Abstract

A clear understanding of stem cell differentiation pathways is important to advance regenerative medicine therapies using stem cells. An incorporation knowledge base of developmental mechanisms is essential for stem cell research and innovation. The iDifferentiate system is a genetic engineering platform that may be used to elucidate differentiation pathways of any cell type for which there is a known lineage-specific cis-regulatory element. To demonstrate this system we developed the SAVE Assay, which uses visual cues to indicate the overall quantity and relative percentage of atrial and ventricular cardiomyocytes among differentiated stem cells. This assay uses a dual-plasmid system that allows for successful transduction into mammalian cells via amphoteric and protoplast transduction along with fluorescent reporter genes regulated by atrial and ventricular promoters. Allowing the basic protocol by using different inducers and induction factors will allow scientists to quickly and accurately determine differentiation pathways of two or more related cell types. Future goals are to develop a system that allows for negative selection of nondifferentiated and misdifferentiated stem cells in vivo. Furthermore, we plan to develop a system to quickly and accurately determine differentiation pathways of any cell type for which there is a known lineage-specific cis-regulatory element.

Goals of iDifferentiate

We will integrate many areas of biotechnology to develop a system that facilitates the study of stem cell differentiation.

This is done by engineering multiple cell imaging assays—the SAVE assay—to speed the progress of our understanding of stem cell differentiation and embryonic development.

This will save time and lower the cost of research. Thus, getting novel cell-based therapeutics to the mass faster.

Benefits of iDiff

Lower Cost

Stably transfected cell lines

Real Time Science!

Quick

Real-time analysis of results

Power reagents

Real-time procedure alterations

Repeatable

Track cells

Spatiotemporal cell differentiation

Random Mutations

All cells acquire mutations as they divide. The higher the passage number the more likely a harmful mutation could cause massive damage to the host after implantation. Furthermore, it could fundamentally change the characteristics of the cell.

Necessity for Multiple Cell Lines

Currently with embryoid stem cells, multiple cell lines need to be maintained and new cell lines formed. This increases the chance for HLA matching for implantation into a host.

Time

Human cells have slower division rates and are harder to pass down. This leads to increased demands on samples. This time investment further increases the need for high efficiency protocols, especially when patients live at stake.

Labor

- We can add multiple assays and test them in parallel within a day.
- We can use Hepatic cells to determine cell types.
- We can also test multiple cell lines.

Cost

- We can create and test the assay in parallel.
- We can use Hepatic cells to determine cell types.
- We can also test multiple cell lines.

Methods

Plasmid Engineering

- Grow bacteria containing CTS promoter vectors in nutrient broth and IPTG

- Purify the plasmid

- Extract CTS promoter and associated reporter gene from the vectors using a restriction digest

- Characterize the RNA by electrophoresis

- Purify the digested fragments from the gel

- Clone the CTS promoter and reporter gene into the multiple cloning site of a maintenance vector

- Select for successfully transfected cells using gentamycin and puromycin

- Image the cells using a fluorescent microscope

- Analyze photomicrographs using ImageJ and LIPS

Cell Engineering

- Grow a feeder layer of irradiated fibroblasts for stem cell culture

- Plate stem cells onto feeder layer and expand to appropriate quantity

- Initialize differentiation of stem cells by forming embryoid bodies

- Dissect gel-embryoid bodies and plate the single cell suspension onto a new dish for transfection

- Transfect the cells with both plasmids using cationic lipid

- Image the cells using a fluorescent microscope

- Analyze photomicrographs using ImageJ and LIPS

References


Acknowledgements

The authors thank the Department of Biology at Wisconsin Lutheran College for funding the project; Dr. Robert Balza, and Dr. Efte for their contribution as mentors. They would also like to thank Dr. Michel Pecaut from INSERM for donating the MLc2s ventricular cardiomyocyte specific promoter, as well as Dr. Paul Krieg for donating the ANF atrial cardiomyocyte specific promoter to the WLC-Milwaukee Stem Team.

In summary, we have outlined a strategy to efficiently and effectively determine the differentiation pathways of cardiomyocytes. This strategy can easily be expanded to determine similar pathways in other cell lineages. The method of identification of differentiation pathways is based on fluorescent reporter gene activation. This occurs when a cell type specific promoter is bound by transcription factors that then transcribes an associated reporter gene. The efficiency of the differentiation protocol is then analyzed based on photomicrographs taken of the transfected cells.