



2008 Research Grant Program Winning Abstract

Surface Proteins in Stem Cells

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Regenerative medicine, through the use of stem cells, has the potential to repair the heart after a heart attack and to restore cardiac function in patients with heart failure, presently an incurable condition. Hurdles currently faced in the development of these clinical therapies include the limited availability of cell surface protein markers for selecting appropriate, therapeutically viable cell populations. To address this need, we will carry out in-depth analyses of cell surface proteins using novel analytical methods coupled with biochemical and functional characterization during stem cell differentiation into cardiomyocytes, ultimately correlating the function of a pure population of stem cells, or their derivatives, with a panel of defined, accessible, naturally occurring cell surface protein markers. A marker panel for the specific selection, enrichment, and tracking of stem cell populations appropriate for cardiovascular regenerative medicine will be generated.

In order for stem cells to be used in cardiac cell therapy, the cell type (e.g. differentiation stage, lineage) that is best suited for regenerating myocardium must be defined, preferably, using a panel of naturally occurring, accessible proteins located on the cell surface. To date, it has been challenging to identify and quantify proteins specifically located on the cell surface, in part due to a lack of suitable technology that specifically targets these hydrophobic, low abundance proteins. An approach for efficiently discovering new markers, which does not rely on a priori knowledge of the cell surface or the availability/specificity of an antibody, is essential. To achieve this, we are employing a new suite of tools, termed cell surface capturing (CSC-technology), which includes quantitative mass spectrometry and sophisticated bioinformatics. The CSC-technology circumvents the challenges typically posed in the global proteomic analysis of the cell surface by taking advantage of the fact that many, if not most, cell surface proteins are glycosylated. We can selectively and specifically place a chemical tag on the extracellular domain of a cell surface glycoprotein which allows us to purify it away from other, intracellular proteins. The CSC-technology allows for rapid identification of bona fide cell surface proteins, validation of the protein orientation within the membrane, and identification of occupied glycosylation sites; the latter two points being critical for antibody selection and design. Our results to date using this technology to study both embryonic stem cells as well as muscle progenitor cells have revealed more than 200 cell surface glycoproteins, including 75 CD molecules. Importantly, in 90% of the proteins, the glycosylation sites identified were novel. To address our specific aims, we will use the CSC-technology to identify proteins of interest for downstream validation and cell selection using FACS. Additionally, FACS will be used to assess the protein copy number per cell with differentiation. As well, we will move on to assess and characterize selected pure populations both in vitro and in vivo.

Our overall hypotheses are that there are 1) unique sets of protein markers that are expressed on specific cardiomyogenic stem and progenitor cells, and 2) these protein markers can be used for isolating cells that are therapeutically viable. We will use the



model of embryonic stem cell differentiation towards cardiomyocytes to test these hypotheses and identify the marker panels. To achieve this goal, we will undertake a multi-step process, beginning with the identification of cell surface proteins using the CSC-technology. Then, using both antibody-dependent and antibody-independent methods, we will determine how the proteins change with differentiation, isolate cell populations based on these marker panels, characterize the isolated cell populations, and finally, validate that these populations isolated in vitro correlate to functionally equivalent populations in vivo.

In the next two years, we will focus on the initial stages of this process, as outlined in the aims below:

Aim 1: To identify early markers of differentiation which are cardiac lineage restricted or markers of multipotency. We will determine which cell surface glycoproteins are different between undifferentiated mouse embryonic stem cells and their derived cardiomyocytes, and subsequently determine when the glycoproteins that change on the cell surface appear/disappear in the time course of in vitro differentiation.

Aim 2: To determine whether the cell surface protein markers that are specific for the mesodermal lineage in vitro are also present in vivo. We will determine when and where the cell surface glycoproteins are present during in vivo development of the mouse.

The knowledge generated here will be important for future studies that seek to further investigate the therapeutic potential of stem cells. For example, the markers identified in the current study could be used for the selection of specific cell stages for investigating regenerative medicine in an in vivo mouse disease model. More broadly, it might be possible to apply the same strategy used here to adult-derived cardiomyocyte progenitor cells, which would allow for short-term ex-vivo expansion to generate a sufficient number of therapeutically viable cells.

Role of BD reagents: This project will require numerous antibodies to cell surface proteins and known markers for mesoderm and cardiac lineage for use by FACS, immunofluorescence, and western blots; BD Matrigel Matrix Growth Factor Reduced; SPHERO calibration particles; and 96 well plates for the forced aggregation method of differentiating mESC to cardiomyocytes.

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