PROJECT TITLE: Characterization of Stem Cells using CDy 1 a Stem Cell-specific dye

AUTHOR: Rhoda Mondeh-Lowor

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THE PROJECT HAS BEEN ACCEPTED BY THE PROJECT COMMITTEE IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOTECHNOLOGY.

Dr. Terry Kelly
PROJECT COMMITTEE CHAIR

Dr. Betsy Read
PROJECT COMMITTEE MEMBER

Dr. John Drewe
PROJECT COMMITTEE MEMBER

Dr. Julie Jameson
PROJECT COMMITTEE MEMBER
Abstract

Characterization of Stem Cells using CDy1 a new fluorescence stem cell dye

Active Motif

Rhoda Mondel-Lowor

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Master of Biotechnology, Professional Sciences Master’s Program

California State University, San Marcos

Stem cells are undifferentiated cells in an organism and have the potential to differentiate into all cell types. Stem cells have the ability to turnover and regenerate organs such as blood, skin, and intestinal tissues. Stem cells are capable of both self-renewal and differentiation. These cells are able to give rise to all cell types found in the embryo and adult animal. Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cell artificially derived from non-pluripotent cell, typically an adult cell with regeneration capability. Active Motif, a biotechnology company that specializes in supplying epigenetic resources and high quality antibodies involved in the regulation and signaling in stem cell biology is looking to extend their product offerings by establishing a stem cell core facility to advance the development of stem cells related products. This project was aimed at establishing a stem cell core facility and validating CDy1 a pluripotent stem cell dye currently sold by Active Motif. This project expands on recent on findings on the use of CDy1 dye for stem cell sorting. Three different stem cell lines, human embryonic stem cells (hESCs), mouse embryonic stem cells (mESCs) and induced pluripotent stem cells (iPSCs) as well as a new batch of CDy1 dye was used for this project. A CDy1 dye that was licensed from a collaborator was used as a control for CDy1 dye produced by Chromeon to test the new batch’s biological equivalency. The results indicate that Chromeon dye is biologically equivalent to the licensed dye and is comparable to live cells alkaline phosphatase staining and fixed cells alkaline phosphatase staining. Based on Immunocytochemistry and Flow cytometry analysis data of CDy1 co-stained with various pluripotency markers, stem cell colonies expressing CDy1 also expressed high levels of pluripotency markers. However the result is inconsistent in iPSC culture as the percentage of cells that expressed both CDy1 and various pluripotency markers were lower than expected. Future work would need to be done to optimize the condition for the iPSCs. The use of CDy1 dye for stem cell isolation and characterization is an invaluable tool for stem cell research.
Rhoda Mondeh-Lowor
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Characterization of Stem Cells using CDy 1 a new fluorescent stem cell-specific dye
Main objectives

- To establish a stem cell culture system.
- To validate CDy 1 a stem cell specific-dye
Established stem cell culture system

- **ES cells require unique conditions**
  - Incubator
  - Tissue culture
  - Unique media/supplements

hESC  mESC
Currently available antibodies stained pluripotent antibodies as expected.
Pluripotent stem cells stained positive for CDy1
CDy 1 staining is comparable to Alkaline Phosphatase staining
Conclusions

- Stem cell culture system was successfully established at Active Motif.
- CDy 1 selectively stained pluripotent stem cell colonies.
- CDy 1 staining is comparable to Alkaline Phosphatase staining and might be a better indicator for pluripotency than AP staining.
- CDy 1 does not stain differentiated stem cells.
Future Direction

- Repeat co-staining by ICC and FACs analysis using different secondary antibodies.
- Perform differentiation analysis to determine if CDy 1 co-stains with early differentiation markers.
- Determine if CDy 1 staining corresponds to a specific lineage during stem cell differentiation.
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Introducing the LumaScope™

Sanford Consortium
For Regenerative Medicine