Signaling Pathways during Newt Heart Regeneration

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Abstract

Substantial injury to mammalian hearts results in the loss of contractile tissue, fibrosis and scar formation due to the hearts' limited ability to form new cardiac tissue. Unlike mammalian hearts, certain non-mammalian vertebrates, like the red-spotted newt (*Notophthalmus viridescens*), have the ability to regenerate myocardium and restore functionality. Previous studies have shown that the process of newt heart regeneration is associated with the strong downregulation of sarcomeric proteins and cardiac-specific genes, suggesting that the cardiomyocytes dedifferentiate in order for the proliferation of new cardiomyocytes to occur. In order to further examine the regenerative properties of the newt heart, we will isolate and culture cardiomyocytes from newt ventricles to investigate the signaling pathways involved in this regenerative process. This will be accomplished utilizing the different inhibitors for specific pathways such as Sonic hedgehog and Notch pathways. Furthermore, we will perform BrdU incorporation studies in the presence/absence of these inhibitors and evaluate the proliferative potential of the cardiomyocytes. It is assumed that the cardiomyocytes undergo dedifferentiation followed by proliferation to accomplish the regeneration. To validate this, we will perform immunohistochemical analysis for MyHC, cTnT and Nkx2-5. Our study would provide interesting insights about regenerative pathways which may be utilized to enhance the mammalian heart regeneration.

Hypothesis

Cardiomyocytes isolated from adult newt have an increased proliferative capacity.

To test this hypothesis, we will isolate cardiomyocytes from the adult newt and evaluate their proliferative capacity *in vitro*. Therefore our protocol is outlined below.

Research Protocol

- Surgically dissect hearts from newts under sterile conditions. Remove the bulbus cordis and auricle. Place ventricles in 70% Leibovitz-15 medium supplemented with penicillin-streptomycin.
- Transfer to an enzyme solution of amphibian PBS containing 50 µg/ml gentamicin, 0.5% trypsin, 380 U/ml collagenase, 0.15% bovine serum albumin, and 0.3% glucose.
- Collect cells at 15 minute time intervals for 10 intervals. Pre-plate collected cells on 6-well tissue culture dishes for 16 hours or 48 hours.
- Transfer unattached cells to laminin coated plates.
- Add bromodeoxyuridine to culture medium and stain with antibody to determine baseline value for proliferation and over time course.
- Add cyclopamine (a Sonic hedgehog signal inhibitor) or DAPT (a Notch signal inhibitor) and observe the changes in proliferation.
- Perform immunohistochemical analysis using antibodies to Nkx2-5, MyHC, cTnT. After staining protocol is established, collect cells over time course for Western blot analysis and quantitate signal.

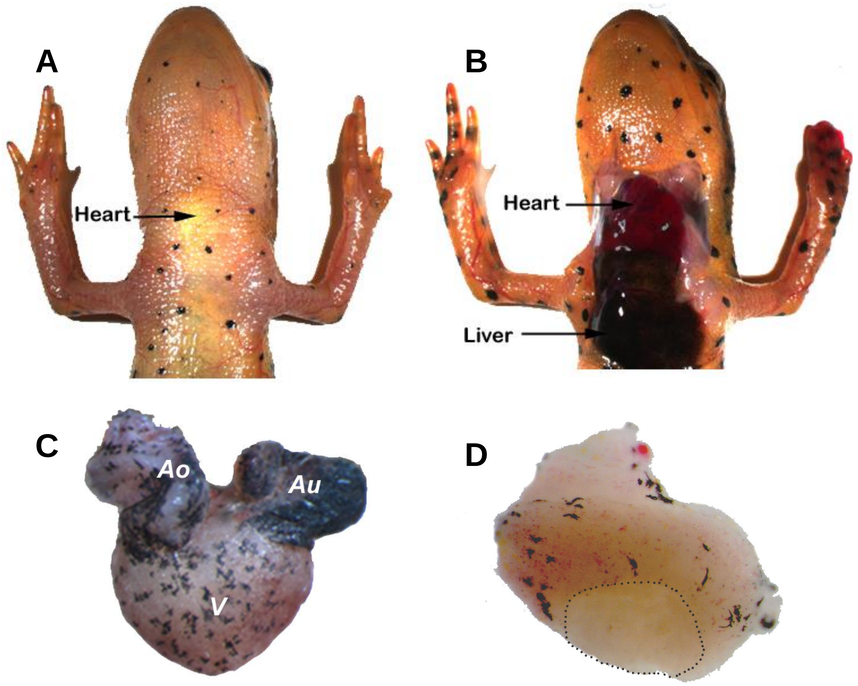

Figure 1. Newt heart. Panel A The ventral view of the adult newt. The location of the heart is shown by black arrow. **Panel B** shows the exposed heart and liver located near the throat. **Panel C** depicts the different parts of the heart, namely **Ao**: Aorta (bulbus cordis); **Au**: Auricle; **V**: Ventricle. For cardiomyocyte isolation, the aorta and auricle were removed prior to enzymatic digestion. **Panel D** 1 day old culture of resected (~30%) newt ventricle.

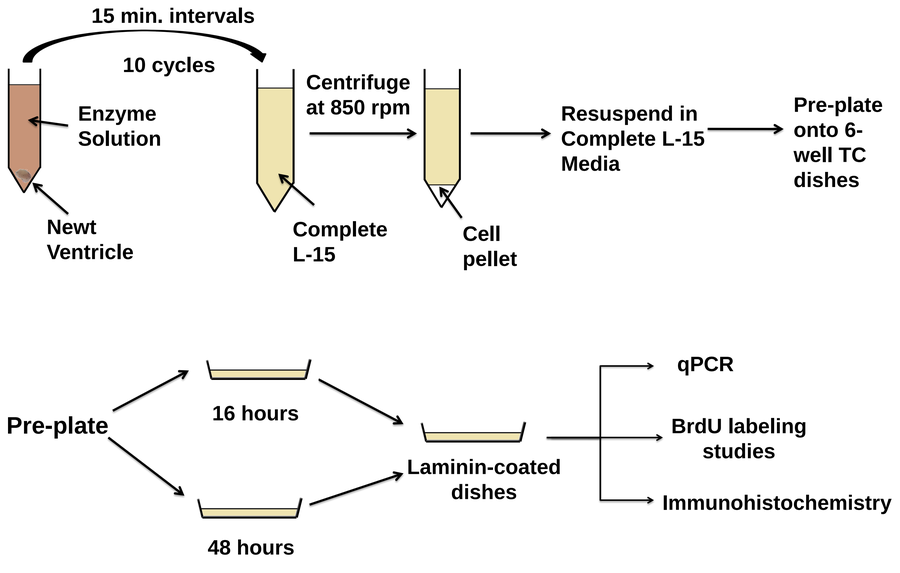

Figure 2. Isolation of cardiomyocytes from whole ventricles.

Primary Cultures of Newt Cardiomyocytes

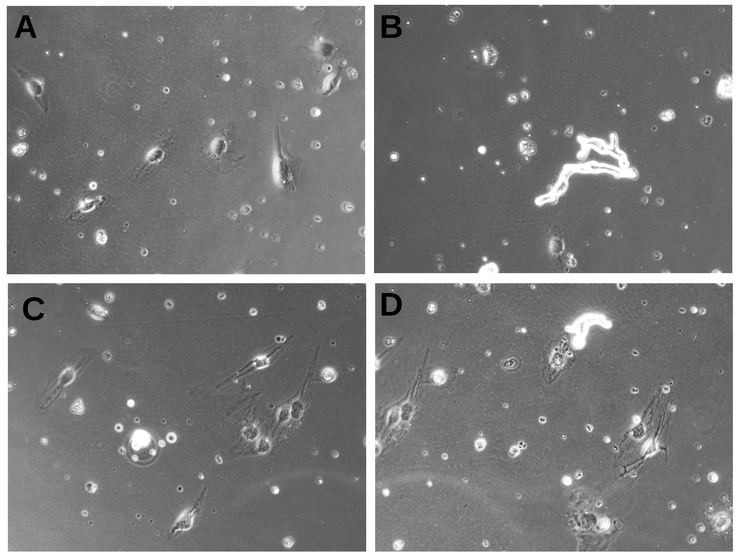

Figure 3. Primary cultures of newt cardiomyocytes. Panel A and C show the 4 day old culture of attached ventricular myocytes. **Panel B and D** depict the cells in suspension after 4 days of culture.

Signaling Pathways Involved in Heart Regeneration

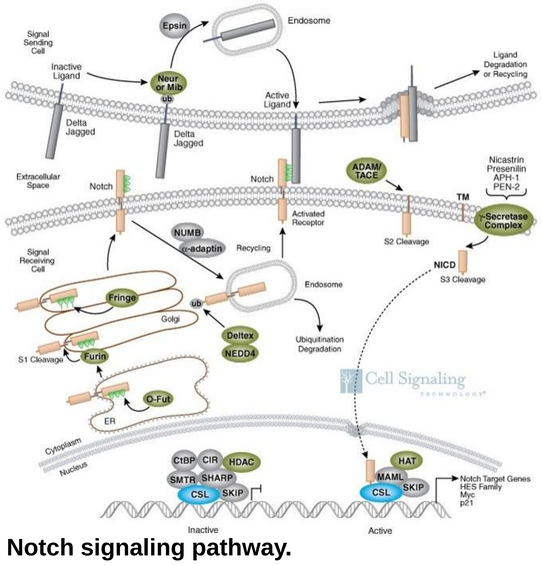

Figure 4. Notch signaling pathway.

Future Experiments

- To determine the proliferative capacity of newt cardiomyocytes *in vivo* and *in vitro.*
- To determine the functional role of the Notch signaling pathway in myocardial regeneration (*in vivo*).
- To determine the functional role of the Notch signaling pathway on cardiomyocyte proliferation (*in vitro*).