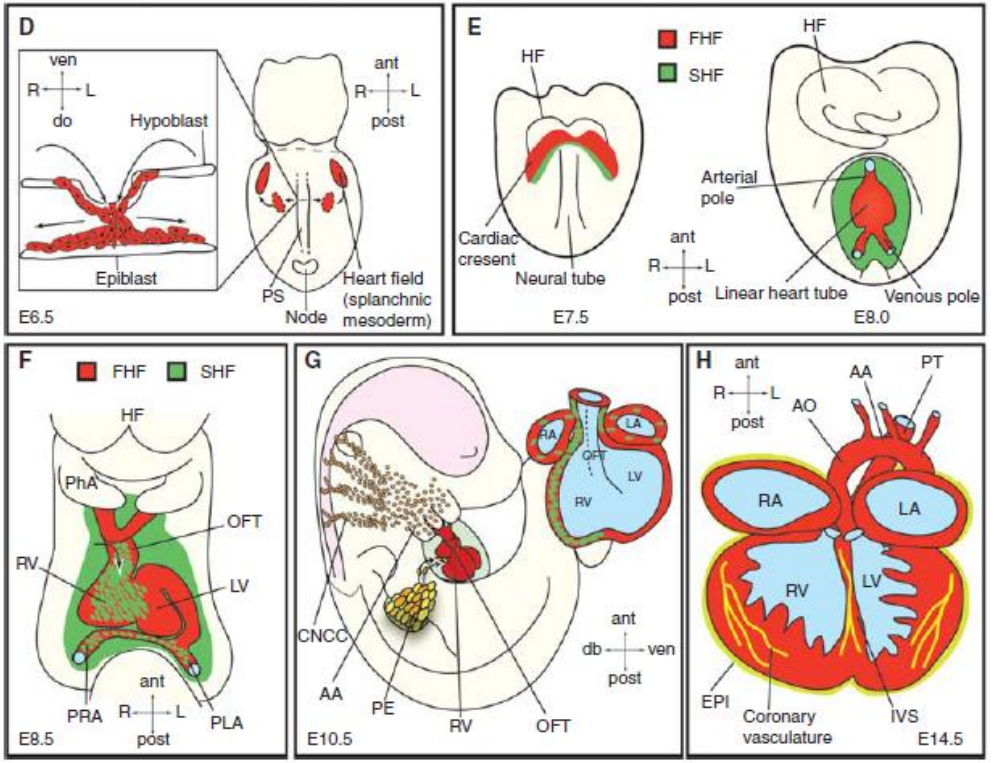
## Pericardium: What We Still Do Not Know?

# Epicardium in Cardiac Development and Disease

Jung-Sun Kim, MD, PhD
Department of Pathology Samsung Medical Center
Sungkyunkwan University School of Medicine, Seoul, Korea

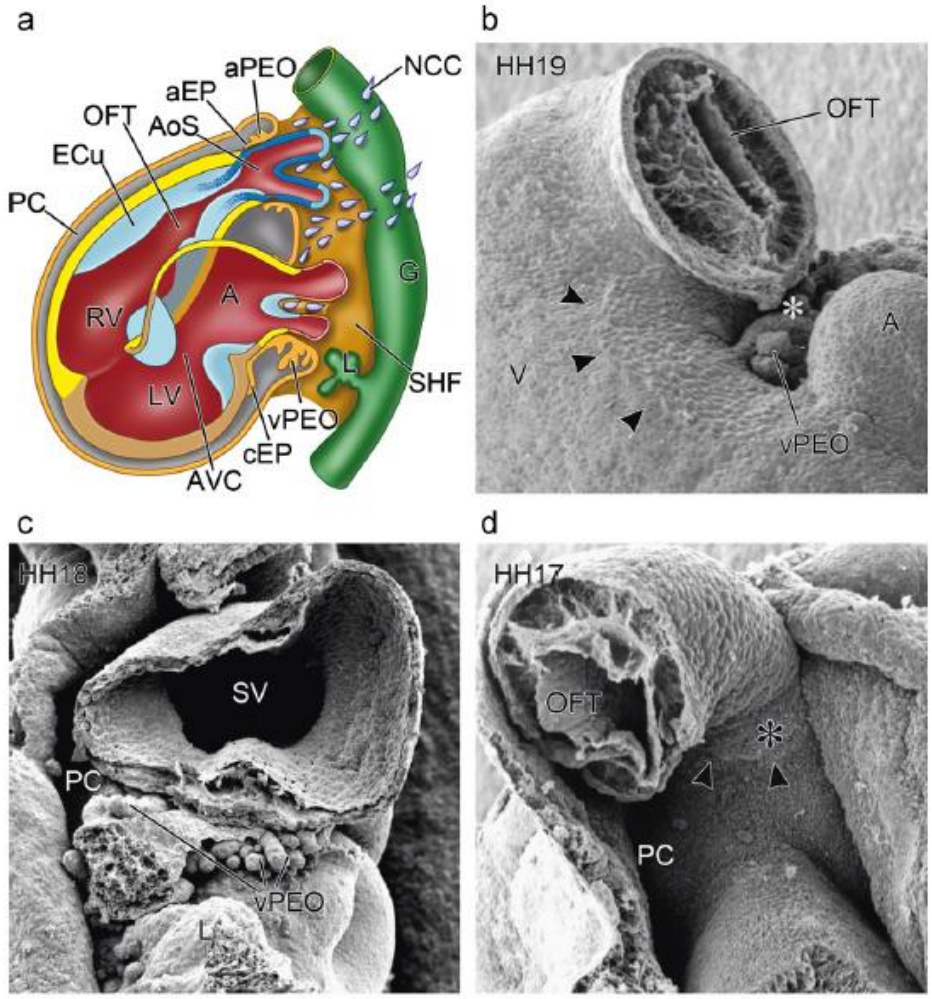
# Cellular Contribution to Heart Development

- Heart field
  - First / Second
  - Cardiomyocytes
  - Endocardial cells
- Proepicardium
  - Epicardium
  - Cardiac fibroblasts
  - Coronary SMC and endothelial cells
  - Some cardiomyocytes
- Cardiac neural crest cells
  - Cardiac outflow tracts
  - Aortic smooth muscle




*Brade et al., Cold Spring Harb Perspect Med 2013;3:a013847*

# Proepicardial Organ




Chick embryo at HH stage 17
Mouse embryo at ED 9.75


*Schlueter and Brand. J Cardiovasc Trans Res 2012;5:641-653*

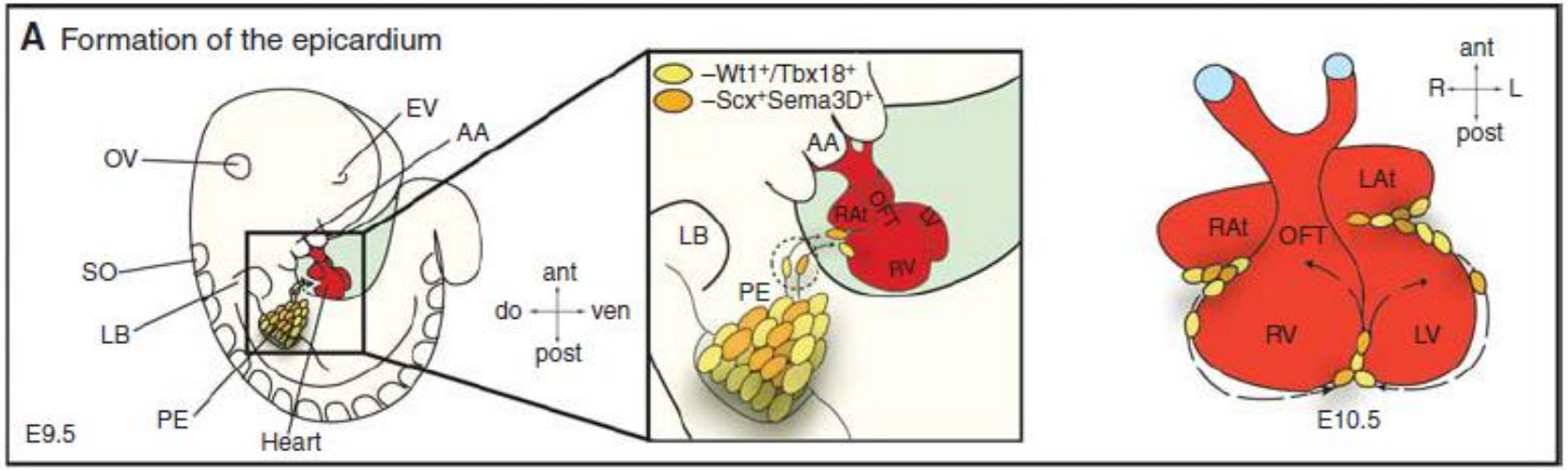
*Gittenberger-de Groot et al., Differentiation 2012;84:41-53*

# Molecular Characterization of Proepicardium

- Tbx18
- Wt1: prevent precocious differentiation
- Tcf21:repressor of cell differentiation, interstitial fibroblasts
- CFC1:PE to fully formed epicardium
- Raldh2: RA as a survival factor for PE
- Nephs1, Flrt, Ccbe1, Scx, Sema3D
- Also expressed in the developing kidney
  - Wt1, Tbx18, Tcg21, nephrin

# Epicardium Formation

- Freely floating PE cell vesicles released from the PE enlage
- They flatten and spread out on contact to the naked myocardium, forming the epicardium (ED9.5-11.5 in mouse)
- VCAM, b4-a1-integrin

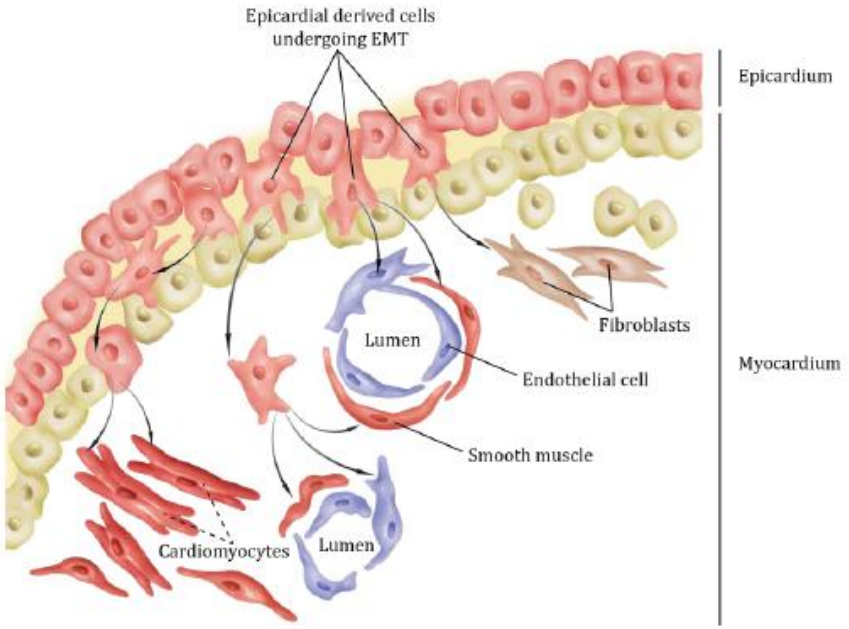



*Brade et al., Cold Spring Harb Perspect Med 2013;3:a013847*

# Epicardial Derived Cells (EPDCs)

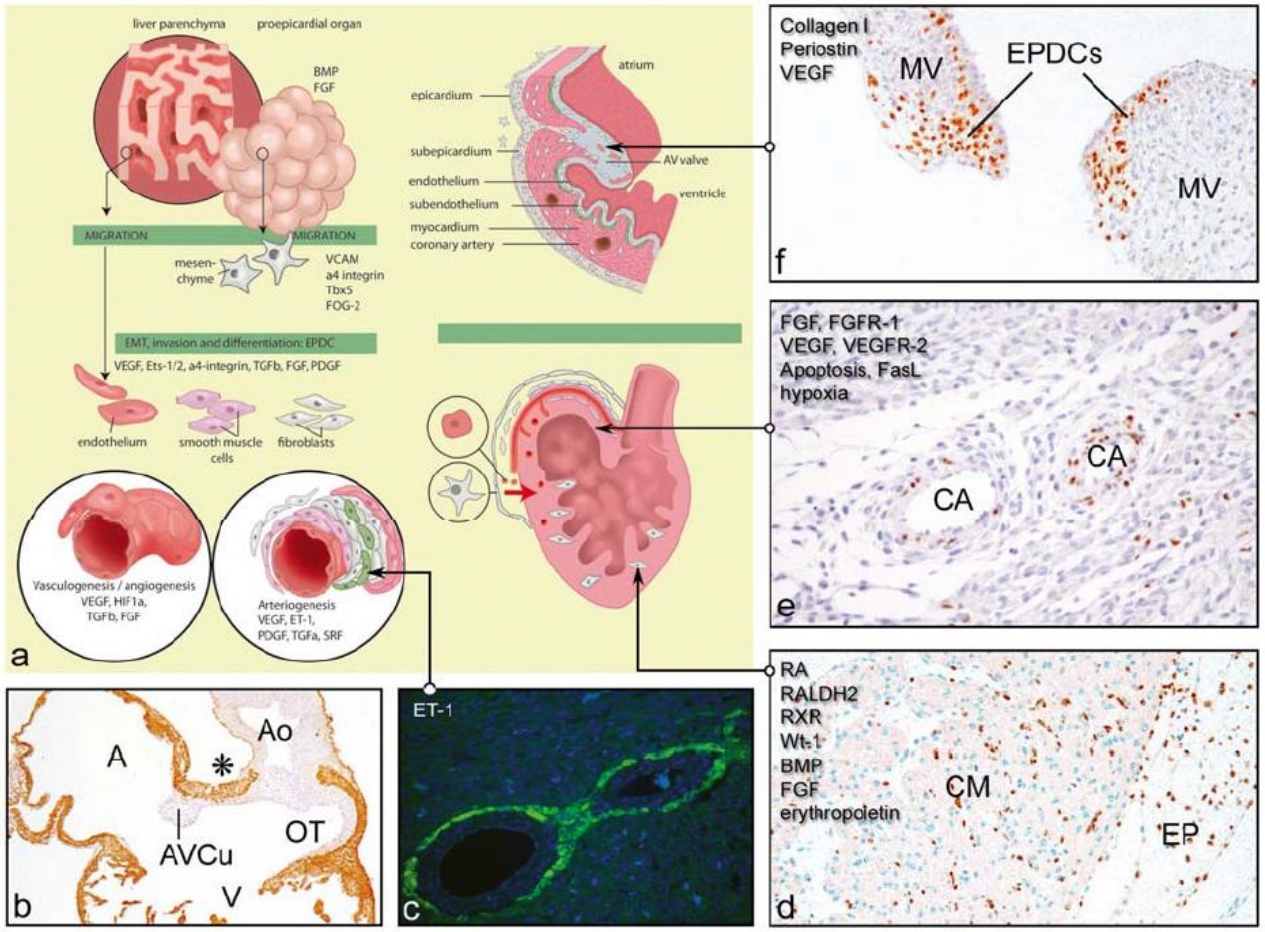
- Epithelial-to-mesenchymal transition (mouse ED11)
- EPDCs migrate into the subepicardial space - myocardial layer - subendocardial area.

- E-cadherin-podoplanin
- VCAM1-PDGFR$\alpha$
- $\alpha 6\beta 4$ integrin - fibronectin




*Singh et al., J Dev Biol 2013;1:141-158*

# Fate of EPDCs




*Lie-Venema et al TheScientificWorldJournal 2077;7:1777-1798*

# Heterogeneity and Differentiation of the EPDCs

- Interstitial cardiac fibroblasts
- Coronary smooth muscle cells
- Adventitial fibroblasts

- Remained to be confirmed
  - Coronary endothelium
  - Myocardial cells
  - Purkinje fiber differentiation

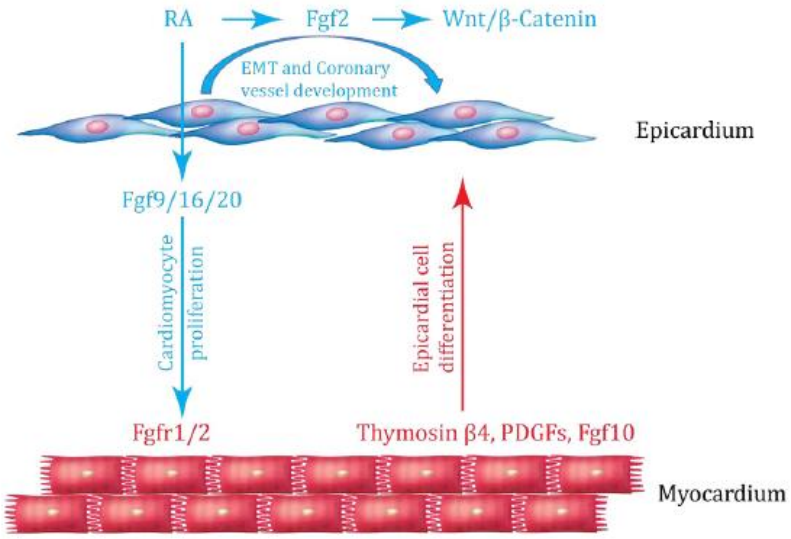
- Predestined heterogeneous population vs. multipotent cell population

# Myocardial Maturation

- Proliferation of myocytes
  - Raldh2 from EPDCs
- Compact layer formation
  - RA induced liver endothelium-derived EPO stimulating Igf in epicardial cells
- Spatio-temporal difference between developing right and left ventricle

# Factors Regulating Myocardial-epicardial Interaction

- FGF2, FGF9, IGF2, PDGF from EP stimulate MC growth during development
  - RA and EPO signaling of EP is involved in expansion of MC compact layer
    - RA (liver) - EPO (liver) – EPO receptor (EP) – Igf2 (EP) – MC compact layer expansion
  - RA –FGF (EP) - FGFR signaling in MC proliferation



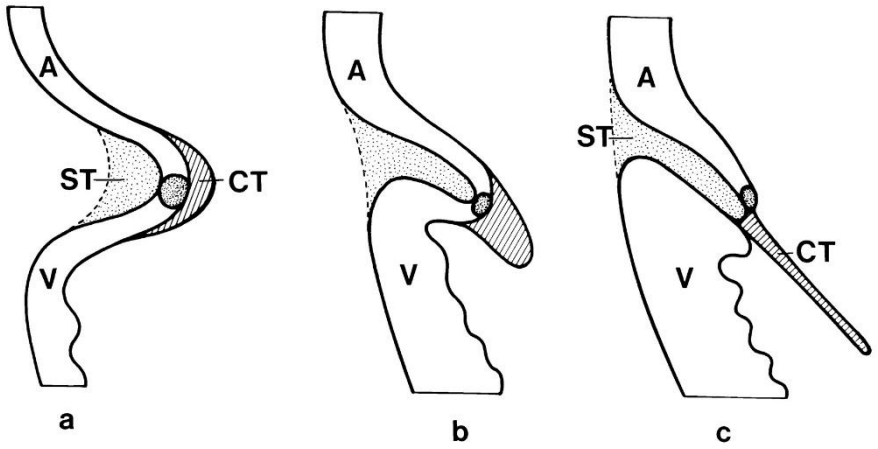

Singh and Epstein. J Dev Biol 2013;1:141-158

# Factors Regulating Myocardial-epicardial Interaction

- Adhesion molecules to modulate the cell-cell interaction
  - Itga4 in PE, Vcam in MC
- PAR3
  - cell polarity of PE cells
  - determine whether cells remain part of the epicardium or undergo EMT to migrate into MC

# Contribution of Epicardium in Cardiac Valve Formation

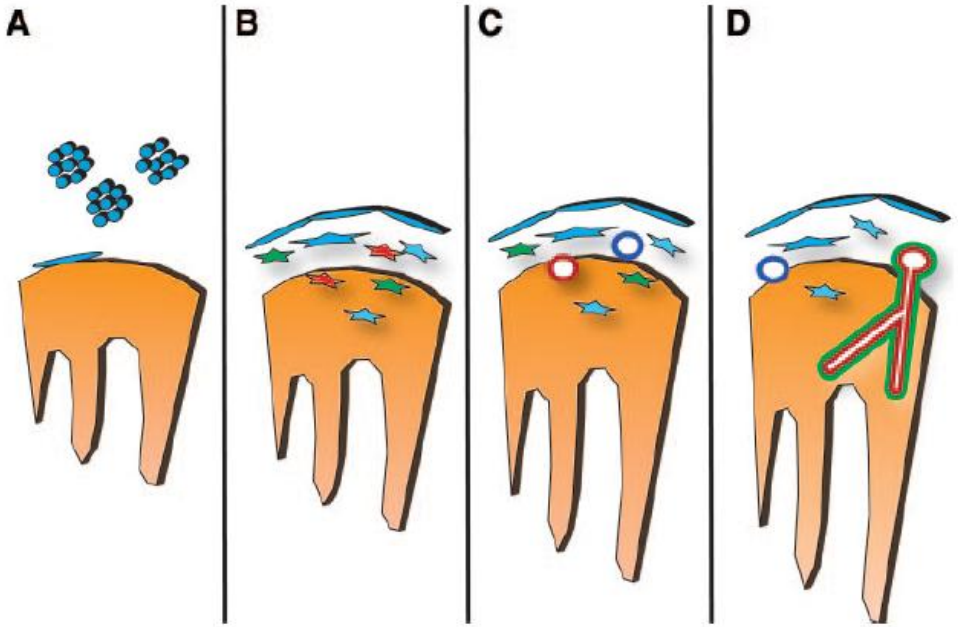
- EMT in the atrioventricular and ventriculoarterial grooves
- Formation of the fibrous atrioventricular annulus
- Annuli and semilunar valves




*Wessels et al., Circ Res 1996;78:110-117*

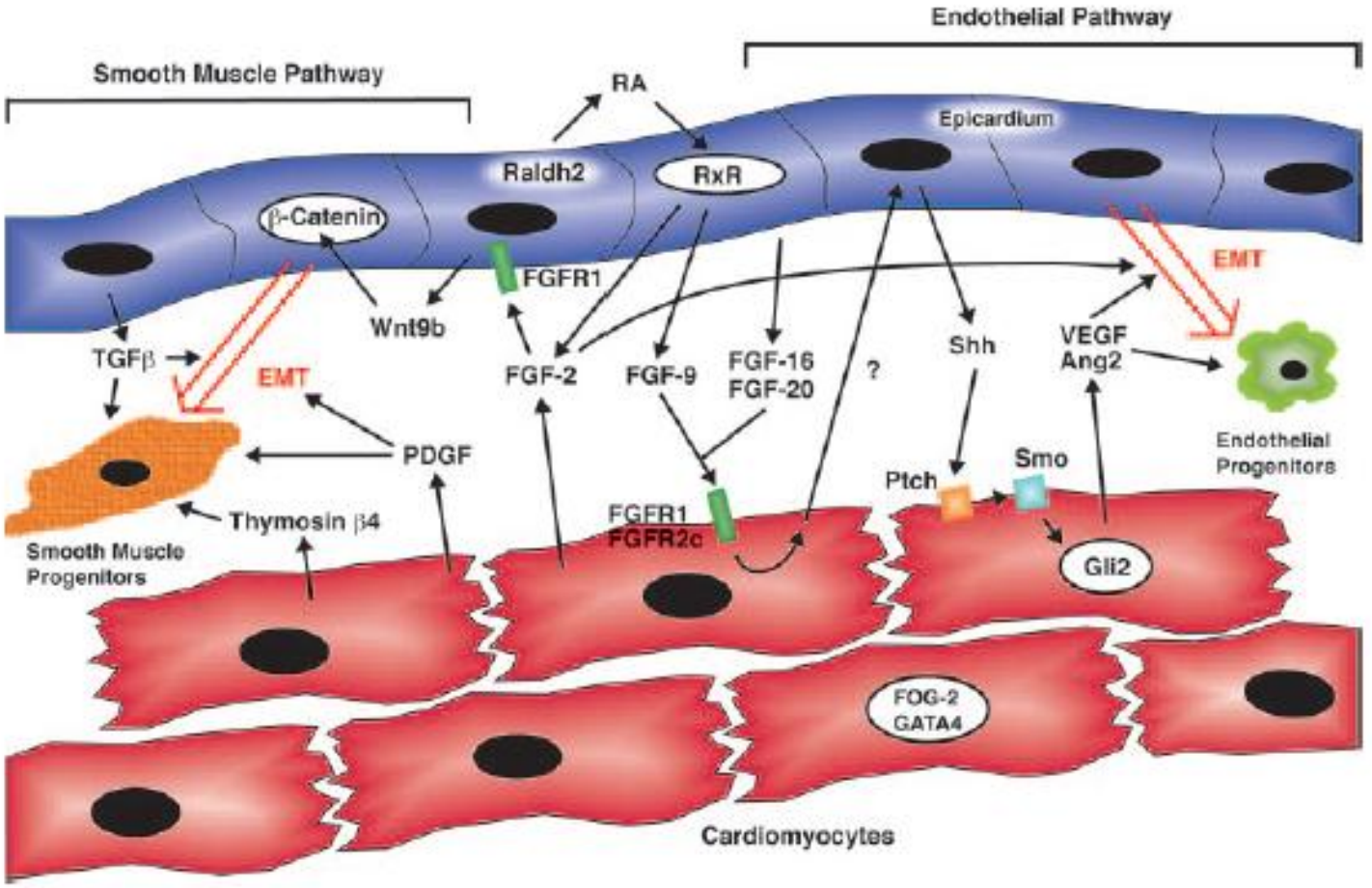
# Formation of Coronary Vessels

- Primitive coronary plexus around E11.5 (mouse)
- Primary coronary vessels spread over the VT at E13.5
- EPDCs surround main coronary vessel to differentiate into smooth muscle cells




*Olivery and Svensson Circ Res 2010;106:818-832*

# Epicardial-myocardial Signaling Pathways in Coronary Vascular Development

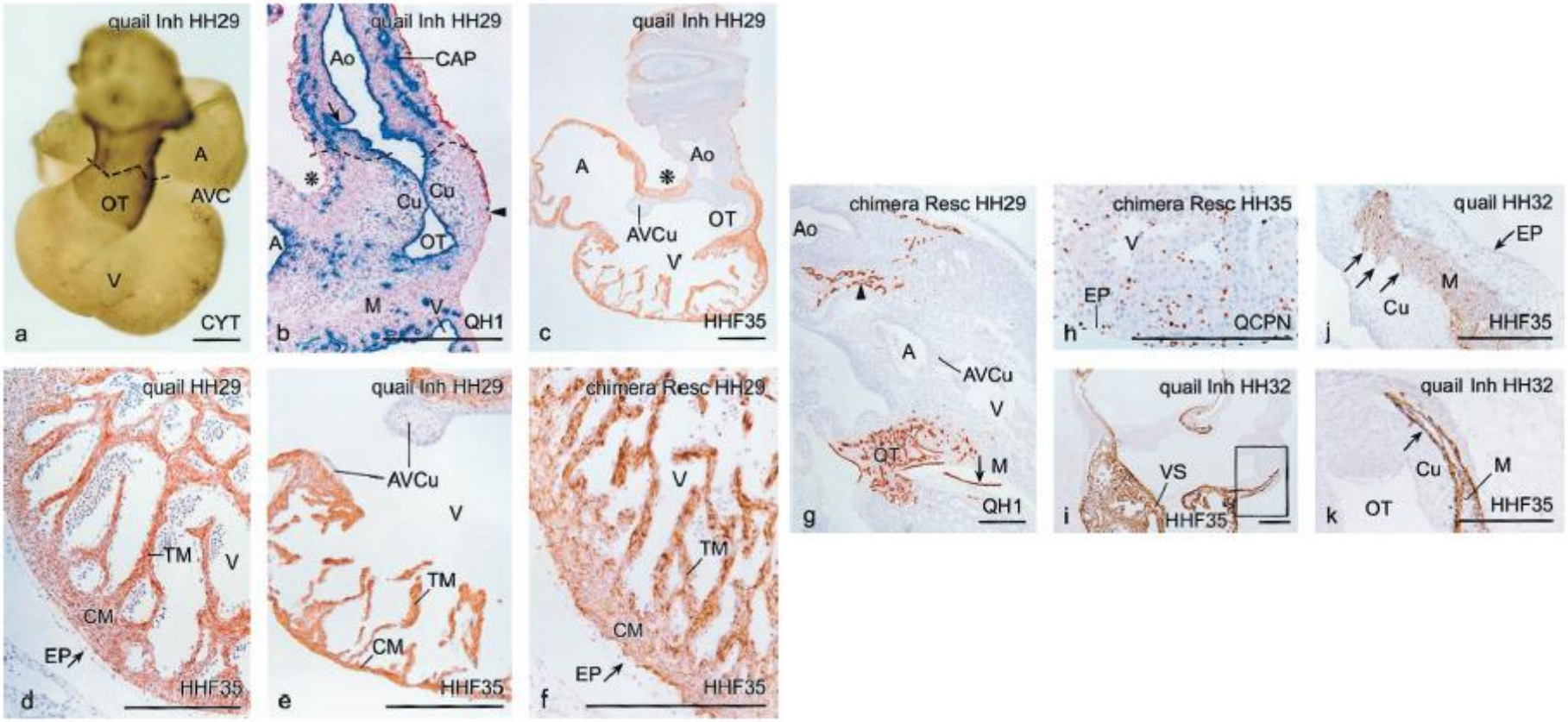



*Olivery and Svensson Circ Res 2010;106:818-832*

# Epicardium in Congenital and Adult Heart Disease

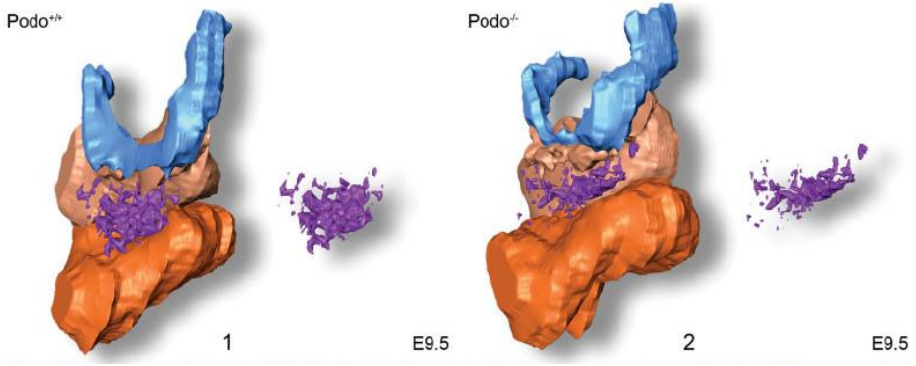
- Non-compaction cardiomyopathy
- Deficient annulus fibrosis and valve formation
- Coronary arterial abnormalities

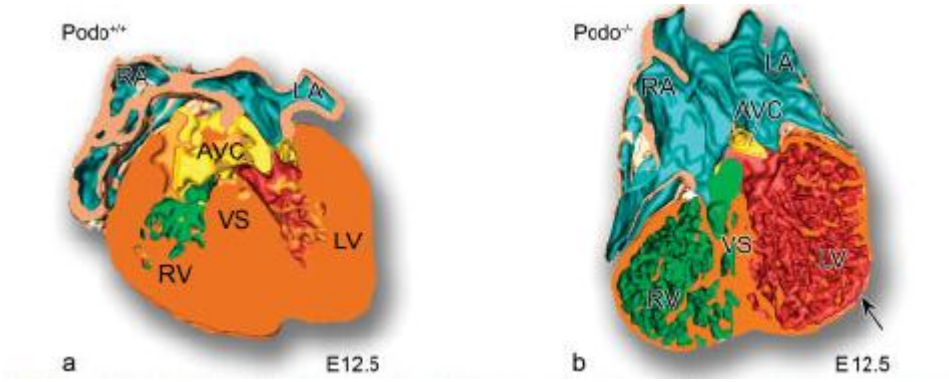
# Epicardial Outgrowth Inhibition in Chick Embryos

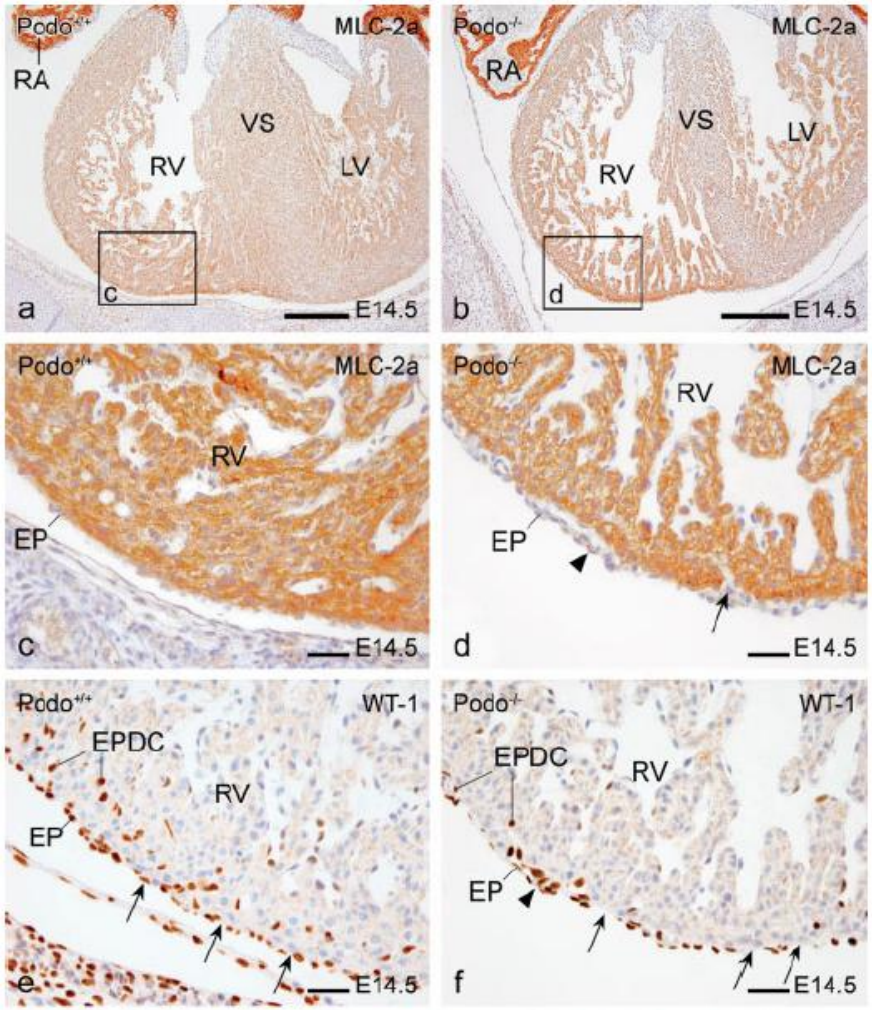



*Gittenberger-de Groot et al. Circ Res 2000;87:969-971*

# Abnormal Epicardial Development and Cardiac Malformation in Pod KO mice

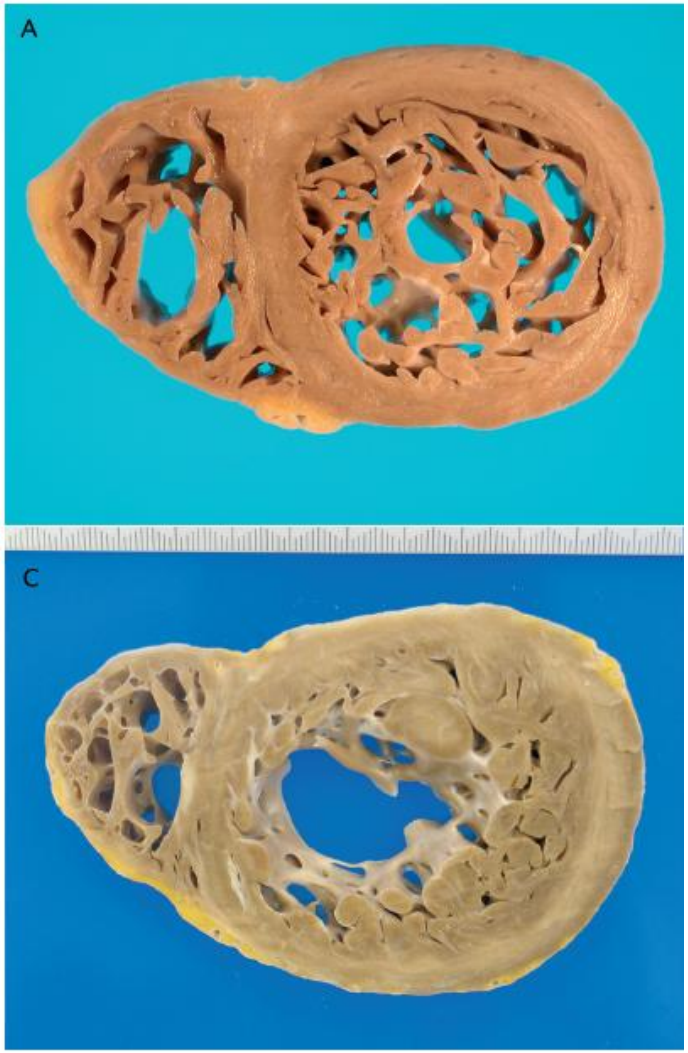


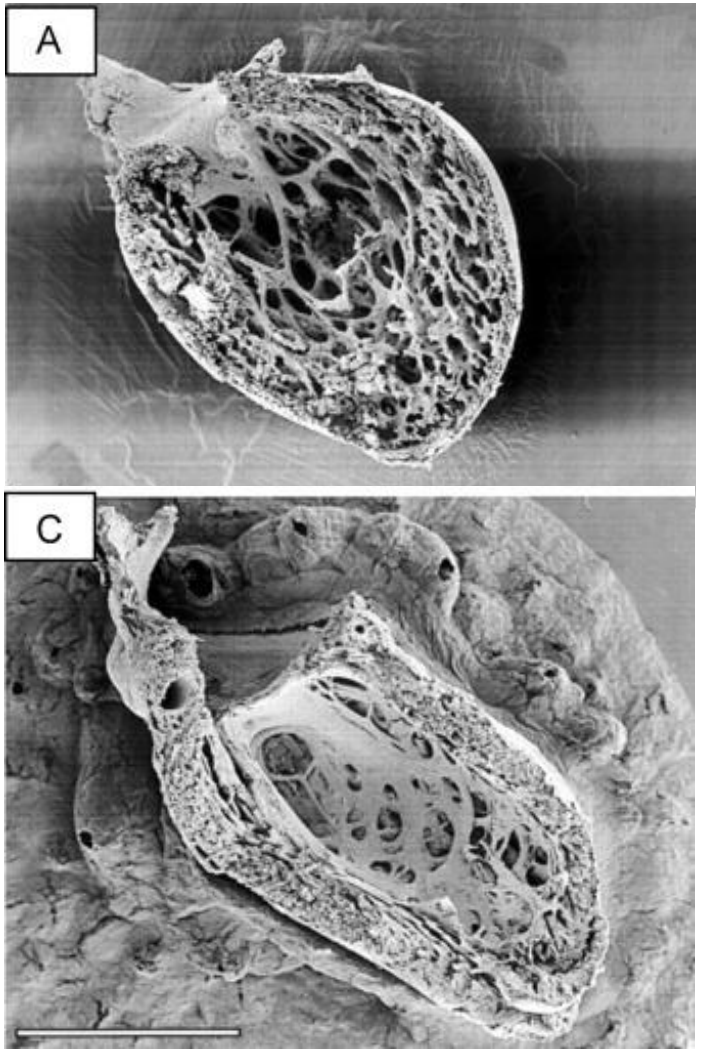




*Mahtab et al., Dev Dyn 2008;237:847-857*

# Non-Compaction Cardiomyopathy




*Ival-Bernal Histol Histopathol 2010;25:495-503*




*Ikeda et al J Cardiol 2015;65:91-97*

# Valvulopathies

- Chick model of PEO inhibition
  - Absence of AV valve
  - Ebstein's anomaly
- Bicuspid aortic valve
  - Notch 1 mutation

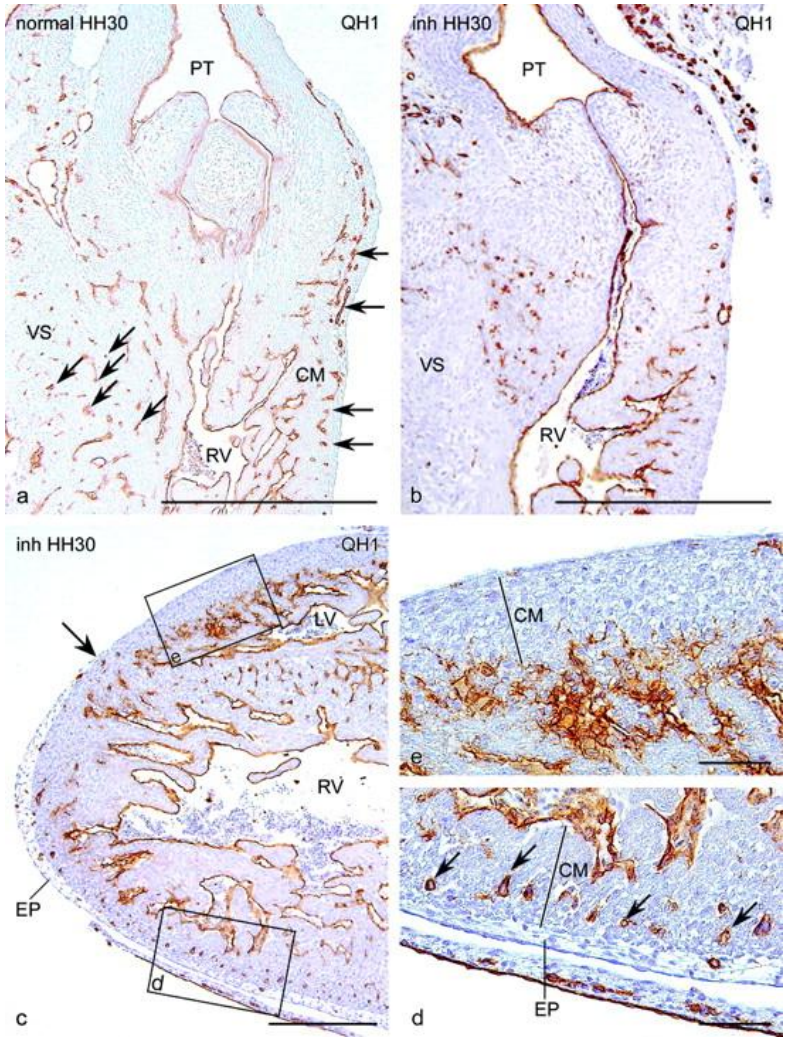
# Cardiac Conduction System Anomalies

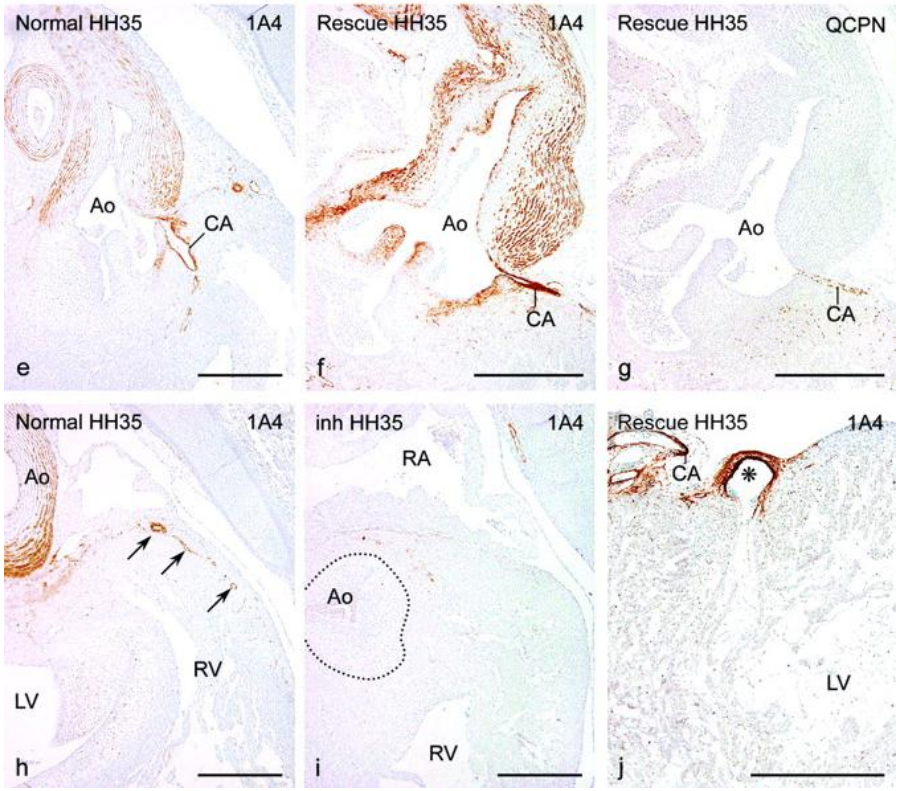
- Purkinje fiber differentiation
- Deficient formation of the fibrous annulus – accessory pathways
- Demonstrated in avian embryos after PEO inhibition
- No reports on mouse models with disturbed epicardial development and deficient annulus fibrosis formation

# Coronary Vascular Anomalies

- Undifferentiated microvascular endothelial plexus to differentiated coronary vessels
- Congenital pattern variations
- Abnormal ventriculo-coronary-arterial communications
- Single coronary ostia
- Pinpoint coronary orifice formation

# Coronary Vascular Anomalies



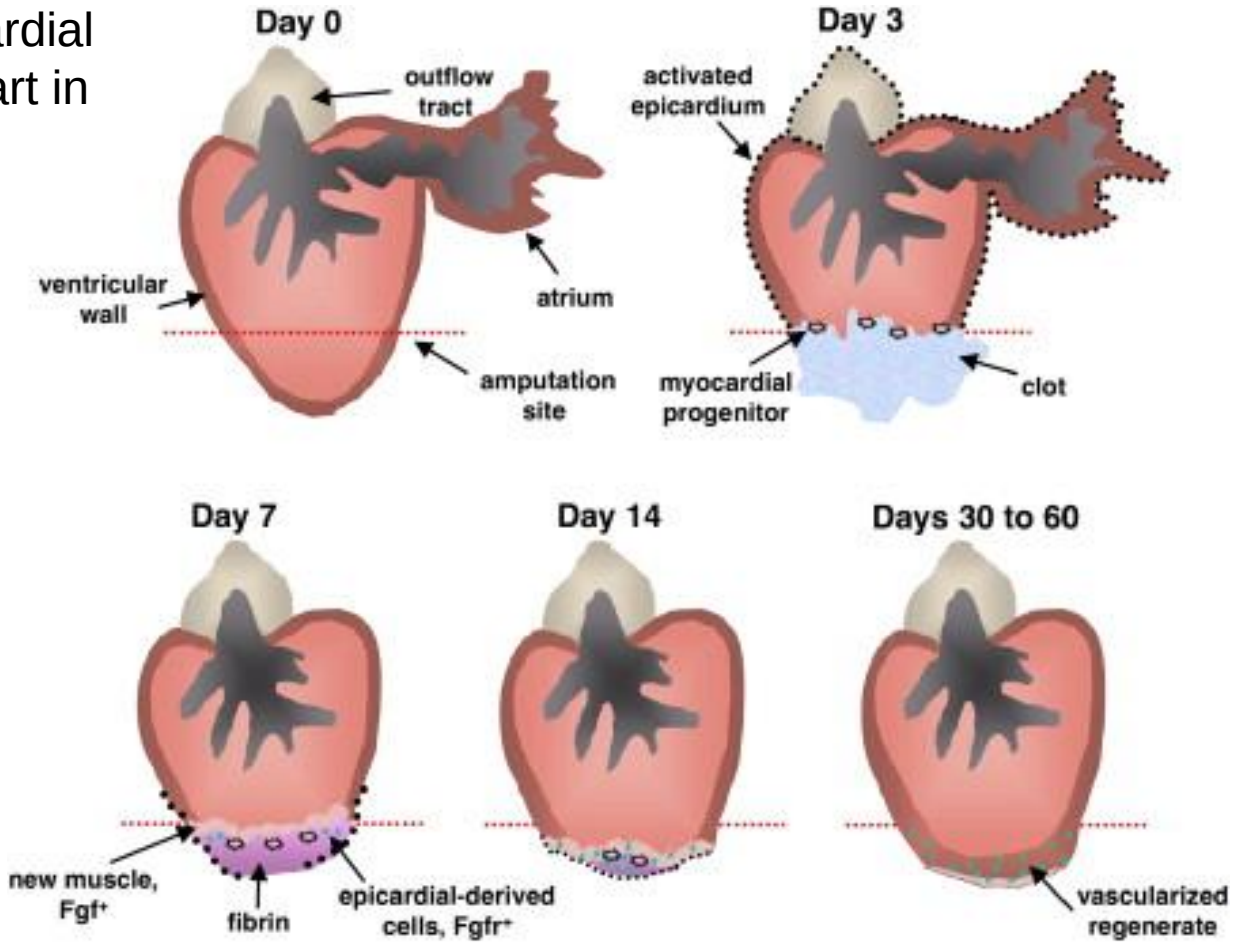

Ismail Eralp et al. Circ Res. 2005;96:526-534

# Quiescent Epicardium in Adult Heart

- Early embryonic marker genes (Raldh2) switched off
- Loss of epicardial potential by P4 in the mouse
- Loss of myocardial responsiveness to epicardial paracrine secretions

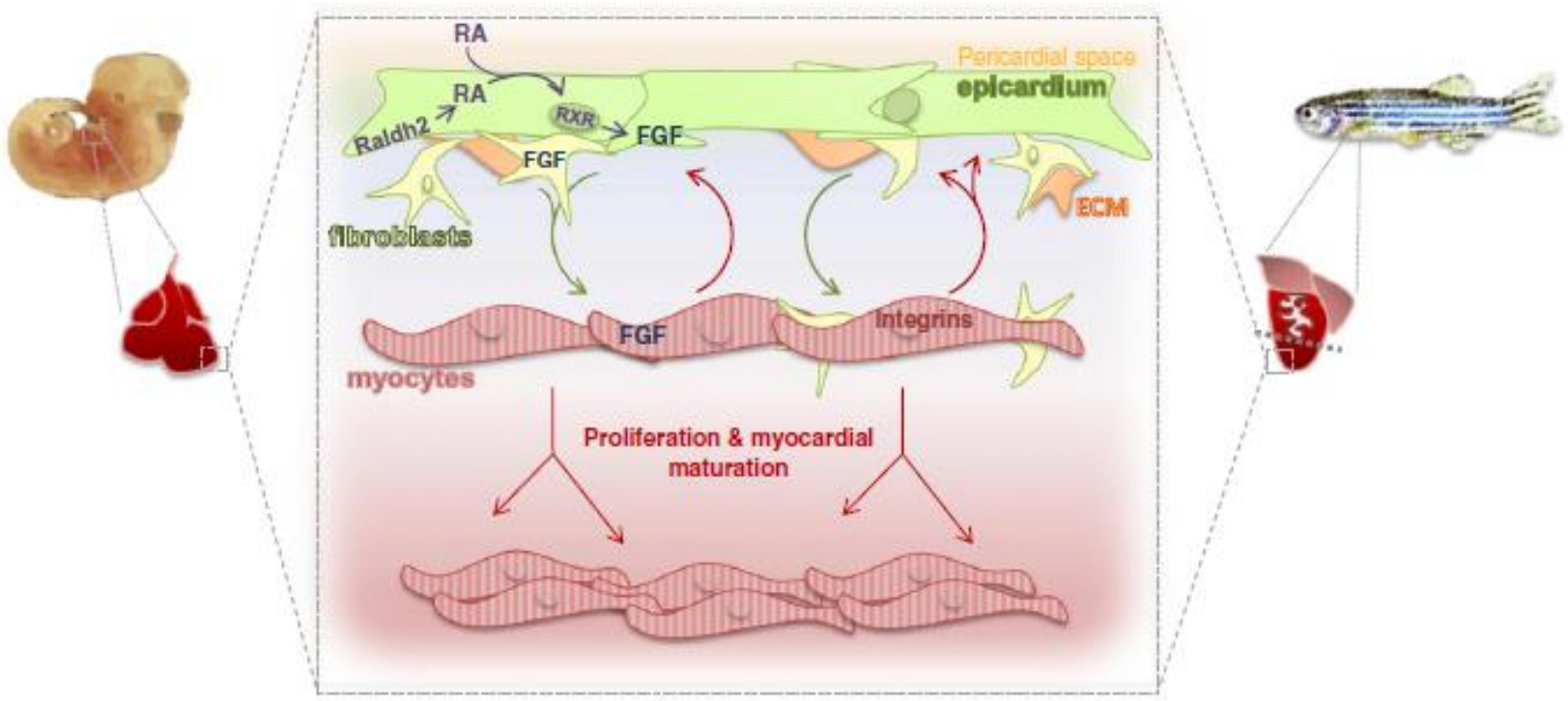
# Epicardium in Cardiovascular Repair

Rapid activation of epicardial cells in the zebrafish heart in response to ventricular wounding




Lepilina et al., cell 2006;127: 607-619

# Epicardial Signals in Mammalian Heart Development and Lower Vertebrate Heart Regeneration




*Masters and Riley. Stem Cell Res 2014;13:684-692*

# Epicardium in Cardiovascular Repair

- The potential of the adult epicardial cells after myocardial infarction
- Mouse model of MI
  - C-kit+ subepicardial EPDCs
    - Renewed epicardial activity
    - Stem cell characteristics
  - Reactivation of epithelial to mesenchymal transition
  - No differentiation into a myocardial or endothelial phenotype
  - Paracrine factors stimulating angiogenesis
  - Neonatal regeneration window

# Summary

- Epicardium represents a critical developmental source of cells and signals
- Epicardium in heart regeneration
  - Therapeutic potential of modulating epicardial signals to instruct heart repair in adult mammals