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Factors that enhance Sendai virus mediated somatic reprogramming

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ABSTRACT

Improvements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the generation of patient-derived stem cells from various genetic backgrounds, creating valuable tools in drug discovery and future cell therapies. These advances have increased the demand for iPSC generated from diseased cells and from various somatic cell sources, some of which have an intrinsic resistance to reprogramming. In such cases, factors that overcome reprogramming barriers are necessary to enhance its efficiency.

Sendai viruses are non-integrating RNA viruses that enable a safe, efficient and consistent way to generate iPSC from a wide variety of cell types. Using sendai virus mediated reprogramming, we explored the effect of three different factors that are known to influence reprogramming efficiency. Small molecules such as epigenetic modulators, pathway inhibitors and hypoxia mimics were examined for their effect on the efficiency of fibroblast mediated reprogramming. The class of molecules that had a consistent effect on both feeder-dependent and feeder-free reprogramming were HDAC inhibitors such as sodium butyrate. Additionally, evaluation of different matrices for feeder-free reprogramming indicated Ln521 as a robust component that enabled more efficient reprogramming. Further more, reprogramming in the presence of hypoxia was also shown to be beneficial for higher reprogramming rates. Finally, we expanded our study to see if these enhancers could improve iPSC generation in Xeno-free media conditions. These factors, alone or in combination, can be used to enhance efficiencies of hard to reprogram somatic cell types, enable iPSC generation from diverse somatic cell sources, and potentially in other species.

INTRODUCTION

Since the discovery of iPSC in 2006 by Shinya Yamanaka(1), they have been used as a novel tool in disease modeling. The ability to generate patient specific disease models provides the potential for developing more physiologically relevant models than those previously developed in animals. A key factor in the generation of patient specific iPSC is the ability to efficiently reprogram multiple somatic cell types to an iPSC.

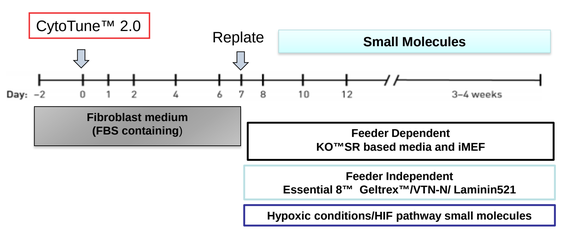
The goal of this study determine factors that may enhance the reprogramming efficiency using CytoTune™-iPS 2.0 Sendai kits to generate iPSCs human fibroblasts . Enhancing the reprogramming efficiency becomes an important factor in successfully generating iPSC from disease patient cells, which may have an intrinsic resistance to being reprogrammed. Here we look at three different methods to enhance reprogramming efficiency.

Previous studies have proposed that HDAC inhibitors, which may alter the epigenome, could play a role in enhancing the ability to reprogram somatic cells into iPSCs (2). Additionally the use of modulators and inhibitors of the TGF beta pathway may also be used to enhance reprogramming efficiencies. Here we investigated the effects of these small molecules on the reprogramming efficiency of CytoTune™. Additionally, we compared multiple matrices for iPSC generation using CytoTune™ in feeder independent conditions. We compared reprogramming efficiencies using the traditional murine derived Geltrex™ basement membrane matrix against the recombinant human proteins rhLaminin-521(3) or Vitronectin (VTN-N). It has also been shown that hypoxic conditions can have a beneficial effect (4) on iPSC generation using retroviral mediated reprogramming. We investigated if any beneficial effects of hypoxic conditions could be observed during CytoTune™ mediated reprogramming. Further, we tested to see if small molecules as enhancers could boost the low efficiencies obtained when trying to reprogram using CytoTune™ Sendai in xeno-free media conditions.

MATERIALS AND METHODS

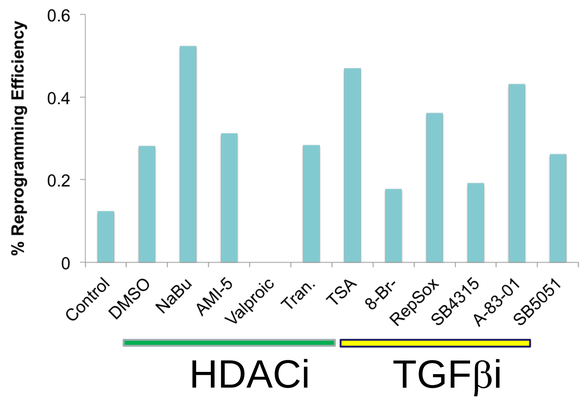
Vector® Red Alkaline Phosphatase kit was obtained from Vector Laboratories. Incucyte® ZOOM Instrument was obtained from Essen Biosciences. BJ HDFn were obtained from ATCC. Small molecules were obtained from various commercial sources. All other reagents were obtained from Thermo Fisher Scientific. Colony counts were performed using Image J software. Percent reprogramming efficiency was calculated as the total number of AP positive colonies relative to the number of cells re-plated at day 7 post transduction.

BJ HDFn fibroblasts were cultured in fibroblast medium (DMEM containing 10% FBS and 1% NEAA). On the day of transduction CytoTune™-iPS 2.0 was added at an MOI of 5-5-3 (KOS, cMyc, and Klf4). Virus was removed the following day. At 7 days post transduction, cells were plated onto either inactivated mouse embryonic fibroblasts (iMEF) for feeder-dependent conditions; or Geltrex™ matrix, Vitronectin (VTN-N) ,or rhLaminin-521 for feeder-independent conditions. The next day medium was changed to human iPSC medium (DMEM/F-12 with 20% KO™SR, 1% NEAA, 55 mM 2- mercaptoethanol, 4 ng/mL bFGF) for feeder-dependent conditions, or Essential 8™ Medium for feeder-independent conditions. Small molecules were added to either media as indicated, from Day 8 to Day 21, and then stained with Vector® Red Alkaline Phosphatase stain for efficiency determination.



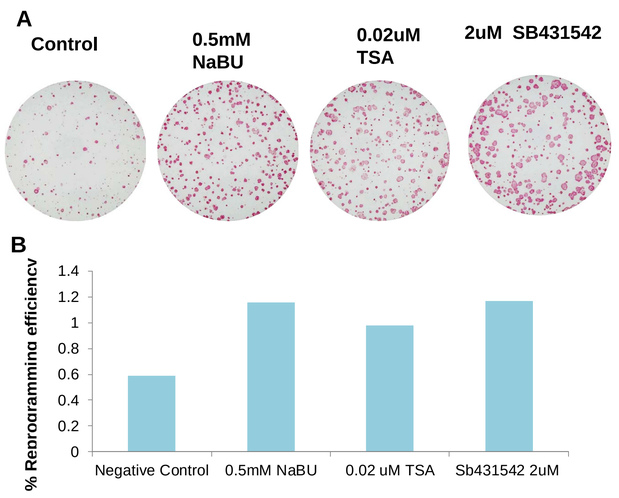
RESULTS

Figure 1. Use of small molecules shows various effects during reprogramming of human fibroblasts



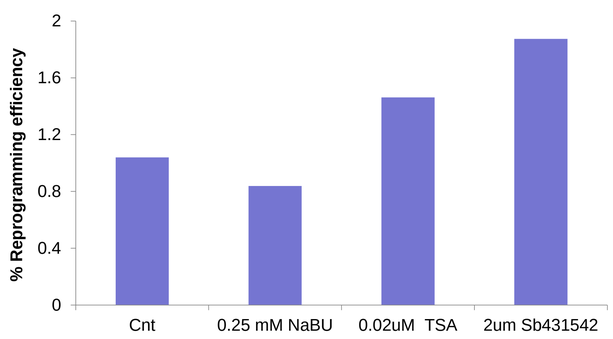
Reprogramming efficiencies from an initial screen of various small molecules. The cells were transduced according to the CytoTune™-iPS 2.0. protocol and at day 7 post transduction, the cells were replated onto iMEF and transferred the following day to KO™SR based media containing various small molecules from day 8 onward. Cells were stained for Alkaline Phosphatase at day 21 post transduction.

Figure 2. Small Molecules selected from initial screen show enhanced reprogramming of human fibroblasts in feeder dependent conditions



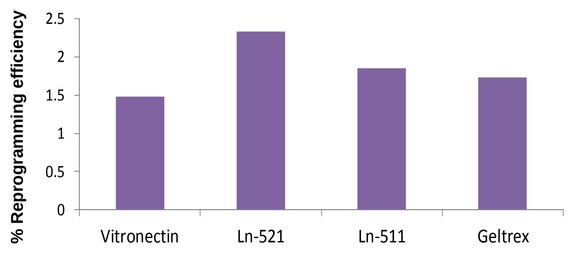
(A) AP stain of iPSCs via feeder dependent conditions at day 21
(B) Reprogramming efficiencies with various small molecule enhancers.
HDFn were transduced according to standard protocol with CytoTune™-iPS 2.0 as previously described. Negative control media contained no small molecule enhancers . 0.5mM NaBU , 0.2uM TSA or 2uM SB431542 were added to KSR based media from day 8-21.

Figure 3. Small molecule enhancers also show improved reprogramming efficiencies in feeder free conditions



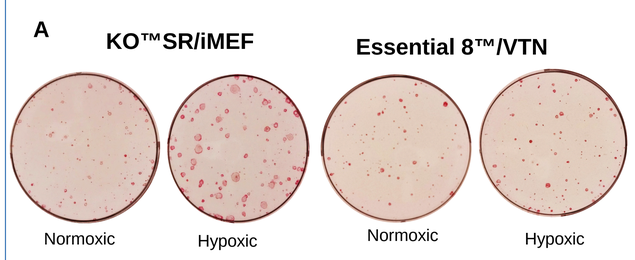
Reprogramming efficiencies with various small molecule enhancers.
HDFn were transduced according to standard protocol restated above. Negative control media contained no small molecule enhancers . 0.5mM NaBU , 0.2uM TSA or 2uM SB431542 were added to Essential 8™ media from day 8-21.

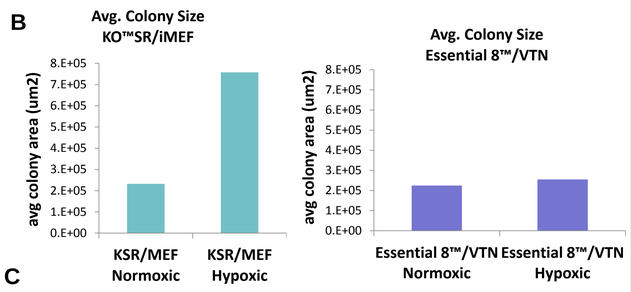
Figure 4. rhLaminin-521 shows improved reprogramming efficiency over other standard matrices for feeder independent reprogramming

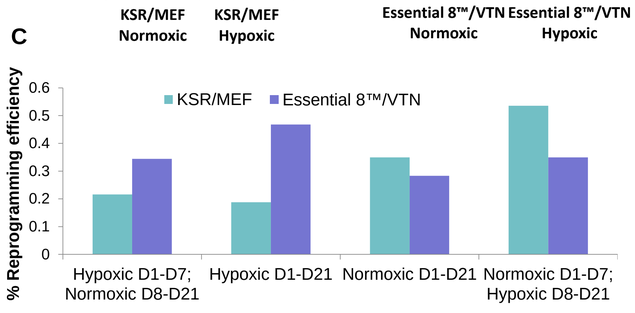


Reprogramming efficiencies on various matrices. The cells were transduced according to the CytoTune™-iPS 2.0. protocol. At day 7 post transduction, the cells were replated onto Vitronectin (VTN-N), rhLaminin-521, Laminin 511 or Geltrex™. The following day, media was changed to Essential 8™ medium. Cells were stained for Alkaline Phosphatase at day 21 post transduction:

Figure 5. Hypoxic conditions during reprogramming results in higher reprogramming efficiencies and larger iPSC colony sizes.

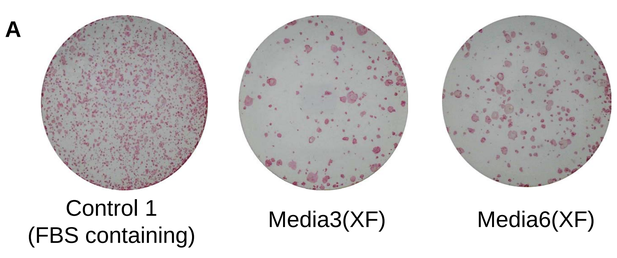


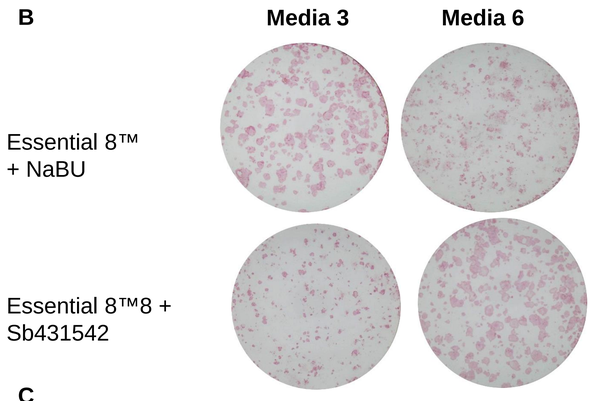


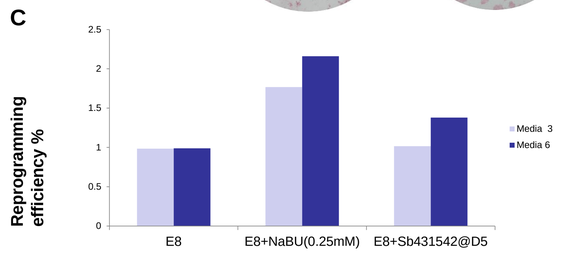


(A) AP stain of iPSC's under hypoxic and normoxic conditions at day 21 post transduction.
(B) Average colony size of reprogrammed iPSC's in normoxic and hypoxic conditions in KO™SR/iMEFs and Essential 8™/VTN
(C). Introduction of Hypoxic conditions at multiple intervals during reprogramming shows various effects on efficiency.
Reprogramming efficiencies with various small molecule enhancers.
HDFn were transduced according to standard protocol with either CytoTune™-iPS 2.0 as previously described. Cell were re-plated onto Geltrex™ on day 7 post transduction. Negative control media contained no small molecule enhancers . 0.5mM NaBU , 0.2uM TSA or 2uM SB431542 were added to Essential 8™ media from day 8-21.

Figure 6. Use of xeno-free media in Sendai based reprogramming.







(A) AP stain of iPSC's reprogrammed in FBS containing fibroblast media (Media 1) and two separate and distinct formulations of Xeno free media, Media 3 and Media 6
(B) AP stain of iPSC's reprogrammed in Media 3 and Media 6 and then transitioned to Essential 8™/Laminin 521 with two small molecule enhancers. Sodium butyrate and Sb431542.
(C) Reprogramming efficiencies between Xeno-free Medias 3 and Media 6 with small molecule enhancers, sodium buytrate(NaBu) and Sb431542
(HDFn were transduced according to standard protocol with either CytoTune™-iPS 2.0 as previously described. Cell were re-plated onto Laminin 521 on day 7 post transduction).

CONCLUSIONS

•Multiple small molecules show an improvement in reprogramming efficiency in KO™SR/iMEF and feeder independent systems when compared to control.

•rhLaminin-521 shows an improvement in reprogramming efficiency as compared to Geltrex™ and Vitronectin (VTN-N).

•Higher reprogramming efficiencies and larger average colony size were observed under hypoxic conditions

•.It is possible to reprogram in xeno-free media and culture conditions and achieve similar reprogramming efficiencies that traditional non-xeno-free medias' s and systems obtain.

REFERENCES

1. Takahashi, Kazutoshi et al. (2007) Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors
Cell , Volume 131 , Issue 5 , 861 – 872
2. K Kim, A Doi, B Wen, K Ng, R Zhao, P Cahan, J Kim, MJ Aryee, H Ji, L Ehrlich. (2010) Epigenetic memory in induced pluripotent stem cells. *Nature* **467**, 285–290.
3. Rodin, S., Antonsson, L., Niaudet, C., Simonson, O. E., Salmela, E., Hansson, et al., (2014). Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. *Nature communications*, 5-3195
4. Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S. Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell. 2009;5:237–241

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