Predicting Successful Pluripotent Stem Cell Differentiation Using Non-invasive Multi-analyte Luminex® Assays

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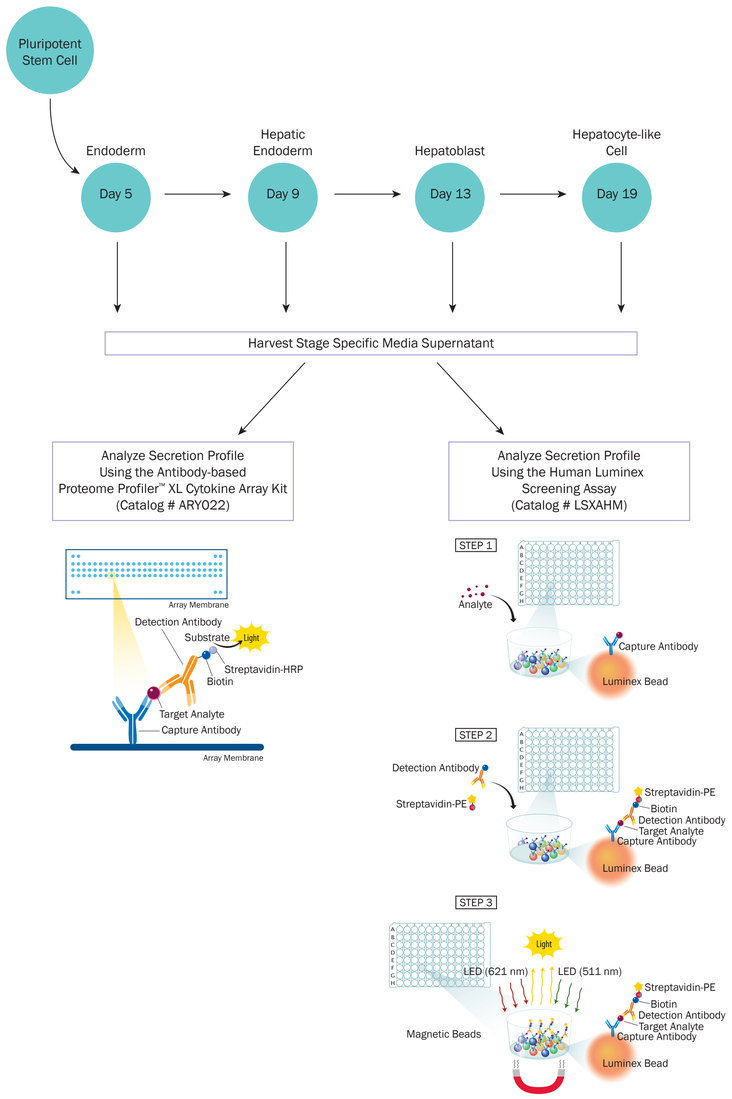
Abstract

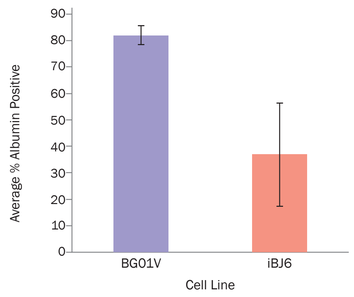
The ability of pluripotent stem cells to differentiate into any tissue of the body has the potential to revolutionize medicine. To fully realize this potential, robust and standardized differentiation and characterization protocols are necessary. In this study, we describe the use of Luminex® multi-analyte technology for non-invasively characterizing stem cells undergoing differentiation as well as for optimizing individualized differentiation protocols. Luminex® technology allows for the simultaneous quantification of up to 100 proteins within a single small-volume sample. We utilized the multi-analyte screening power of Luminex® assays to profile the levels of cytokines and growth factors in cell culture media at key stages during the differentiation of pluripotent stem cells into hepatocytes. Cytokine and growth factor expression profiles were obtained from human induced pluripotent stem (hiPS) and human embryonic stem (hES) cell lines with known differences in hepatocyte 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 albumin expression. We hypothesize that the multi-analyte profile of cell lines with robust differentiation efficiency will differ from cell lines with lower efficiencies. Using Luminex® multi-analyte technology we will be able to identify particular analytes that are predictive of differentiation success. Additionally, this data can be used to identify alternate factors that enhance differentiation and/or maturation of the differentiated cells.

Experimental Outline

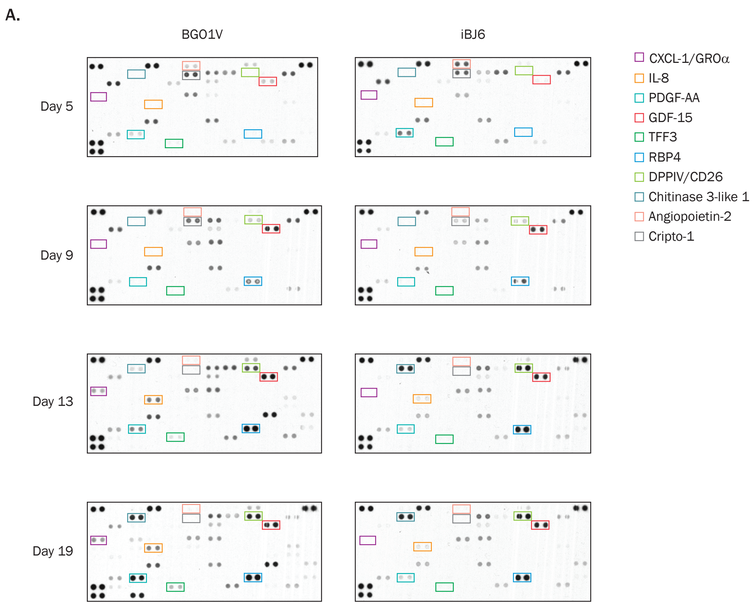
Hepatocyte Differentiation of Pluripotent Stem Cells using the StemXVivo® Hepatocyte Differentiation Kit (Catalog # SC033).

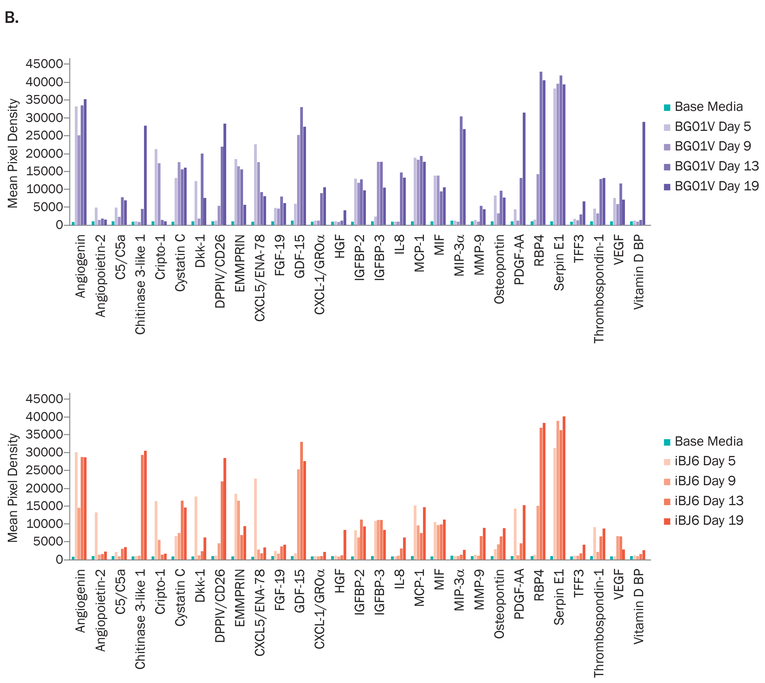


Qualitative Profiling of Hepatocyte Differentiation Using Cell Culture Supernates



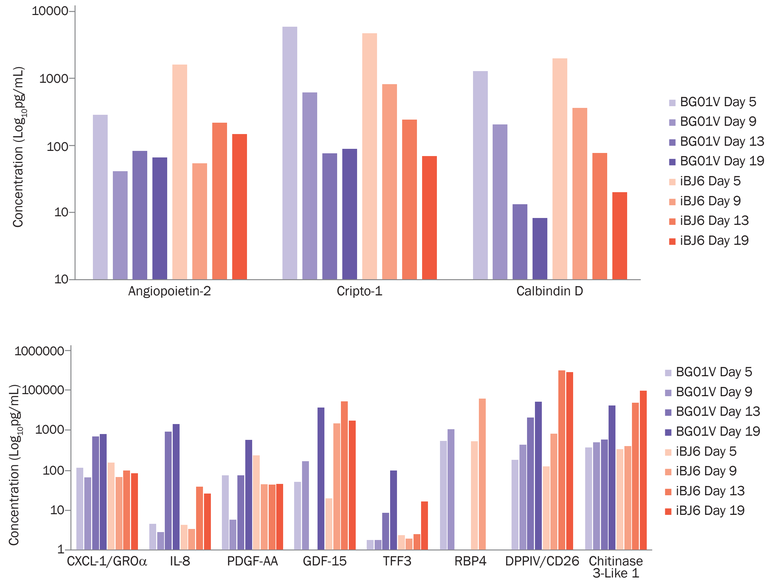
Cell Line Differences in Efficiency of Hepatocyte Lineage Differentiation. BG01V hES and iBJ6 hiPS cells were differentiated into hepatocyte-like cells using the StemXVivo® Hepatocyte Differentiation Kit. Differentiation efficiency was assessed by immunocytochemical staining for Albumin (Catalog # MAB1455). Percent positive cells were quantitated using the Operetta® High Content Imaging System. Error bars indicate the standard deviation.



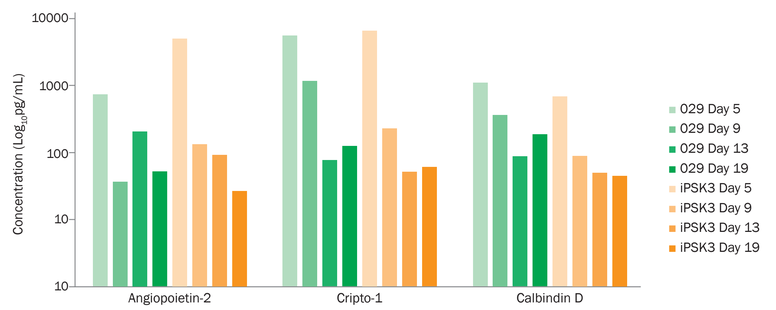


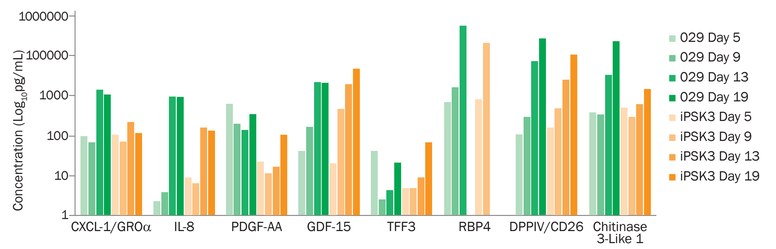
Cytokine Levels in Culture Supernates During Hepatocyte Differentiation of Pluripotent Stem Cells. BG01V hES and iBJ6 hiPS cells were differentiated into hepatocyte-like cells using the differentiation kit. Cell culture supernates taken at select time points during differentiation were analyzed semi-quantitatively using the Proteome Profiler™ Human XL Cytokine Array Kit (Catalog # ARY022). (**A**) Arrays incubated with supernates collected on days 5, 9, 13, and 19 of differentiation are shown (1 minute X-ray film exposure). Colored boxes highlight select analytes that change expression through differentiation. (**B**) Histogram profiles of mean spot pixel density for select analytes at the specified stage of differentiation are shown for BG01V (top) and iBJ6 (bottom) cell lines.

Quantitative Profiling of Hepatocyte Differentiation Using Cell Culture Supernates



Quantitation of Cytokine Levels in Culture Supernates during Hepatocyte Differentiation of Pluripotent Stem Cells. BG01V hES and iBJ6 hiPS cells were differentiated into hepatocyte-like cells using the differentiation kit. Cell culture supernates from days 5, 9, 13, and 19 were analyzed quantitatively using the Human Luminex® Screening Assay. Analytes were chosen based on qualitative analysis with the Proteome Profiler™ XL Cytokine Array. Subsets of analytes displayed decreasing (top histogram) or increasing (bottom histogram) levels through differentiation in both cell lines.





Hepatocyte Differentiation Cytokine Profile is Consistent across Multiple Cell Lines. Human iPS cell lines, 029 and iPSK3, were differentiated into hepatocyte-like cells using the differentiation kit. Cell culture supernates from days 5, 9, 13, and 19 were analyzed quantitatively using the Human Luminex® Screening Assay. 029 and iPSK3 cells showed similar changes in analyte expression as those observed for BG01V and iBJ6 cells. Analyte subsets with decreasing (top histogram) and increasing (bottom histogram) were maintained in both cell lines.

Conclusions

- The Proteome Profiler™ and Luminex® Screening multi-analyte assays detect changes in cytokine expression from cell culture supernates throughout the progression of stem cell differentiation into hepatocyte-like cells.
- Secretion profiles provide a non-invasive method for the characterization of differentiation.
- These signature differences may provide a method for prediction of differentiation success and cell line specific optimization of differentiation protocols.