Characterizing the Transcriptional Regulation of Cardiac Fibroblast Activation

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INTRODUCTION

- Congenital heart defects (CHDs) are a group of conditions where an infant's heart is not properly formed at birth. CHDs cause almost half the stillbirths in North America each year but only 25% of all cases have a known cause[1].
- Heart valve formation depends on cardiac fibroblast (CF) differentiation[2]. Two related transcription factors, zinc finger E-box binding protein-1 (ZEB1) and ZEB2, drive CF differentiation[3].
- ZEB2 also contributes to CF activation, which causes CFs to adopt a myofibroblast phenotype that is linked to cardiovascular disease[4].
- Myofibroblasts have increased size, contractile proteins like α-smooth muscle actin (α-SMA), and secretion of extracellular proteins like periostin[4].

Objective: Gain a deeper understanding of ZEB1 and ZEB2 signalling in cardiac fibroblast activation to illuminate how CHDs develop.

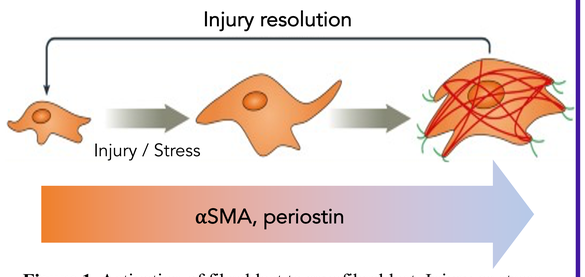

Figure 1. Activation of fibroblast to myofibroblast. Injury or stress trigger activation, which causes expression of αSMA and periostin. Image source: Tallquist and Molkentin, 2017.

METHODS

- NIH 3T3 cells will be used to verify ZEB1 and ZEB2 antibody specificity for Western Blot experiments and siRNA molecules for knockdown experiments.
- ZEB1 and ZEB2 protein levels will be analyzed by Western Blot in activated and non-activated primary rat cardiac fibroblasts (PRCFs), which model human CFs. The effects of ZEB1, ZEB2, and ZEB/ZEB2 knockdown on PRCF activation will be measured by Western Blot.
- PRCF activation will be measured by monitoring extracellular periostin and intracellular α-SMA protein levels by Western Blot.

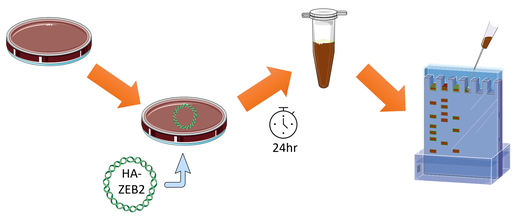

Figure 2. Verification of anti-ZEB1 and anti-ZEB2 antibodies. NIH 3T3 cells were transfected with a tagged-Zeb1 or tagged-Zeb2 DNA vector using Lipofectamine 3000. Cells were harvested after 24 hours by lysis and manual scraping. Lysates were probed by Western Blot for FLAG- and HA-tags and ZEB1 or ZEB2, respectively.

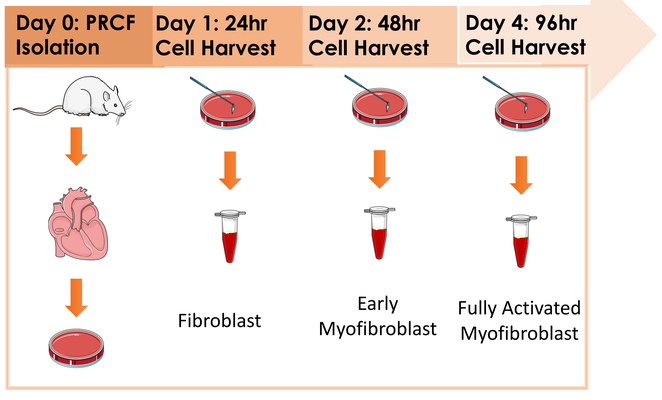

Figure 3. Primary rat cardiac fibroblast isolation and harvest. Hearts were harvested from male Sprague-Dawley rats then digested in collagenase to isolate single cells. Cells were activated by plating on hard tissue culture plates. After 24hr, 48hr, or 96hr, cells were lysed and stored in 1.5mL tubes.

RESULTS

- A band in the ZEB2 treatment lanes is seen at the same weight (~170kDa) when probing for HA-tag and ZEB2 (see Figure 4).
- PRCF lysates have been harvested at 24hr, 48hr, and 96hr timepoints (see figure 5).
- Intracellular levels of α-SMA at 24hr, 48hr. and 96hr timepoints were measured by Western Blot (see Figure 6).

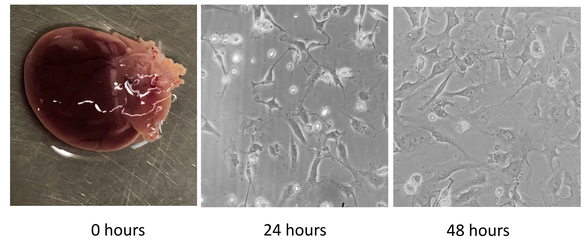

Figure 5. Light microscope images of a male Sprague-Dawley rat heart (A) and primary rat cardiac fibroblasts (B-C). Cells were plated on hard plastic. Primary rat CFs were imaged at 24 and 48 post harvest of the rat heart.

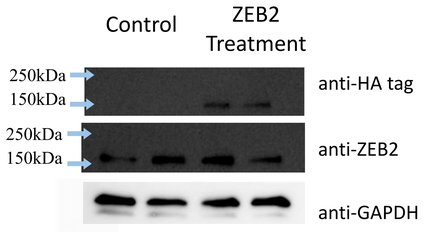

Figure 4. Western Blot of clarified NIH 3T3 cell lysates. A control (empty DNA vector) and a ZEB2 treatment (DNA vector containing HA-tagged ZEB2) are compared. Transfection performed using the Lipofectamine 3000 protocol from ThermoFischer Scientific. GAPDH is shown as a loading control.

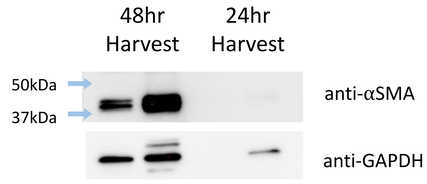

Figure 6. Western Blot of primary rat cardiac fibroblasts at 48hr and 24hr timepoints. αSMA was only detected in the 48hr harvest. GAPDH is shown as a loading control.

CONCLUSION

- Anti-ZEB2 antibody has been successfully verified.
- Acquisition of myofibroblast phenotype in PRCFs has been demonstrated.

NEXT STEPS

- Verify the anti-ZEB1 antibody.
- Examine ZEB1, ZEB2, and periostin levels in PRCFs at 24hr, 48hr, and 96hr by Western Blot.
- Knockdown ZEB1, ZEB2, and ZEB1/ZEB2 in PRCFs using siRNA specific to the two genes.
- Perform experiments in PRCFs isolated from female Sprague-Dawley rats.

ACKNOWLEDGEMENTS

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