Predicting Cell Line Variability in Cardiomyocyte Differentiation Efficiency Using Non-invasive Multi-analyte Luminex® Assays

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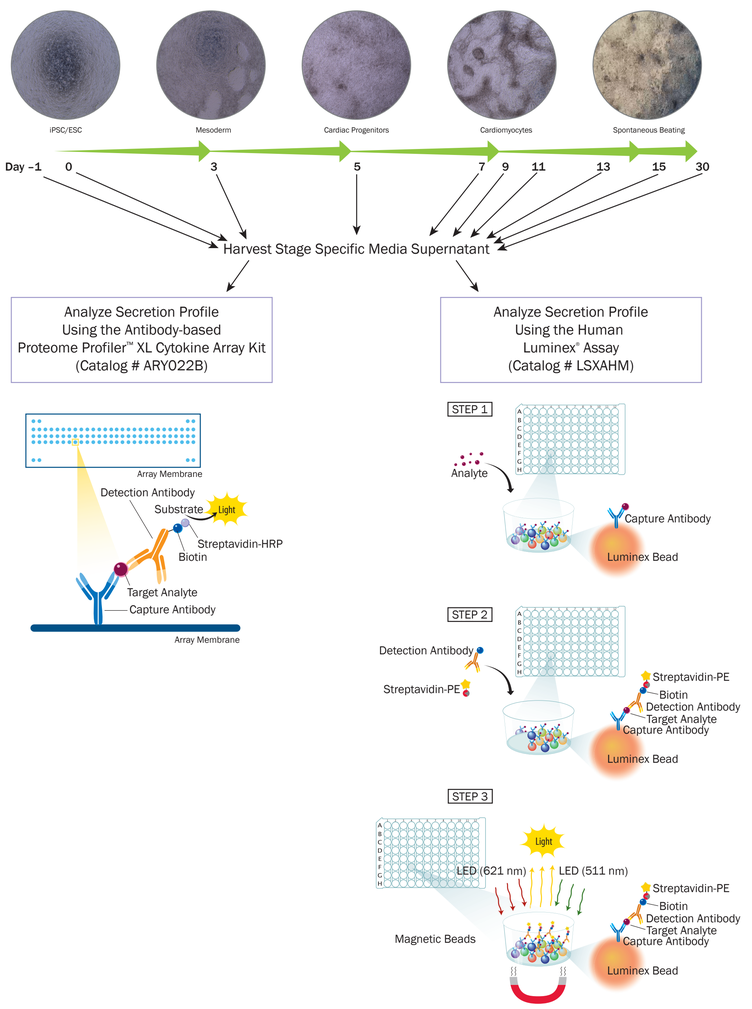
Abstract

The ability of pluripotent stem cells to differentiate into any tissue of the body has the potential to revolutionize medicine. One technical challenge that must be overcome to fully realize this potential is generating patient-derived pluripotent stem cells that can efficiently and robustly differentiate into specific tissues. Even using standardized differentiation protocols, differentiation efficiency is highly variable across cell lines from both human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells. In this study, we demonstrate the power of Luminex® Assays as an early detection method to assess pluripotent cell line variability and to determine cell line differentiation efficiency. Various hES and hiPS cell lines were differentiated into cardiomyocytes using the standardized protocol and reagents in the StemXVivo® Cardiomyocyte Differentiation Kit. Combining our standardized differentiation protocol with multi-analyte Luminex® assays enabled us to profile the changes in cytokine and growth factor levels in cell culture media at key stages during the differentiation. We found that cytokine and growth factor expression profiles varied across hiPS cell lines with known differences in cardiomyocyte differentiation efficiency. Because analytic samples are obtained from culture media, the cells are able to continue through the differentiation process and be analyzed for efficiency by assessing beating and cardiac-specific marker expression via immunocytochemistry. We demonstrate that the multi-analyte profile of cell lines with robust differentiation differs from cell lines with lower efficiencies. Using Luminex® multi-analyte technology we identified particular analytes that are predictive of differentiation success. Additionally, this information can be used for identification of important pathways involved in stem cell differentiation and/or maturation into cardiomyocytes.

Experimental Outline

Induced pluripotent stem cells were differentiated into cardiomyocytes using the StemXVivo® Cardiomyocyte Differentiation Kit (R&D Systems; Catalog # SC032B). Cell culture supernatants were collected at select time points during the differentiation and analyzed using either the Proteome Profiler™ XL Cytokine Array (R&D Systems; Catalog # ARY022B) or the Human Luminex® Assay (R&D Systems; Catalog # LSXAHM).



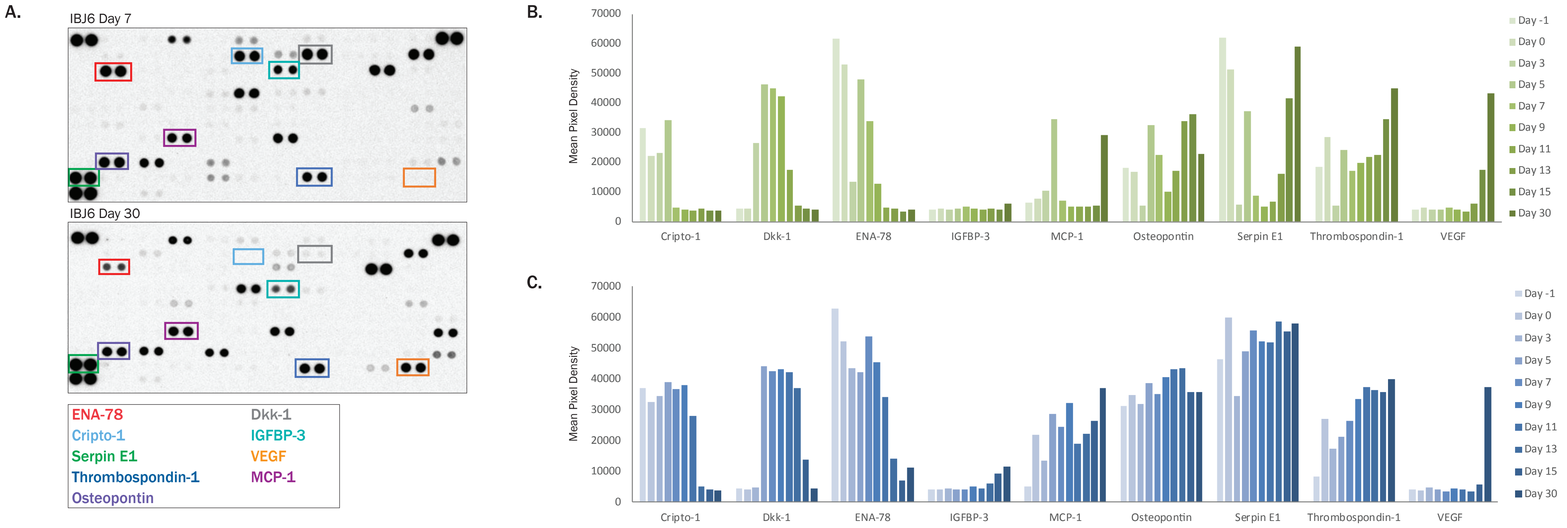
iPS Cell Lines Exhibit Variability in Their Differentiation Capacity

Cell Line Name	Origin	Clones	Ectoderm	Endoderm	Mesoderm	Cardiac	Hepatocyte	Neural
JOY	PBMC	6	+++	+++	++	++	+++	++
FAB	PBMC	6	+++	+++	++	+++	–	++
ADLF	PBMC	6	+++	+++	+	–	++	++
KF	PBMC	6	++	+++	+	++	–	–
MF	PBMC	6	++	+++	++	+++	–	++
IBJ6	Fibroblast	1	+++	+++	+	+++	+++	++
029	Fibroblast	1	++	+++	+	+	++	++
iPSK3	Fibroblast	1	++	+++	+	+	+++	++
BG01V	ES cells	1	++	+++	++	++	++	++

Ectoderm = StemXVivo® Ectoderm Kit (Catalog # SC031B)
Mesoderm = StemXvivo® Mesoderm Kit (Catalog # SC030B)
Endoderm= StemXVivo® Endoderm Kit (Catalog # SC019B)
Cardiac = StemXVivo® Cardiomyocyte Differentiation Kit (Catalog # SC032B)
Hepatocyte = StemXVivo® Hepatocyte Differentiation Kit (Catalog # SC033)
Neural = StemXVivo® Neural Progenitor Differentiation Kit (Catalog # SC035)

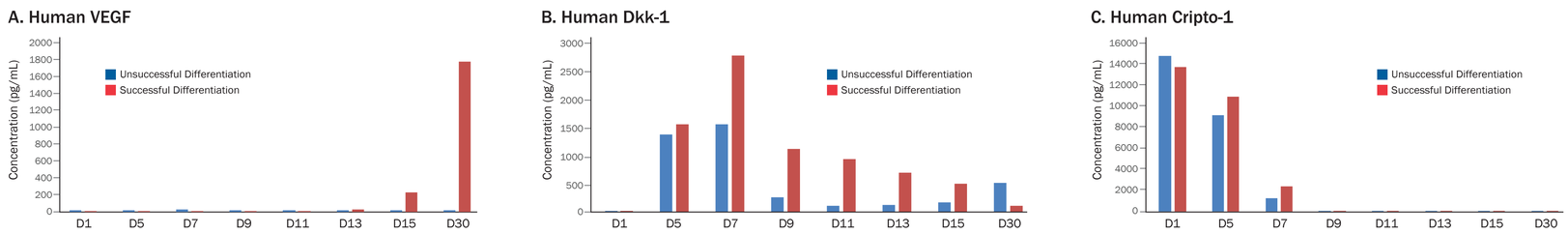
Pluripotent Stem Cell Lines Exhibit Variability in Differentiation into Cardiomyocytes. Multiple established human iPS cell lines and one embryonic stem cell line were differentiated into germ layer or terminal cell types using StemXVivo® Differentiation Kits. This table shows that individual cell lines are variable in their ability to differentiate into specific lineages or cell-types, including cardiomyocytes.

Qualitative Profiling of Cardiomyocyte Differentiation Using Cell Culture Supernates



The Proteome Profiler™ Human XL Cytokine Array Identifies Protein Expression Changes During Stem Cell-derived Cardiomyocyte Differentiation. Two different induced pluripotent stem cell lines (IBJ6 and JOY) were differentiated into cardiomyocytes using the StemXVivo® Cardiomyocyte Differentiation Kit. Cell culture supernates, taken at selected time points during differentiation, were analyzed semi-quantitatively using the Proteome Profiler™ Human XL Cytokine Array Kit. (**A**) Representative images of arrays incubated with supernates collected on Day 7 and Day 30 of differentiation using the IBJ6 cell line. Colored boxes highlight select analytes that changed levels through differentiation. (**B, C**) Histogram profiles of mean spot pixel density for select analytes at the specified time point of differentiation are shown for IBJ6 (**B**) and JOY (**C**) cell lines. Using this data we identified analytes that increased during differentiation (MCP-1, Osteopontin, Serpin E1, Thrombospondin-1, VEGF), decreased during differentiation (Cripto-1, ENA-78), had variable expression during differentiation (Dkk-1), and remained the same throughout differentiation (IGFBP-3). These profile patterns were similar between the different pluripotent cell lines tested.

Quantitative Profiling of Successful and Unsuccessful Cardiomyocyte Differentiation



Comparison of Cytokine Secretion Profiles Between Successful and Unsuccessful Cardiomyocyte Differentiation Experiments. IBJ6 induced pluripotent stem cells were differentiated into cardiomyocytes. The cytokine profiles of cell culture supernatants were compared between differentiation experiments that were identified as “Successful” (> 75% beating cardiomyocytes at differentiation Day 15) versus “Unsuccessful” (< 20% beating cardiomyocytes at differentiation Day 15). Cell culture supernate samples were taken at stage-specific time points throughout differentiation and analyzed quantitatively using our Human Luminex® Assay. Select analytes were chosen based on qualitative analysis with the Proteome Profiler™ XL Cytokine Array. Expression of Human VEGF (**A**) and Human Dkk-1 (**B**) differed between successful and unsuccessful differentiation experiments. Other analytes, such as human Cripto-1 (**C**), showed similar changes in expression regardless of differentiation success.

Conclusions

- Multi-analyte Proteome Profiler™ Arrays and Luminex® Assays are effective tools to detect changes in cytokine expression throughout the progression of stem cell differentiation into cardiomyocytes.
- Changes in analyte expression during cardiomyocyte differentiation were consistent across pluripotent stem cell lines tested.
- Expression of VEGF and Dkk-1 varied between successful and unsuccessful differentiation experiments.
- Proteome Profiler™ Arrays and Luminex® Assays may provide a way to predict differentiation success as well as to optimize cell line-specific differentiation protocols.