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FY08 LDRD Final Report Stem Cell Fate Decisions

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Abstract

A detailed understanding of the biological control of fate decisions of stem and progenitor cells is needed to harness their full power for tissue repair and/or regeneration. Currently, internal and external factors that regulate stem cell fate are not fully understood. We aim to engineer biocompatible tools to facilitate the measurement and comparison of the roles and significance of immobilized factors such as extracellular matrix and signaling peptides, synergistic and opposing soluble factors and signals, and cell-to-cell communication, in stem cell fate decisions. Our approach is based on the development of cell microarrays to capture viable stem/progenitor cells individually or in small clusters onto substrate-bound signals (e.g. proteins), combined with conventional antibody and customized subcellular markers made in-house, to facilitate tracking of cell behavior during exposure to relevant signals. Below we describe our efforts, including methods to manipulate a model epithelial stem cell system using a custom subcellular reporter to track and measure cell signaling, arrays with surface chemistry that support viable cells and enable controlled presentation of immobilized signals to cells on the array and fluorescence-based measurement of cell response, and successful on-array tests via conventional immunofluorescence assays that indicate correct cell polarity, localization of junctional proteins, and phenotype, properties which are essential to measuring true cell responses.

Background and Motivation

A stem cell is generally defined as a clonal precursor to more identical stem cells, as well as specialized or differentiated cells of one or more distinct tissue types (e.g., blood cells, skin cells, etc.). [1] As such, stem cells possess two characteristic features: first, the ability to renew themselves, and second, the capacity for multilineage differentiation into specialized cells. [2] (Figure 1). It is accepted that cell fate decisions are governed by both environmental,

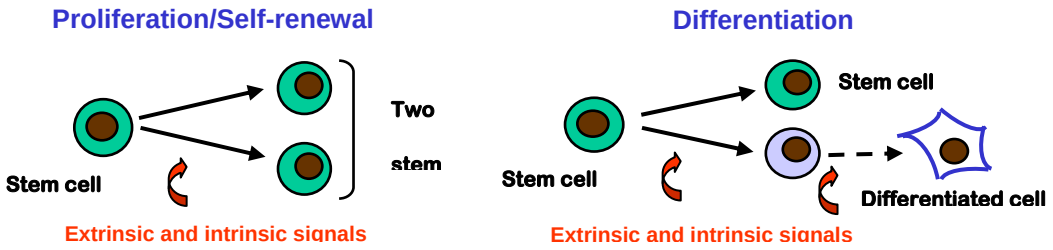

Figure 1. Depiction of the basic concept of stem cell fate decisions.

extrinsic cues (e.g., growth factors, ligands secreted by or attached to other cells, physical stresses, and other external influences) in a deterministic manner, and intrinsic

cell mechanisms (e.g., gene expression) in a stochastic manner. A major goal of current stem cell research is to better understand the molecular factors and signaling pathways that guide stem cell fate transitions. This knowledge will help to determine, ultimately, if intrinsic events place a limit on the ability to control the fates of cells in order to use them for regenerative medicine.[1] Furthermore, this knowledge will help to inform of molecular pathways which contribute to tumorigenesis, as it is well documented that signaling pathways which govern normal cellular development are often dysregulated in cancer. [3]

Stochastic and deterministic regulation of stem cell fate is not well understood. Recent microarray data suggests that stem cells are transcriptionally active and that differentiation occurs by reducing expression of stem cell related genes while increasing lineage-specific gene expression. [4] Interactions between the stem cell and its extracellular environment, or “niche”, which consists of insoluble factors, such as the extracellular matrix (ECM) peptides fibronectin, laminin, and collagen, and soluble signals, such as cytokines and growth factors, are also known to play an essential role in the fate choices of stem cells. [5] There is considerable evidence that cell-cell interactions play an essential role in regulating balance between self-renewal and differentiation and subsequently the long-term fate of cells. [6] Gene expression microarray data also indicates that cell-to-cell contact can lead to the upregulation of genes which regulate cell adhesion and cytoskeletal rearrangement, further suggesting that cross-talk between stem cells and their extracellular environment may be key to the ultimate choice of signaling and differentiation pathways. [5] Adding further to the complexity is recent evidence that both mechanical [7, 8] and electrical [9] signals can also regulate stem cell fate. For instance, a recent report observed changes in gene expression and protein synthesis by mechanical modulation of nuclear shape. [10]

While it is widely recognized that the interactions between stem cells and their extracellular microenvironment play an essential role in cell function and fate, relative to studies on soluble chemical signals, there have been very few investigations of the effects of insoluble factors on stem cell fate. Two studies [11, 12] have demonstrated that it is possible to recreate the cell microenvironment in vitro and measure responses to varied concentrations and combinations of ECM peptides or to receptor-specific ligands for clusters of stem cells in spotted microarray or 96-well formats, respectively. Yet, if we are to fully understand the molecular mechanisms controlling cell fate, measurements at the single cell level, where fate decisions are made, are needed to supplement information gained from cell population level studies. One of the major challenges to accomplishing studies at the single cell level has been a lack of physical tools to complement the powerful molecular biological assays which have provided much of what we currently know about stem cell behavior. Thus, our goal for this project is to combine biocompatible chemical approaches for arraying stem/progenitor cells or cell clusters with the expertise of stem cell biologists who to establish tools for better measurement of cell fate decisions down to the single cell level.

Technical Activities and Results

The Hiddessen laboratory at LLNL has been collaborating with the Pallavicini Lab at University of California, Merced (Merced, CA, USA) to develop compatible cell arraying chemistry and molecular biology tools to study cell fate decisions of model mammary epithelial stem cells on a single cell level. At LLNL, we have developed chemically patterned, microcontact printed arrays that enable experiments where immobilized signals can be presented to cells in a registered array manner in order to modulate signaling pathways that regulate mammary cell fate determination. Our collaborators at UC Merced, graduate researcher H. Bryan and Prof. M. Pallavicini, experts in stem cell biology, have been developing tools to manipulate and track cell fate in a model mouse mammary epithelial stem cell system. Of particular interest with this model cell system is manipulation of the Notch signaling pathway in mammary cells via the use of a Delta-like-1 ligand (which we often refer to more generally in this report as an immobilized signal).

One experimental design consideration important to understanding how extrinsic and intrinsic signals control stem cell fate, and in developing experimental tools to perform such studies, is the fact that many stem cells and their environments have not been characterized. For this reason, our team selected a mouse mammary epithelial cell line as an in vitro model of cell fate decisions. This cell line has been previously characterized and exhibits bipotent differentiation into two distinct lineages, myoepithelial and luminal epithelial. [13] Importantly, markers are available to discriminate among undifferentiated cells and cells that have differentiated into different phenotypes. These markers include cytokeratin 14 (K14) for the myoepithelial type and cytokeratin 8 (K8) for the luminal epithelial type. At Merced, off-array experiments were performed to establish the bipotentiality of a cell line in vitro using cell-type specific immunofluorescent markers prior to experiments on the array platform. Additional off-array experiments to measure the different effects of extracellular matrix peptides (or more generally referred to in this report as immobilized signals) on cell differentiation were conducted with our chosen cell system. The results suggested that, as observed elsewhere [11], different ECM peptides can lead to different cell fates. In our experiments, for example, we observed an increase in differentiation toward the myoepithelial type when cells were grown on fibronectin-coated glass substrates as compared to untreated or laminin-coated substrates.

During the course of this project, as part of our efforts to build a suite of molecular tools, a Notch reporter that can be used to track cell signaling during exposure to extracellular signals in parallel with phenotypic marker expression was constructed by our teammates at UC Merced. The epithelial cells were successfully transected with this construct, as determined by microscopy and fluorescence flow cytometry measurements (data not shown) that showed a fraction cells as GFP+ (indicating active Notch signaling). A custom fusion protein construct containing the Delta-like-1 ligand was also created by our team at Merced. These molecular tools are being applied in continuing and future experiments.

At LLNL, as part of our engineered array development goals, we developed a patterning chemistry that was compatible with mouse mammary epithelial stem cells, which had

previously proven resistant to directed patterning. Using standard microcontact printing techniques, we tested various PEG polymer derivatives as suitable matrices to prevent the mammary epithelial cells from overgrowing their intended capture sites in the array. We identified one polymer that made it possible to maintain an array of mouse mammary epithelial cells for capture sites ranging from 10 μm to 200 μm in diameter.

Using this patterning chemistry, we introduced the epithelial stem cells to our arrays and tested for cell viability, polarity and phenotype. We verified that these critical cell properties could be successfully assessed and also successfully maintained after cell capture onto the array. We have performed assays with arrayed cells and demonstrated that the mammary cells are able to grow on the array and exhibit proper cell polarity and correct localization of junctional proteins. Additionally, cell phenotype can be assessed by immunofluorescence (images not shown). To the best of our knowledge, this is the first demonstration of viable arraying and on-array, fluorescence based assays of this particular model mammary epithelial stem/progenitor cell line. The array capability combined with the aforementioned molecular tools holds promise for more detailed studies into the molecular control of stem cell fate down to the single cell level.

Conclusions and continuing research

We developed many custom tools during the course of this project, including custom molecular biology tools, arrays with surface chemistry that support viable model stem/progenitor cells, and demonstrated on-array immunofluorescence assays that indicate cell viability, correct cell polarity and phenotype, properties which are essential to measuring true cell responses. Additional details and images for the findings documented in this report are being included in a manuscript that is currently in preparation.

The completion of this project has resulted in several important tools (some of which were lacking or needing improvements) that will be applied in continuing and future work. The efforts contributed to one successful, collaborative (Merced/LLNL) extramural grant and are being used to compete for additional funding. Future research plans will focus on building a more detailed understanding of how mammary stem/progenitor cell fate decisions are controlled and influenced by their extracellular environment. Such knowledge holds promise for determining if and/or how dysregulation of mammary cell fate decisions plays a role in breast cancer development.

References

1. O'Neill, A. and D. Schaffer, *The biology and engineering of stem cell control.* Biotechnology and Applied Biochemistry, 2004. **40**: p. 5-16.

2. Preston, *The new stem cell biology: something for everyone.* Journal of Clinical Pathology: Molecular Pathology, 2003. **56**: p. 86-96.

3. Liu, S., *Mammary stem cells, self-renewal pathways, and carcinogenesis*. Breast Cancer Research, 2005. **7**: p. 86-95.

4. Akashi, K., *Transcriptional accessibility for genes of multiple tissues and hematopoietic lineages is hierarchically controlled during early hematopoiesis.* Blood, 2003. **101**: p. 383-390.

5. Ho, A., *Kinetics and symmetry of divisions of hematopoietic stem cells.* Experimental Hematology, 2005. **33**: p. 1-8.

6. Punzel, M., *The symmetry of initial divisions of human hematopoietic progenitors is altered only by the cellular microenvironment.* Experimental Hematology, 2003. **31**: p. 339-347.

7. Estes, B., *Mechanical signals as regulators of stem cell fate.* Current Topics in Developmental Biology, 2004. **60**: p. 91-126.

8. McBeath, *Cellshape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment.* Developmental Cell, 2004. **6**: p. 483-495.

9. McLeod, K., *Electric fields modulate bone cell function in a density dependent manner.* Journal of Bone and Mineral Research, 1993. **8**: p. 977-984.

10. Thomas, C.H., et al., *Engineering gene expression and protein synthesis by modulation of nuclear shape.* Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(4): p. 1972-1977.

11. Flaim, C., *An extracellular matrix array for probing cellular differentiation.* Nature Methods, 2005. **2**: p. 119-125.

12. Beckstead, B., *Mimicking cell-cell interactions at the biomaterial-cell interface for control of stem cell differentiation.* Journal of Biomedical Materials Research Part A, 2006: p. 94-103.

13. Stingl, J., Phenotypic and functional characterization in vitro of a multipotent epithelial cell present in the normal adult human breast. Differentiation, 1998. **63**: p. 201-213.