Identification of two distinct pro-epicardial Populations during development

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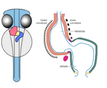
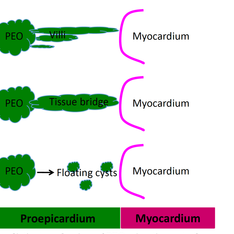
Introduction

The epicardium is the outer most layer of the heart and plays crucial roles in cardiac development and cardiac wound healing. The epicardium arises from precursor cells in the proepicardial organ (PEO) that forms around the base of the cardiac inflow tract. The location and initial symmetry is conserved across species despite morphological differences. Three cellular mechanisms of proepicardial migration have been proposed. Using zebrafish as a model organism, we have identified a combinatorial mechanism of PEO migration along with two molecularly and behaviorally distinct PEO populations.

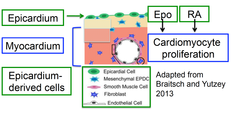
Background



During zebrafish development, the myocardium and endocardium for a contractile tube with a distinct atrium and ventricle. At about 32 hour development, the proepicardial organ (PEO) can be seen at the back of the pericardial cavity as a cluster of cells at the boundary between the atrium and ventricle. By 6 days post fertilization the myocardium is fully encapsulated in an epicardial layer. These cells will go on to produce a wide array of cell types in the adult fish.



Three distinct mechanism of PEO migration onto the myocardium have been proposed in an array of organism. A villi model, a tissue bridge model and a floating cyst model. We show evidence for both a villi and floating cyst model in zebrafish. Interestingly, these two behaviors drive coverage of the ventricular myocardium. We also identify a population of PEO that migrates directly onto the atrial myocardium to for the atrial epicardium.



Early Atrial and Late Ventricular PEO Migration

At 35 hours post fertilization (hpf), the PEO can be seen (green) to cover the base of the atrium, over the course of the next several hours, the atrium becomes progressively covered with epicardium, in an atrial to ventricular direction. The majority of the atrium is covered with epicardial cells before any cells appear on the ventricular surface. At 47 hpf, cells can be seen migrating up the back of the pericardial cavity and forming villi. Shortly after, epicardial cells can be seen on the surface of the ventricle. These cells appear as individuals. The heart is fully covered in epicardial cells at 6 days post fertilization (dpf).

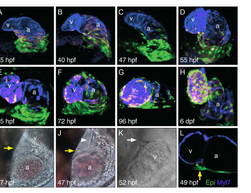
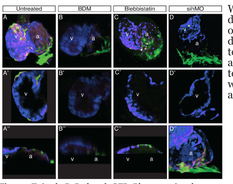
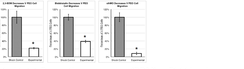


Figure: (A) Atrial proepicardium has begun to migrate. (B) Near full coverage of the atrial myocardium. (C) Proepicardial cells near the atrial-ventricular junction. (D-H) Partial to full ventricular epicardium coverage. (I-K) Multi-cellular proepicardial villi (yellow arrows), white arrowhead and arrows indicate transferred proepicardial cell and villi respectively. (L) proepicardial villous (yellow arrow). hpf=hours post fertilization, v=ventricle, a=atrium.

Inhibition of Cardiac Contractions Disrupts Villi Transfer



We hypothesize that the ventricular epicardial cells derive from the villi and are dependent on contraction of the heart for their migration while the atrial epicardial cells migrate directly onto the atrial surface. To test this we use two chemical (BDM and Blebbistatin) and one genetic mechanisms (*silent heart* morpholino) to inhibit contraction. Regardless of which mechanism we use, the ventricular epicardium is reduces. Is the atrial epicardium also impacted?



Figures: Tg(myl7:DsRed;wt1b:GFP- Blue; green) embryos were treated with 2,3-Butanedione monoxime (BDM) or Blebbistatin or sihMO to inhibit cardiac contractions. Significantly fewer wt1b:GFP positive PEO cells migrate to the ventricular myocardium than control. However, villi and atrial epicardium persist.

Novel Atrial Mechanism Independent of Cardiac Contractions

To assess the requirement for cardiac contractions for atrial epicardial formation, we repeated the experiment above and evaluated atrial epicardial formation. We find little to no impact of loss of contraction on formation of the atrial epicardium.

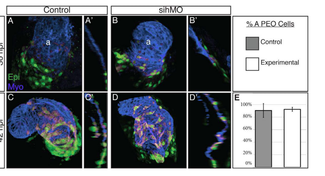
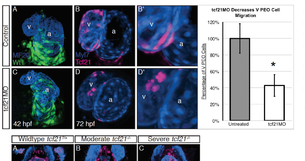
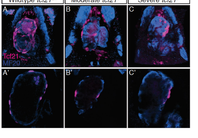


Figure: (A-D) Ventral views, (A'-D') Optical sections through atrium. Atrial proepicardium cells migrate to the atrial myocardium in sihMO embryos. Early atrial proepicardium migration is independent of cardiac contractions and ventricular epicardium formation. Proepicardial migration to the atrium has not previously been described in zebrafish.

tcf21 Promotes Ventricular Epicardium Coverage





We identified a gene predominantly expressed in the ventricular epicardium. *tcf21* is ventricular epicardium specific until late in epicardial development at which point its expression increases in the atrial epicardium. To explore the role of *tcf21* in epicardial development we examined epicardial development in both the *tcf21* morpholino and the *tcf21* mutant embryos. While the epicardium is present, ventricular epicardium is very patchy and the cells appear less tightly adhered to the myocardial surface suggesting *tcf21* plays a role in cell migration or adhesion but is not necessary for ventricular epicardial fate.

Figure: (A,C) Early atrial proepicardium migration is independent of *tcf21*. (B,D) when *tcf21* is knocked down, ventricular epicardium is significantly reduced (Lower Panel). Similar ventricular epicardium defects are found in *tcf21* null embryos.

Right-sided Asymmetry of Ventricular Proepicardial cells

In some organisms, the PEO is bilaterally symmetrical while in others it is restricted to the right side. In zebrafish we found that the atrial epicardium is symmetrically organized while the ventricular epicardium is restricted to the right side. This finding has interesting implications for evolution and PEO specification.

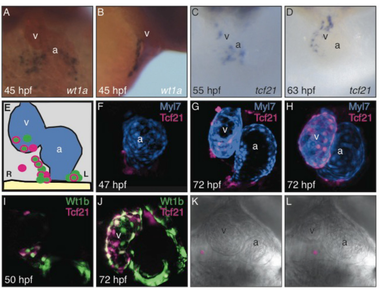
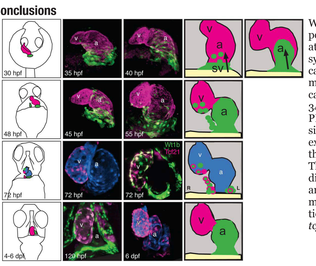


Figure: (A-D) in situ hybridization and reporter protein expression (F-J) indicate that ventricular PEO displays a right-sided asymmetry. (K,L) floating transgenic proepicardial cells are tcf21:DsRed positive.

Conclusions



We have identified two distinct populations of epicardium. The atrial population is bilaterally symmetrical and migrates in a cardiac contractile independent mechanism directly onto the myocardial surface starting at about 34 hpf. The second population of PEO cells is located on the right side of the primitive heart tube, expresses *tcf21* and migrates up the back of the pericardial cavity. The cells form villi, that via a cardiac contractility dependent mechanism transfer to the ventricular myocardial surface. This migration is disrupted in the absence of *tcf21* expression.

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