Deciphering Roles of Zinc Finger E Homeobox Binding-1 (ZEB1) and ZEB2 in Cardiac Fibroblast Activation

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INTRODUCTION

- Cardiac fibroblasts (CFs) have a large role in heart valve formation (Figure 1).
- The myofibroblast is a contractile, secretive CF phenotype that produces heart valve proteins and mechanically regulates valve structure during heart development.
- Dysregulated myofibroblasts pathologically remodel the heart from excess protein secretion, possibly causing congenital heart defects (CHDs) during development.
- Understanding CF activation will increase the understanding of the genetic mechanisms underlying CHD development.
- **Rationale**: ZEB1 and ZEB2, transcription factors essential for EMT and downstream targets of TGFβ (a known activator of CFs), may control CF activation.
- **Objective**: decipher the roles that ZEB1 and ZEB2 have in CF activation.

CHDs are the **#1** cause of infant mortality, while **80%** of CHD origins are unknown

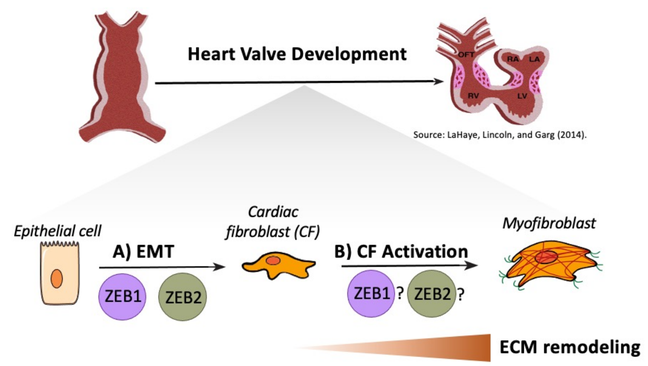


Figure 1. Heart valve development uses epithelial-to-mesenchymal transition (EMT) to make cardiac fibroblasts (CFs), which when activated to myofibroblasts through CF activation, secrete proteins needed for valve structure. αSMA and periostin are markers of myofibroblasts.

METHODS

1. Overexpress and knock down ZEB1 and ZEB2 in embryonic fibroblast.

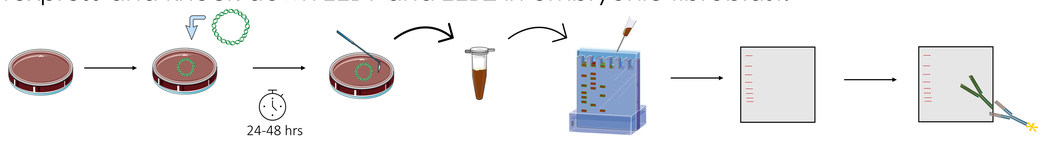


Figure 2. Process of knocking down or overexpressing ZEB1 or ZEB2 in the NIH3T3 or primary rat CF (PRCF) model. NIH 3T3 cells (a common embryonic fibroblast model) and PRCFs were transfected with HA-ZEB2 for ZEB2 overexpression or siRNA specific for ZEB1 or ZEB2. Cells were harvested and analyzed by Western blot 24-48hrs after transfection.

2. Compare ZEB1 and ZEB2 protein expression trends during primary rat CF activation.

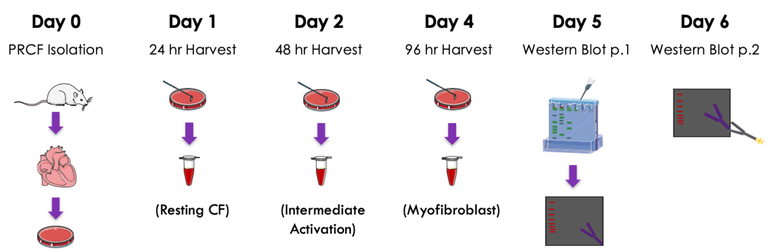


Figure 3. PRCFs were isolated by digesting rat heart ventricles harvested from female or male Sprague-Dawley rats in collagenase Type II. PRCFs were activated by plating on hard tissue culture plates. Cells were harvested at 24hr, 48hr, or 96hr timepoints, representing 3 stages of CF activation, then analyzed by Western blot.

RESULTS

1. Overexpression and knockdown of ZEB1 and ZEB2.

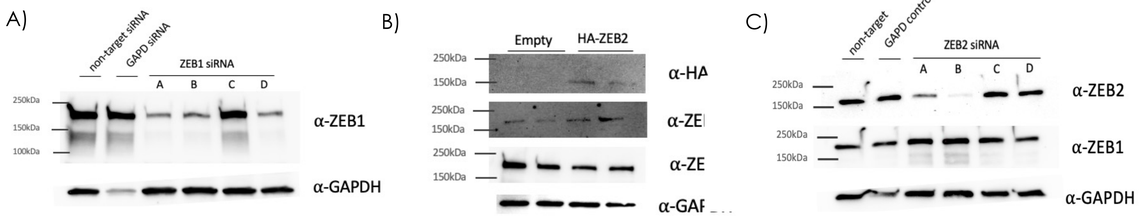


Figure 4. Western blot images of A) ZEB1 knockdown using ZEB1A-D siRNA, non-target siRNA (negative control), and mouse GAPDH siRNA (positive control) in NIH 3T3s, B) ZEB2 overexpression using pcDNA3.1 Empty and HA-ZEB2 vectors in PRCFs, and C) ZEB2 knockdown using ZEB2A-D, non-target siRNA, and rat GAPD siRNA in PRCFs. GAPDH is shown as a loading control.

2. ZEB1 and ZEB2 protein expression trends during PRCF activation.

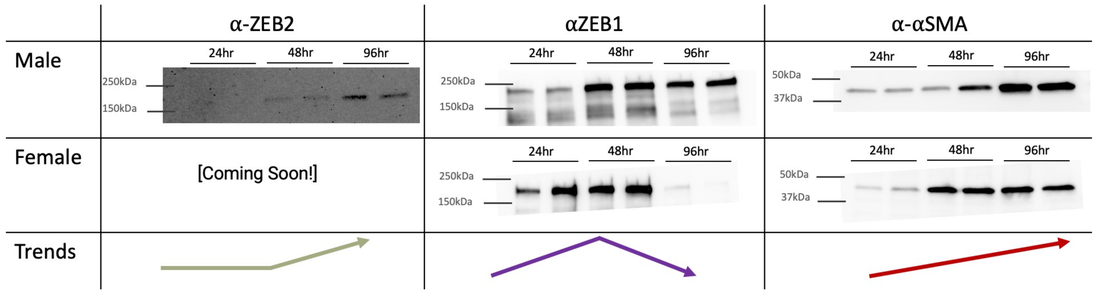
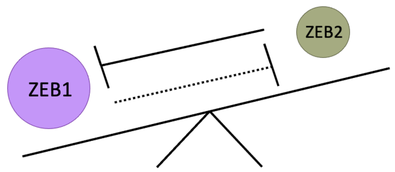


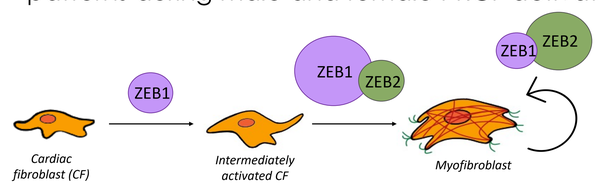
Figure 5. Western blot images and trends of α-ZEB1, α-ZEB2, and α-αSMA proteins in PRCFs isolated from male and female ventricles at three timepoints (24hr, 48hr, and 96hr) representing resting, intermediately activated, and activated CFs. Biological timepoints were run in duplicate.

CONCLUSIONS

1. ZEB2 displays negative autoregulation of ZEB1 protein expression.



2. ZEB1 and ZEB2 have different protein expression patterns during male and female PRCF activation.



FUTURE DIRECTIONS

1) Identify specific ZEB1 and ZEB2 roles in CF activation.
2) Alter Zeb1 and Zeb2 *in vivo* in disease and development models.

ACKNOWLEDGEMENTS

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