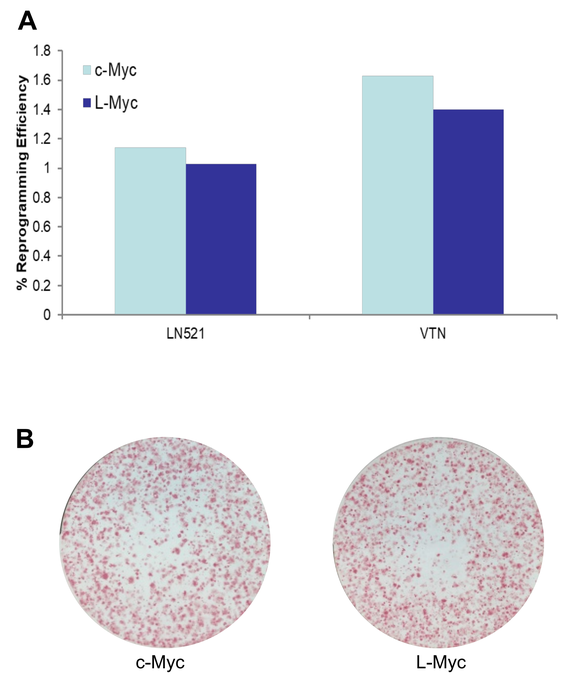
RESULTS

Figure 1: PBMCs reprogrammed with L-Myc yielded lower reprogramming efficiencies.



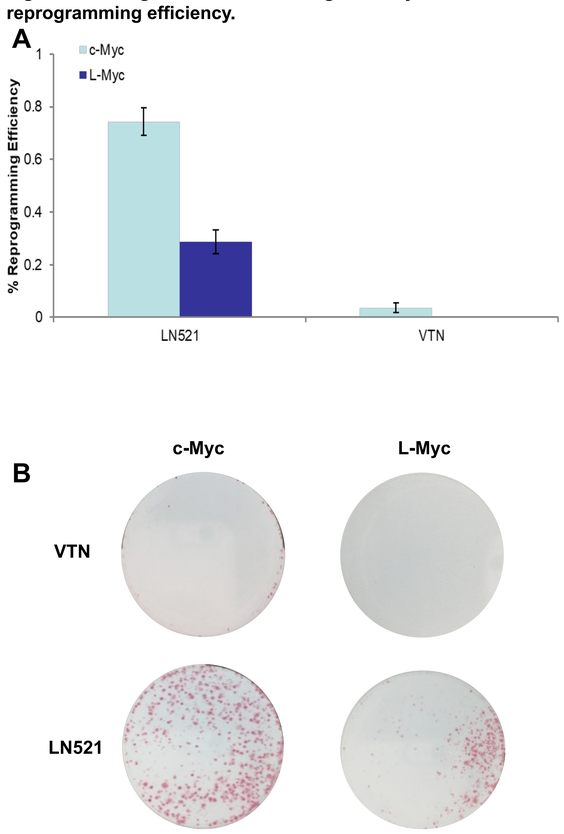
(A) PBMCs from two donors were cultured for 4 days post thaw, then 300,000 cells were reprogrammed using each CytoTune™ kit as described in methods. After the reprogramming process was complete cells were AP stained and the number of AP positive colonies and relative reprogramming efficiencies were determined. The graph is shown as a mean of the two donors. **(B)** Representative images of AP stained colonies on LN521 are shown.

Figure 2: CD34+ cells reprogrammed with c-Myc and L-Myc yielded similar reprogramming efficiencies.



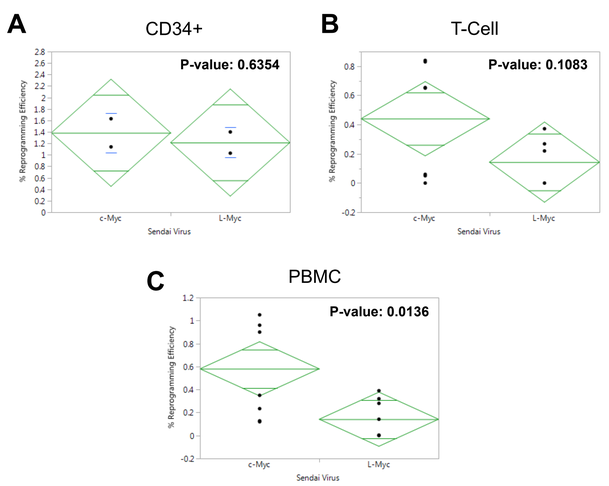
(A) StemPro CD34+ cells were cultured for 3 days post thaw, then 250,000 cells were reprogrammed using each CytoTune™ kit as decribed in methods. After the reprogramming process was complete cells were AP stained and the number of AP positive colonies and relative reprogramming efficiencies were determined. The graph is shown as a mean of the two donors. **(B)** Representative images of AP stained colonies on LN521 are shown.

Figure 3: Plating T-Cells on LN521 significantly increases reprogramming efficiency.



(A) T-Cells were isolated from two different PBMC donors using the Dynabeads™ FlowComp™ Human CD3 Kit, cultured with CD3/CD28 Dynabeads™ for 7 days, then 300,000 cells were reprogrammed using both CytoTune™ kits are described in methods. After the reprogramming process was complete cells were AP stained and the number of AP positive colonies and relative reprogramming efficiencies were determined. The graph is shown as a mean of the two donors. **(B)** Representative images of AP stained colonies are shown.

Figure 4: CD34+ cells and T-Cells do not show statistically significant difference between the reprogramming efficiencies of c-Myc and L-Myc.



(A) Data analysis was carried out using JMP software. Oneway ANOVA gave a p-value of 0.6354 for CD34+ cells indicating that there is no statistically significant difference between the reprogramming efficiencies of c-Myc and L-Myc. **(B)** Oneway ANOVA gave a p-value of 0.1083 for T-Cells indicating that there is no statistically significant difference between the reprogramming efficiencies of c-Myc and L-Myc. **(C)** Oneway ANOVA gave a p-value of 0.0136 for PBMCs, indicating that there is a statistically significant difference between the reprogramming efficiencies of c-Myc and L-Myc, L-Myc yielding consistently lower reprogramming efficiencies.

CONCLUSIONS

LN521 and VTN were compared as feeder-free matrices for reprogramming. Plating T-Cells on LN521 significantly increased reprogramming efficiency as T-Cells did not reprogram well on VTN. However, both PBMCs and CD34+ cells yielded similar reprogramming efficiencies when plated on either VTN or LN521.

For the CD34+ reprogramming, the sample size was small compared to PBMCs and T-Cells, therefore definitive conclusions cannot be drawn.

Significant variability in reprogramming efficiencies was observed between cells from different donors, one donor yielding consistently lower reprogramming efficiencies for both PBMCs and the T-Cells isolated from PBMCs.

Although L-Myc yielded lower reprogramming efficiencies overall, its ability to reprogram blood cells into iPSCs while reducing the oncogenic risk makes it a good candidate for the generation of clinical-grade iPSCs.

For future studies, protocols can be optimized in order to maximize the reprogramming efficiency of L-Myc.

REFERENCES

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76. Epub 2006 Aug 10. PubMed PMID: 16904174.
2. Kanemura H, Go MJ, Shikamura M, Nishishita N, Sakai N, Kamao H, Mandai M, Morinaga C, Takahashi M, Kawamata S. Tumorigenicity studies of induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) for the treatment of age-related macular degeneration. PLoS One. 2014 Jan 14;9(1):e85336. doi: 10.1371/journal.pone.0085336. eCollection 2014. PubMed PMID: 24454843; PubMed Central PMCID: PMC3891869.
3. Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka S. Promotion of direct reprogramming by transformation-deficient Myc. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(32):14152-14157. doi:10.1073/pnas.1009374107.
4. Okita K, Yamanaka S. Induced pluripotent stem cells: opportunities and challenges. Philosophical Transactions of the Royal Society B: Biological Sciences. 2011;366(1575):2198-2207. doi:10.1098/rstb.2011.0016.

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