Characterizing the Transcriptional Regulation of Cardiac Fibroblast Activation

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INTRODUCTION

- Congenital heart defects (CHD) are a group of structural malformities in the heart and/or large surrounding blood vessels that appear at birth (Lim et al. 2021).
- CHDs are the most common birth defect, accounting for up to 40% of stillbirths in North America, yet only 20-30% have known causes (Lim et al. 2021).
- Cardiac fibroblasts are support cells required for proper development of the heart, so increasing our understanding of cell signaling in cardiac fibroblasts may illuminate how CHDs develop (Doppler et al., 2017).
- Two related transcription factors, zinc finger E-box binding protein-1 (ZEB1) and -2 (ZEB2), play a role in epithelial-to-mesenchymal transitions in developing heart cells, which is important for heart valve development (Quijada et al. 2020).

AIMS

- The goal of this project is to further elucidate the roles of ZEB1 and ZEB2 in fibroblast activation to better understand the mechanisms leading to CHD development.

METHODS

- Two model systems, the mouse fibroblast cell line NIH 3T3 and primary rat cardiac fibroblasts (PRCFs) were used.
- NIH 3T3 cells were used to verify the specificity of the ZEB1 and ZEB2 antibodies while PRCFs were used to model human cardiac fibroblast activation.
- PRCFs were isolated from male Sprague-Dawley rat hearts and activated by plating cells on hard plastic tissue culture plates. Activation was measured by monitoring the protein levels of the extracellular periostin and intracellular alpha-smooth muscle actin, two markers in activated fibroblasts.

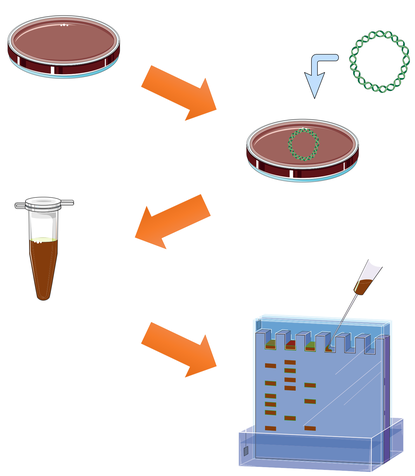


Figure 1. Verification of the anti-ZEB2 antibody. A) NIH 3T3 cells were cultured to 60% confluency. B) Cells were transfected with pcDNA3-ZEB2 vector containing an HA-tag using Lipofectamine 3000. C) After 24 hours, cell lysates were harvested and stored in 1.5mL tubes. D) Lysate samples were then run on SDS-PAGE gels, transferred to a nitrocellulose membrane, and blotted for HA-tagged ZEB2.

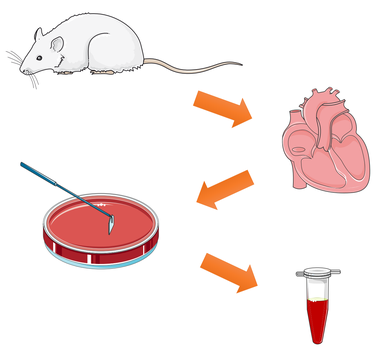


Figure 2. Primary rat cardiac fibroblast studies. Male Sprague-Dawley rats were anesthetized and the hearts harvested under sterile conditions. The heart was digested in collagenase and plated on hard plastic tissue culture plates in DMEM/F12 supplemented with fetal bovine serum and penicillin/streptomycin. After 48hr or 96hr, cells were lysed and manually scraped from the plate. Lysates were clarified by centrifugation.

RESULTS

- The HA-tagged ZEB2 vector was successfully transfected into NIH 3T3 cells and verified by Western Blotting (see Figure 3).
- PRCFs have been successfully isolated by digestion with collagenase type II and subsequently cultured on hard plastic plates.
- Cell lysates have been harvested and media samples collected from these plates at 48hr and 96hr timepoints.
- PRCFs have been imaged on a light microscope (see Figure 4).

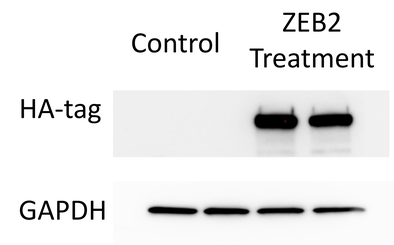
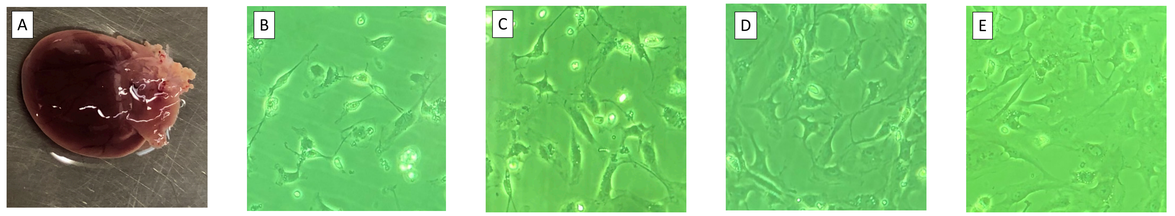


Figure 3. Western Blot of NIH 3T3 whole cell lysates comparing cells transfected with pcDNA3-ZEB2 and a control. Transfection was performed using the Lipofectamine 3000 protocol from ThermoFischer Scientific. The pcDNA3-ZEB2 transfection vector contained an HA-tag that was probed for during Western Blotting. An increase in HA-tagged ZEB2 was found after transfection, confirming that the transfection was successful. GAPDH is used as a loading control.

Figure 4. Light microscope images of a male Sprague-Dawley rat heart (A) and primary rat cardiac fibroblasts (PRCFs) (B-E). Cells were plated on hard plastic in DMEM/F12 media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. PRCFs were imaged at the following timepoints relative to when the rat heart was harvested: B) 22hr, C) 28hr, D) 46hr, E) 50hr.



CONCLUSION and NEXT STEPS

- PRCFs have been successfully isolated and cultured to 48hr and 96hr timepoints.
- Next, ZEB1 and ZEB2 levels in PRCFs at 24hr, 48hr, and 96hr timepoints will be examined by Western Blot.
- Future experiments will include knockout studies of ZEB1, ZEB2, and ZEB1/ZEB2 in PRCFs using siRNA specific to the two proteins.
- Experiments will also be performed in female Sprague-Dawley rats.

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