POTENTIAL ROLE OF TCL1A IN THE PLURIPOTENT REPROGRAMMING OF FIBROBLASTS

TATHIANE M MALTA (1,2); VIRGÍNIA PICANÇO-CASTRO (1); ELISA M S CARBOLANTE (1,2); EVANDRA S RODRIGUES (1,2); RODRIGO A PANEPUCCI (1); LYGIA V PEREIRA (4); DIMAS T COVAS (1,3); SIMONE KASHIMA (1,2).

(1) Center for Cell-Based Therapy, Regional Blood Center of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil. (2) Faculty of Pharmaceutical Science of Ribeirão Preto, USP, Ribeirão Preto, Brazil.
(3) Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, Brazil. (4) Department of Genetic and Evolutionary Biology, Institute of Bioscience, USP, São Paulo, Brazil.
E-mail: tathimalta@hemocentro.fmrp.usp.br

INTRODUCTION and OBJECTIVE

Induced pluripotent stem cells (iPS) derivation is a labor, demanding and extensive process and the reprogramming mechanisms are largely unknown. The main reason of the difficulty is that iPS phenomenon may occur in a stochastic manner. As iPS might be similar to embryonic stem cells (ESC) and offer novel therapeutic strategy to generate patient-specific stem cell lines, it would be essential to elucidate the mechanisms and to improve the efficiency. With this in mind, the aim of this study was to analyze the global expression profile of iPS-TSM, a reprogrammed human fibroblast generated with forced expression of TCL1A, SOX2 and C-MYC, and to compare with the donor fibroblast cell line and with ESCs in order to identify genes that could modulate the reprogramming process.

METHODS

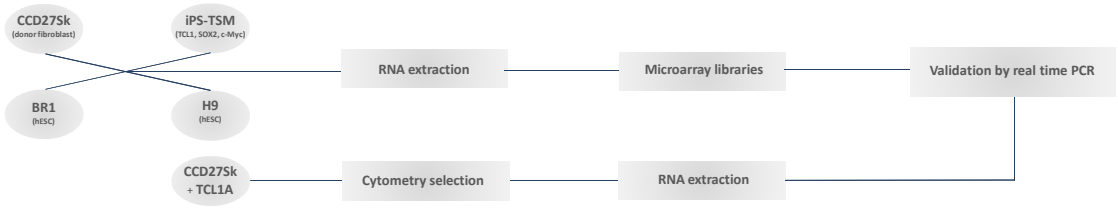

Figure 1. Experimental strategy. We compared the gene expression profile of the donor fibroblast CCD27Sk, the iPS-TSM and the hESC BR1 and H9 using a microarray platform composed by 44,000 human genes . We also established cell lines with overexpression of TCL1A individually in order to find out its contribution to the reprogramming process, as this transcription factor had never been used for reprogramming before. We further validated some gene transcripts levels by quantitative PCR (qPCR).

RESULTS

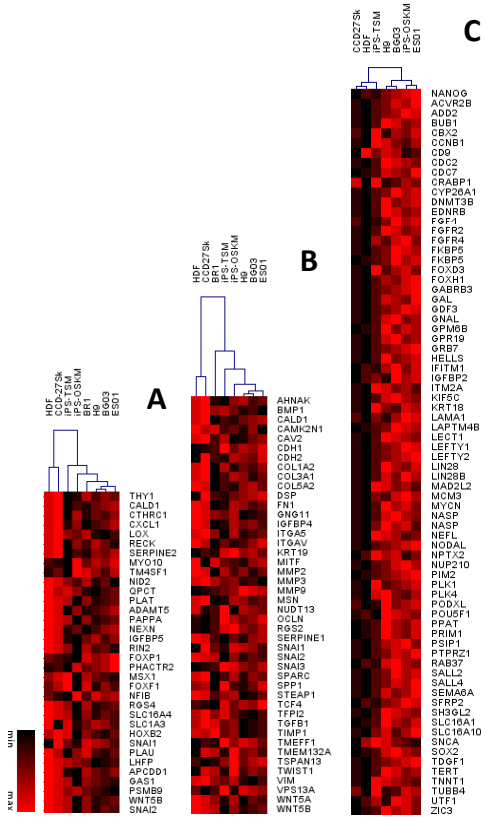

Figure 2 . Transcriptome analysis of the generated iPS-TSM. **(A)** Hierarchical clustering (Average linkage, Euclidean distance) of 34 fibroblast specific genes. We investigated if fibroblast specific genes were correctly silenced in the TSM cells, since this is the first step in the reprogramming process. We selected a group of fibroblast signature genes and analyzed their expression in the TSM cells. The results showed that the introduction of TCL1A, C-MYC and SOX2 into the TSM cells successfully silenced the fibroblast specific genes. **THY1** and **PLAU** genes, which are markers of fibroblast lineage, were down-regulated in TSM cells compare to the donor fibroblast. **(B)** Heatmap generated with values of 44 EMT/MET (Epithelial-mesenchymal transition/mesenchymal-epithelial transition) associated genes. We examined genes involved with MET, since this process had been suggested to be critical in the initial of mouse iPS derivation. We found that these genes had been modulated after the induction and have a similar pattern of expression to the ESCs. Important epithelial markers were activated in the TSM cells, including **CDH1**. Moreover, **COL1A2** and **CDH2**, which are mesenchymal markers, were downregulated in the TSM cells. We also detected that **SNAI1**, **TWIST1**, and **TGFB1**, that are inhibitors of MET, were downmodulated in TSM cells. **(C)** Heatmap of expression values of 74 ESC marker genes. We investigated if the pluripotent genes had been properly activated in TSM cells. We identified a core of pluripotent genes that had low expression levels compared to ESCs, such as **POU5F1**, **NANOG** and **GDF3**, indicating that they had not been correctly turned on. Columns are arranged by cell types. The heatmap is coloured by expression values according to the color key on the left. The analyses were performed using Genesis 1.75 software.

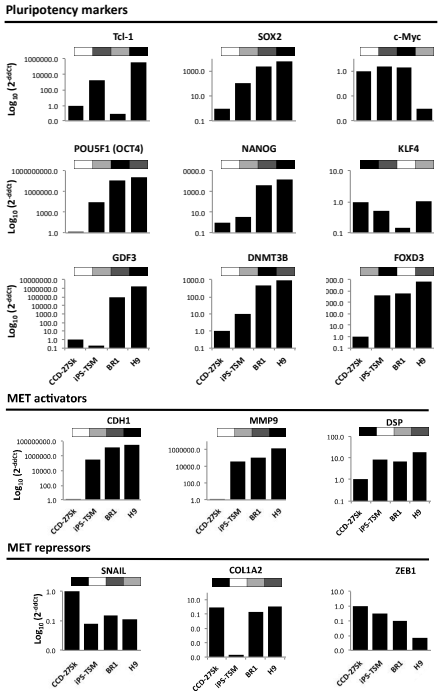

Figure 3. Figure 3. Real-time quantitative PCR analysis of pluripotency genes and MET/EMT markers in CCD27SK fibroblast, derived iPS-TSM, BR-1 and ESC-H9. The horizontal bars above the charts indicate the gene expression levels of the respective genes obtained in the microarray experiment. The bar colors represent the expression intensity and varies form absent (white and light gray) to higher levels (dark gray and black). The relative gene expression was obtained using the 2-ddCt method and the expression level of CCD27Sk was used as calibrator. ZEB1 gene probe was not present in the microarray chip.

CONCLUSION

The results revealed that the TSM cells presented an intermediary profile between the donor fibroblast and the ESCs, but the pattern of gene expression seems to move toward the embryonic-state. Additionally, our results showed that the TSM combination was able to induce MET giving rise additional questions about the role of Tcl1 in this process.

Financial support:

FAPESP INCTC Hemocentro RP