EARLY MARKERS OF REPROGRAMMING IN INDUCED PLURIPOTENT STEM CELLS (IPSCS): A TIMELINE OF KEY STEPS IN THE REPROGRAMMING PROCESS.

Background

A major breakthrough in the field of human embryonic stem cell (hESC) research was the recent discovery that somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by transgenic expression of a key transcription factors (OCT4, SOX2, KLF4 and CMYC) [1]. Methods of transgene delivery utilize viral and non-viral techniques. These iPSCs mimic the pluripotent state observed for hESCs and have the advantage that human embryos are not needed, thus circumventing potential ethical concerns. However, the equivalence between hESCs and iPSCs as well as the mechanisms by which reprogramming occurs have as yet not been established. Research in this field could ultimately lead to new treatment strategies for human diseases through the use of the patient's own somatic cells for stem cell therapy.

Objective

The purpose of this study was to evaluate the appearance of validated markers of pluripotency during the process of early reprogramming in iPSCs in order to elucidate the mechanism of reprogramming and to validate pluripotentiality of the iPSCs.

Materials and Methods

We used nuclear markers (Nanog, OCT4, SOX2, and DNMT3B) and surface markers (SSEA4, GCTM2, ALP, and TRA-1-60) to construct a timeline of new expression during the reprogramming process. A piggyBac (PB) transposon was used to deliver the reprogramming transcription factor transgenes OCT4, SOX2, KLF4 and CMYC to human embryonic fibroblasts via the Amaxa Nucleofector [2]. Cells that had undergone transposon integration were positively selected by a Puromycin resistance cassette, which was part of the transposon plasmid.
Immunohistochemistry (IHC) staining for nuclear pluripotency markers (Nanog, DNMT3B, SOX2, OCT4) and surface pluripotency markers (TRA-1-60, GCTM-2, SSEA4, and Alkaline Phosphatase) was undertaken on days 6,9,12,15,18, and 21 after transposon nucleofection. The amount of positively stained cells for a given marker was expressed as the percentage of positive cells per total cells on the slide: absent (0%), minimal (<10%), moderate (10-50%) or marked expression (>50%).

Results

All studied markers appeared at some timepoint during the course of the experiments. Marked expression of OCT4 and SOX2 was observed at all timepoints, starting day 6, indicating satisfactory integration; these two factors are under constitutive transcriptional control of the transposon. Minimal expression of Alkaline Phosphatase and SSEA-4 was observed on day 6 and advanced to moderate expression on and after day 12, confirming that reprogramming was occurring.
GCTM2 and TRA-1-60 exhibited minimal expression starting day 6. Moderate positivity for the nuclear factor Nanog was observed starting day 9 and remained stable on subsequent timepoints, indicating activation of endogenous pluripotency gene networks present in embryonic stem cells.

Conclusions

In this study we describe the appearance of pluripotency markers during the process of early reprogramming in iPSCs. Whereas an initial expression of reprogramming transcription factors OCT4 and SOX2 was observed as expected, subsequent expression of Nanog, SSEA4, TRA-1-60, Alkaline Phosphatase, and GCTM2 indicates a progression of the reprogramming process, with resultant pluripotency marker expression similar to hES cells. We conclude that successful reprogramming of somatic cells into iPSCs is possible with the method described, and that it may provide a promising alternative to hESCs.

Support

California Institute of Regenerative Medicine (CIRM) grant no. RB1-01372

References:

1. Takahashi, K., et al., *Induction of pluripotent stem cells from adult human fibroblasts by defined factors.* Cell, 2007. **131**(5): p. 861-72.
2. Yusa, K., et al., *Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon.* Nat Methods, 2009. **6**(5): p. 363-9.