IMAGE-BASED ASSESSMENT OF PSC QUALITY DURING EARLY IPSC ESTABLISHMENT

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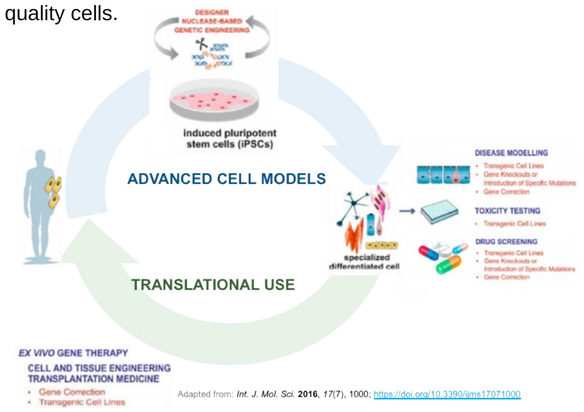
ABSTRACT

Somatic reprogramming for the generation of induced pluripotent stem cells (iPSC) has rapidly evolved with promising applications in disease modeling and translational applications. Genetically diverse iPSC derived from varying somatic sources is enabled by foot-print free reprogramming methods under different culture conditions. Despite these advances, early iPSC identification and clonal expansion continues to be a tedious and laborious process which needs to be further streamlined for high throughput and automated workflows.

Traditional methods of morphological assessment are now complemented with a combination of positive and negative markers albeit with varying levels of success. Previously we had reported the use of CD44 and SSEA1 to distinguish unreprogrammed and partially reprogrammed cells from fully reprogrammed colonies. This and other similar methods have been used to effectively identify and select bona fide iPSC. Here, we extend this approach to monitor subtle differences in early expanding iPSC clones to eliminate unstable clones that have a higher propensity to spontaneously differentiate.

INTRODUCTION

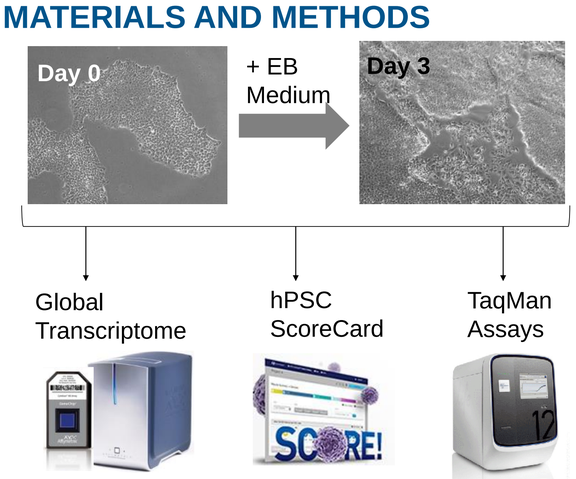
Stem cells offer unprecedented insight into the mechanisms of tissue development and are well suited for the generation of disease models and drug screening platforms. Stem cells hold great potential for therapeutic applications but stem cell culture workflows require extensive characterization, especially early on, to ensure that time and resources aren't wasted on poor quality cells.



Adapted from: *Int. J. Mol. Sci.* **2016**, *17*(7), 1000; https://doi.org/10.3390/ijms17071000

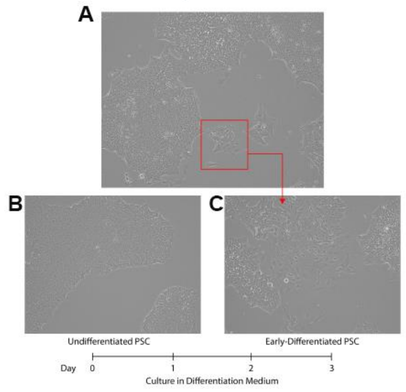
Subtle changes in stem cell morphology indicate the quality of stem cells and their pluripotency state which in turn has tremendous impact on their differentiation potential and genomic stability. Antibodies specific for self renewal and differentiation markers are not sufficiently distinct to distinguish undifferentiated cells from early differentiating cells. The objective of this study is to identify effective early-markers of differentiation.

MATERIALS AND METHODS



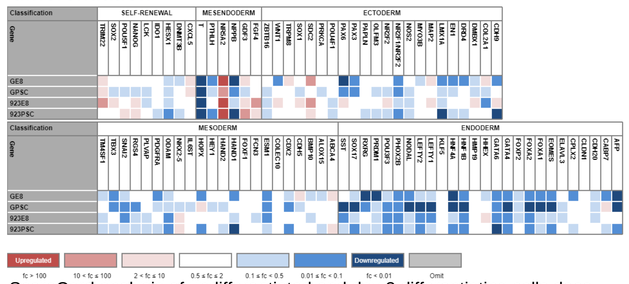
RESULTS

Figure 1. Routine image-based assessment of PSC in culture



A. Representative image of PSC in culture. Majority of the cells have typical PSC morphology with few patches of cells that deviate from that
B. A typical morphology of undifferentiated PSC
C. Subtle morphological changes observed during day 3 of differentiation

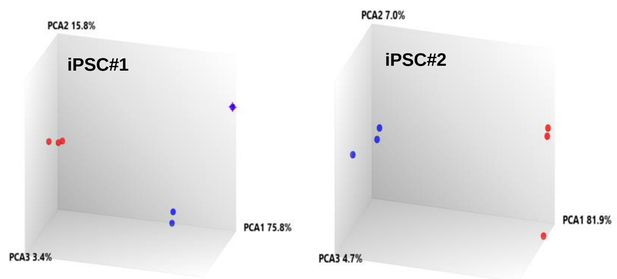
Figure 2. TaqMan™ hPSC ScoreCard™ Analysis showed subtle changes in PSC gene expression at Day 3 of differentiation



ScoreCard analysis of undifferentiated and day 3 differentiating cells does not show significant changes in self renewal or differentiation markers.

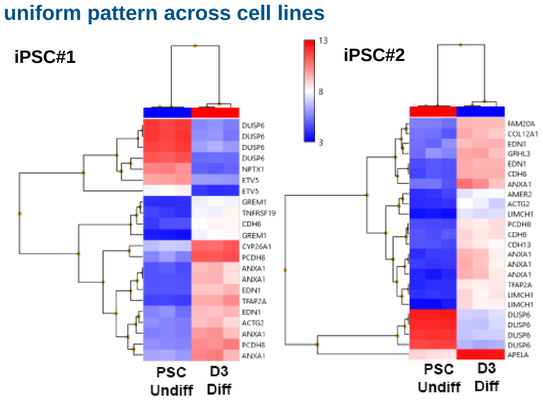
Global Transcriptome Analysis

Figure 3. PCA Plots show that biological replicates cluster



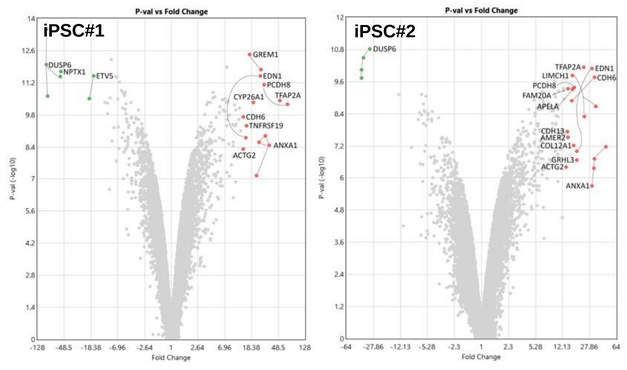
PrimeView 16 Global Gene Expression Profile Assay with triplicate samples of undifferentiated and day 3 differentiating cells shows clustering of undifferentiated cells (Red dots) together and away from day 3 differentiating (Blue dots) cells.

Figure 4. Hierarchical Clustering shows clear differences in early-differentiated PSC gene expression but no uniform pattern across cell lines



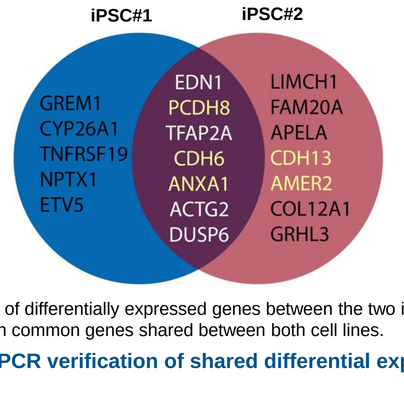
Hierarchical clustering of differentially expressed genes (X-fold) showing grouping of undifferentiated cells and differentiating cells with distinct upregulated (red) and downregulated (blue) genes.

Figure 5. Overview of key differential expressed genes



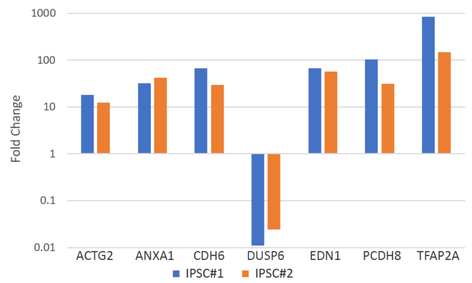
Volcano plot of global gene expression data (Fold change threshold: -13 to +13, P-value: < 0.0005) to identify specific genes that are differentially expressed between undifferentiated cells and day 3 differentiating samples

Figure 6. Differentially Expressed Genes



Comparison of differentially expressed genes between the two iPSC lines shows seven common genes shared between both cell lines.

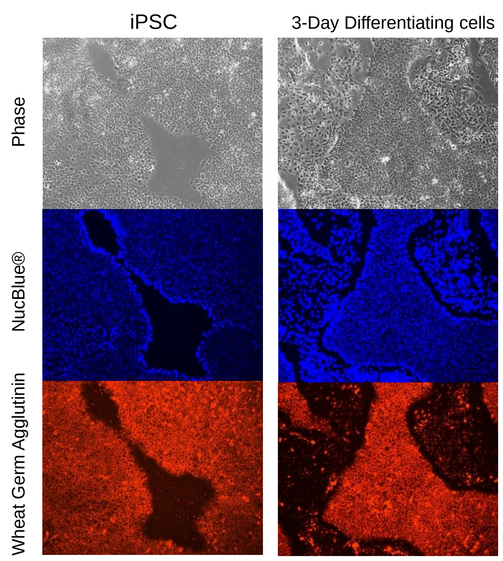
Figure 7. PCR verification of shared differential expressed genes



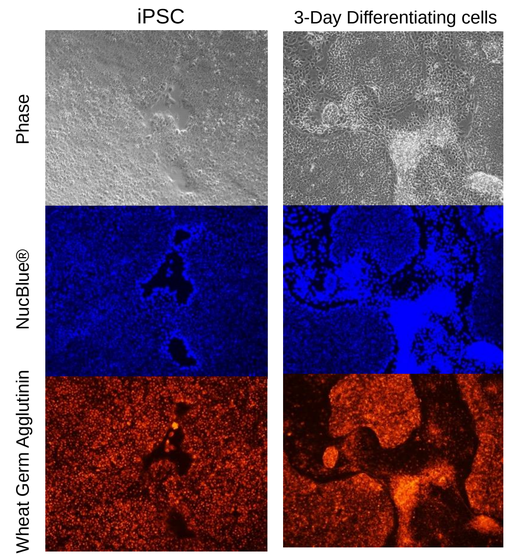
PCR confirms the differential expression of several cell adhesion genes - CDH6, ANXA1,PCDH8 and CDH13.

Figure 8. Membrane and Nuclear Dyes Reveal Morphological Differences Between Stem Cell States

(A) CellMask™ Red Membrane Dye



(B) Wheat Germ Agglutinin Membrane Dye



Cell Mask Red (A) and Wheat germ agglutinin (B), two dyes specific for cell membrane showed distinct intensity and pattern of staining between undifferentiated cells and day 3 differentiating cells

Figure 8. Signal quantification of membrane stained cells

Quantitation of the cell membrane dye signal was carried out using ImageJ Analysis.

CONCLUSIONS

- There is a need for easy methods to distinguish early spontaneous differentiating PSC cells in cultures since current methods used to detect differentiating cells are not sensitive.
- Global gene expression analysis of two independent iPSC lines suggests clear change in gene expression patterns between undifferentiated cells cultured in Essential 8 media and early differentiating cells cultured in EB media for 3 days
- Several differentially expressed genes were identified in the two iPSC lines between undifferentiated and day 3 differentiating cells.
- The differential expression pattern observed with gene array was confirmed using TaqMan PCR analysis. The majority of these genes were associated with cell adhesion

ACKNOWLEDGEMENTS

This study was funded by the California State University-San Marcos CIRM Bridges to Stem Cell Research Training Grant.

TRADEMARKS/LICENSING