Engineering Notch Signaling Activation for Generating Human Embryonic Stem Cell-Derived Cardiomyocytes

Jason C. Tung, Charles E. Murry, Buddy D. Ratner, Cecilia M. Giachelli
University of Washington, Seattle, WA USA

Statement of Purpose: In the fields of regenerative medicine and tissue engineering, control of cell fate by biological surface modification of has garnered attention for the ability to create biomimetic microenvironments. Attention has focused on presenting extracellular matrix proteins and growth factors for control of cellular fate as few studies have reported presenting cell surface ligands on biomaterials. The Notch signaling pathway represents a promising target as this cell-to-cell surface signaling pathway regulates cell fate decisions and tissue formation during development. We have engineered Notch-signaling technologies for use in generating human embryonic stem cell (hESC)-derived cardiomyocytes. These non-genetic, time-specific, and activation-tunable approaches allow us to investigate the role of Notch signaling at key junctions of hESC-derived cardiomyocyte development. By determining the role of Notch signaling in generating hESC-derived cardiomyocytes, these studies aim to provide insight for improving upon current techniques for generating cardiomyocytes and developing new directions for creating treatments for myocardial infarction.

Methods: *Fabrication of Notch-signaling surfaces:* Rat Jagged-1/Fc (R&D Systems, Minneapolis, MN) was indirectly immobilized as follows. A 20 ug/ml solution of anti-polyHistidine (R&D Systems, Minneapolis, MN) was incubated overnight at 4°C on tissue culture polystyrene. After washing, surfaces were blocked with bovine serum albumin. Following washing, surfaces were incubated with 7.5ug/ml solution of Jagged-1 for 2 hours at 4°C. Randomly-oriented Jagged-1 surfaces were generated by incubation with 7.5 ug/ml of Jagged-1 overnight at 4°C.

hESC Differentiation Assay: H7 human embryonic stem cells were plated on Matrigel™, anti-polyHistidine, or oriented Jagged-1 surfaces. RNA isolated over the course of 8 days was used for qPCR analysis of gene expression for HES1, Oct4, BrachyuryT, FoxA2, and Sox1.

Cardiovascular Progenitor Cell Differentiation Assay: Kinase insert domain protein receptor (KDR) cardiovascular progenitor cells were generated, isolated, and cultured using a previously described embryoid body-based differentiation system (Yang L, Nature. 2008; 453:524-528.). Cells were plated on Matrigel™, anti-polyHistidine, or oriented Jagged-1 surfaces for 2 weeks and then dissociated for fluorescence-activated cell sorting to determine the cell population expressing the cardiac lineage marker cardiac troponin T (cTnT).

hESC-Derived Cardiomyocyte Proliferation Assay: Cardiomyocytes generated via the embryoid-body differentiation system were plated on gelatin, anti-polyHistidine, randomly-oriented Jagged-1, or oriented Jagged-1 surfaces. After cells adhered, cultures were serum-starved for 1 day. Groups receiving 5 uM gamma secretase inhibitor (Sigma-Aldrich, USA) were treated for 2 days, and on the 2nd day, all groups received 10 umol/L BrdU. Cells were methanol fixed and double-stained for BrdU and beta-myosin heavy chain (MHC).

Results: *Notch activation in hESCs increases ectodermal gene expression*: Compared to control surfaces, oriented Jagged-1 surfaces induce Notch signaling as demonstrated by a 2-fold increase at day 1 in the expression of the Notch gene, HES1. Significant differences in genes related to pluripotency (Oct4), mesoderm (BrachyuryT), and endoderm (FoxA2) were not observed on the various surfaces. However, at day 2, cells plated on oriented Jagged-1 surfaces exhibited a 3-fold increase in Sox1 ectodermal gene expression. These results demonstrate that Notch activation in undifferentiated hESCs serves to promote ectodermal gene expression, but not maintenance of pluripotency or formation of mesoderm or endoderm.

Notch activation in KDR+ cells promotes cardiac differentiation: Plating KDR+ progenitor cells on oriented Jagged-1 surfaces resulted in roughly 46% cTnT+ cells, nearly a 3-fold increase compared control surfaces. This finding suggests an important role for Notch signaling in guiding mesodermal progenitors towards a cardiac fate.

Notch activation in hESC-derived cardiomyocytes induces cell proliferation: Oriented Jagged-1 surfaces resulted in an increase in proliferation (~56% proliferating compared to ~40% on control surfaces). Treatment with gamma-secretase inhibitor reversed this proliferative effect, demonstrating that the observed increase in proliferation is Notch-mediated. Gamma secretase inhibitor treatment of cells on control surfaces decreased the proliferative population to ~33%, implying that active Notch signaling is partially responsible for endogenous levels of proliferation. In comparison to oriented Jagged-1 surfaces, only ~43% of cardiomyocytes on randomly-oriented Jagged-1 surfaces were BrdU+. This difference between oriented and randomly-oriented Jagged-1 surfaces may be attributed to more effective Notch activation as a result of ligand orientation.

Conclusions: The aim of this study was to engineer Notch-signaling technologies to investigate the role of Notch at key time points of hESC-derived cardiomyocyte development. Oriented Jagged-1 surfaces allowed us to determine that Notch activation promotes ectodermal gene expression in undifferentiated hESCs, improves cardiac differentiation of KDR+ progenitor cells, and induces proliferation of hESC-derived cardiomyocytes. These studies demonstrate a key role for the Notch pathway in generating cardiomyocytes from hESCs. Future studies will investigate the mechanism of Notch-mediated effects, continue working on improving upon current methods for generating cardiomyocytes, and determine the ability of Notch-signaling materials to be used as treatments for myocardial infarction.