Single-cell analysis elucidates cardiomyocyte differentiation from human induced pluripotent stem cells

Introduction

Recent studies have demonstrated high variability in cell cycle and gene expression in cells with identical morphology, indicating that even "homogeneous" cells—such as those within the same tissue or culture vessel—can exhibit transcriptomic heterogeneity. One example of this is induced pluripotent stem cells (iPSCs): when tissue-specific differentiation is initiated, cells do not uniformly transition; rather, the differentiation rate varies from cell to cell. By analyzing single cells, it is possible to capture gene expression changes that would otherwise be masked in bulk analyses and gain a clearer picture of the differentiation process.

To address this challenge, Drs. Akira Watanabe and Yoshinori Yoshida at Kyoto University's iPS Cell Research Institute leveraged our open, flexible ICELL8 Single-Cell System to develop a method for transcriptomically characterizing the differentiation of human iPSCs into cardiomyocytes.

Methods

Following the induction of human iPSCs into cardiomyocytes, cells were sampled at Days 1, 3, 9, and 21. Cell sample concentrations were normalized, and then cells were processed on the ICELL8 Single-Cell System—an advanced, integrated platform that allows for the automated acquisition and imaging of 1,200–1,500 single cells on a nanowell chip (Figure 1). Cell concentrations were normalized, stained with propidium iodide and Hoechst 33342 dyes, and then dispensed onto an ICELL8 3' DE chip. Nanowells were imaged and those containing single, live cells were automatically identified and subjected to reverse transcription and RNA-seq library preparation. Single-cell RNA-seq libraries were sequenced and gene expression analyses were performed on an Illumina HiSeq® sequencer. Gene expression information from each cell was visualized using T-distributed stochastic neighbor embedding (tSNE) analysis.

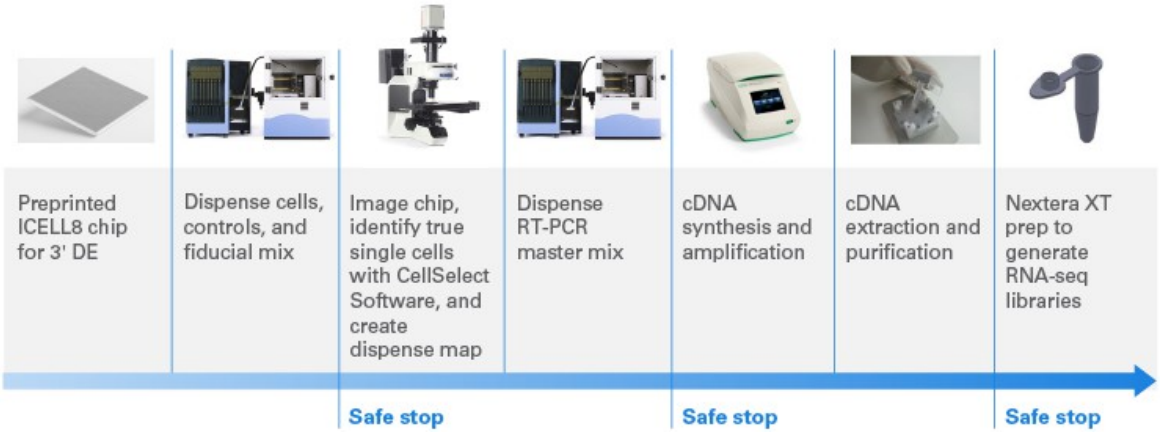

Figure 1. ICELL8 Single-Cell System workflow overview. Cells, controls, and fiducial mix are dispensed onto a 5,184-well chip at an average of one cell per well. CellSelect Software automatically identifies nanowells containing live, single cells and creates a dispense map targeting only these wells, saving time and reagents. Targeted nanowells are then subjected to cDNA synthesis, with a barcode in each well allowing the association of sequencing data with a given cell. cDNA is extracted and purified, RNA-seq libraries are generated via PCR off-chip, and then libraries are subjected to sequencing.

Results

For each time point, a total of ~1,000 single, live cells was obtained, with an average of 120,000 reads (750,000 maximum) and 1,200 genes (4,500 maximum) identified per cell. tSNE analysis of single-cell RNA-seq data showed clustering of cells by transcriptomic signatures along their differentiation pathway, with robust clustering by timepoint (Figure 2) and heterogeneity across all clusters, except Day 21 (i.e., differentiated cardiomyocytes, labeled "cluster 3"). This heterogeneity was particularly notable in cluster 5, which exhibited a high proportion of

both Day 3 and Day 9 cells, and a lower proportion of Day 1 cells. This pattern is consistent with the appearance and disappearance of known markers: as differentiation proceeded, the expression of iPSC-specific genes decreased, while the expression of cardiomyocyte-specific genes increased (Figure 3).

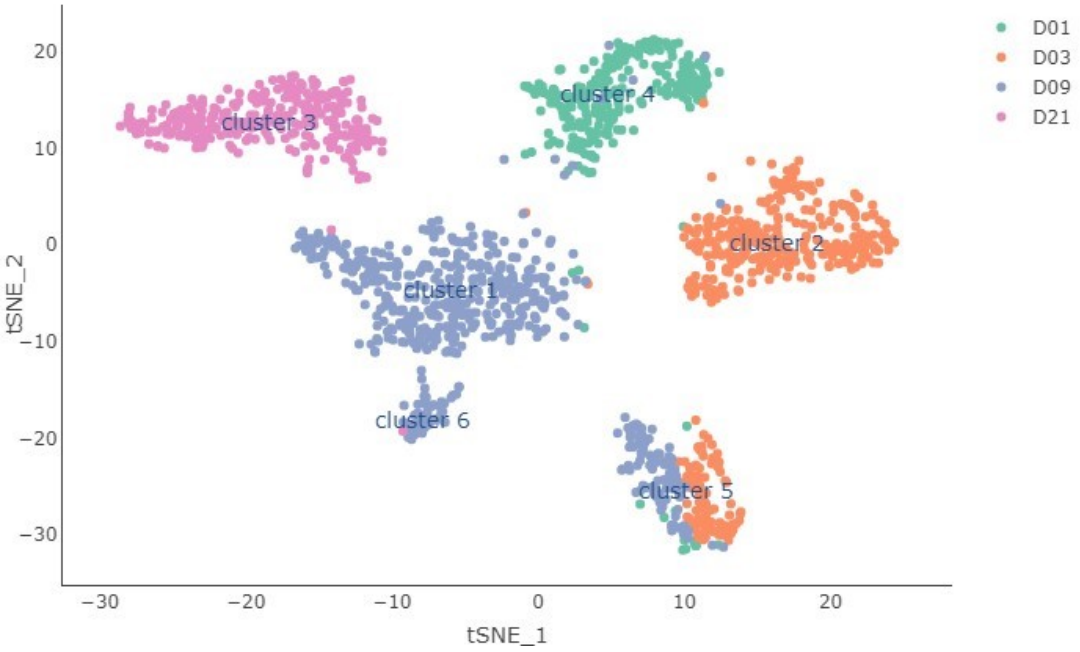

Figure 2. Single-cell RNA-seq of iPSCs reveals robust clustering based upon time post-induction. Cells from several time points were observed in all clusters (with the exception of Day 21, the differentiated cardiomyocytes). This was particularly notable in cluster 5, which contained cells from Days 1, 3, and 9.

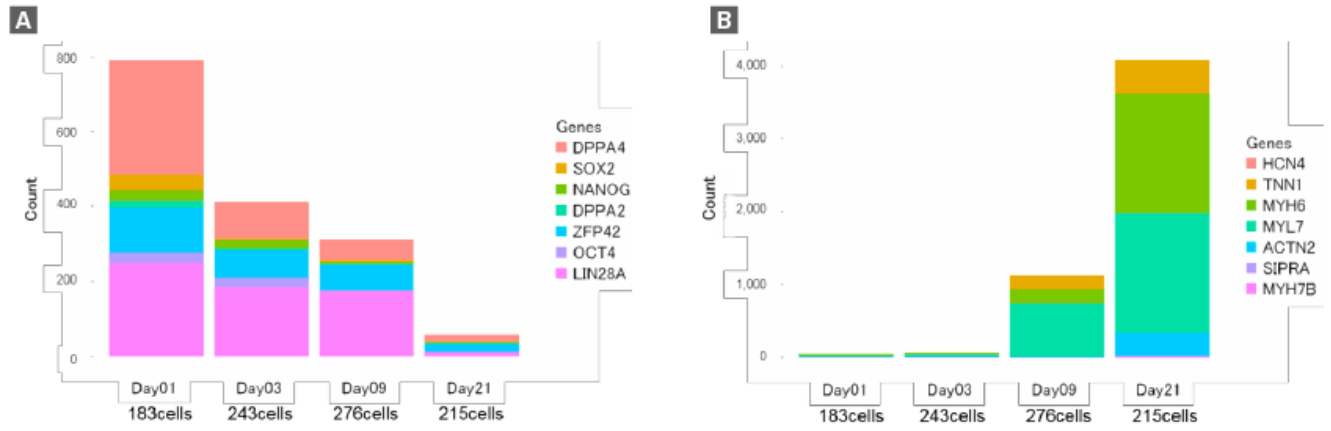

Figure 3. Proportions of cells expressing iPSC and cardiomyocyte markers throughout differentiation. Following induction of iPSCs into cardiomyocytes, expression of iPSC-specific genes tapers off, with a rapid drop at 21 days (**Panel A**). Similarly, cardiomyocyte-specific genes increase expression, with a sharp peak at 21 days (**Panel B**). This corresponds with the relative homogeneity of terminally differentiated cardiomyocytes (Day 21 cells), as compared to cells from earlier time points.

Conclusions

The ICELL8 Single-Cell System is an open platform that inspires the development of new single-cell analysis methods and allows a deeper look inside the transcriptomic programs underlying complex biological phenomena. The ICELL8 system can be used to resolve developmental changes at the single-cell level, which sheds light on processes like differentiation from pluripotent cells to terminally differentiated, mature cells. This study found that, during induced differentiation, transcriptional profiles tended to coincide with certain timepoints (iPSC-specific gene expression decreased, while cardiomyocyte-specific gene expression increased over time), and clustering of single cells via tSNE analysis revealed transcriptional heterogeneity throughout differentiation. While not a part of this analysis, data generated using the ICELL8 system could

enable *de novo* identification of transcripts that characterize each cluster and provide further insight into the mechanics of the iPSC-to-cardiomyocyte differentiation program.

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