Cell

Multipotent Embryonic *Isl1*$^+$ Progenitor Cells Lead to Cardiac, Smooth Muscle, and Endothelial Cell Diversification

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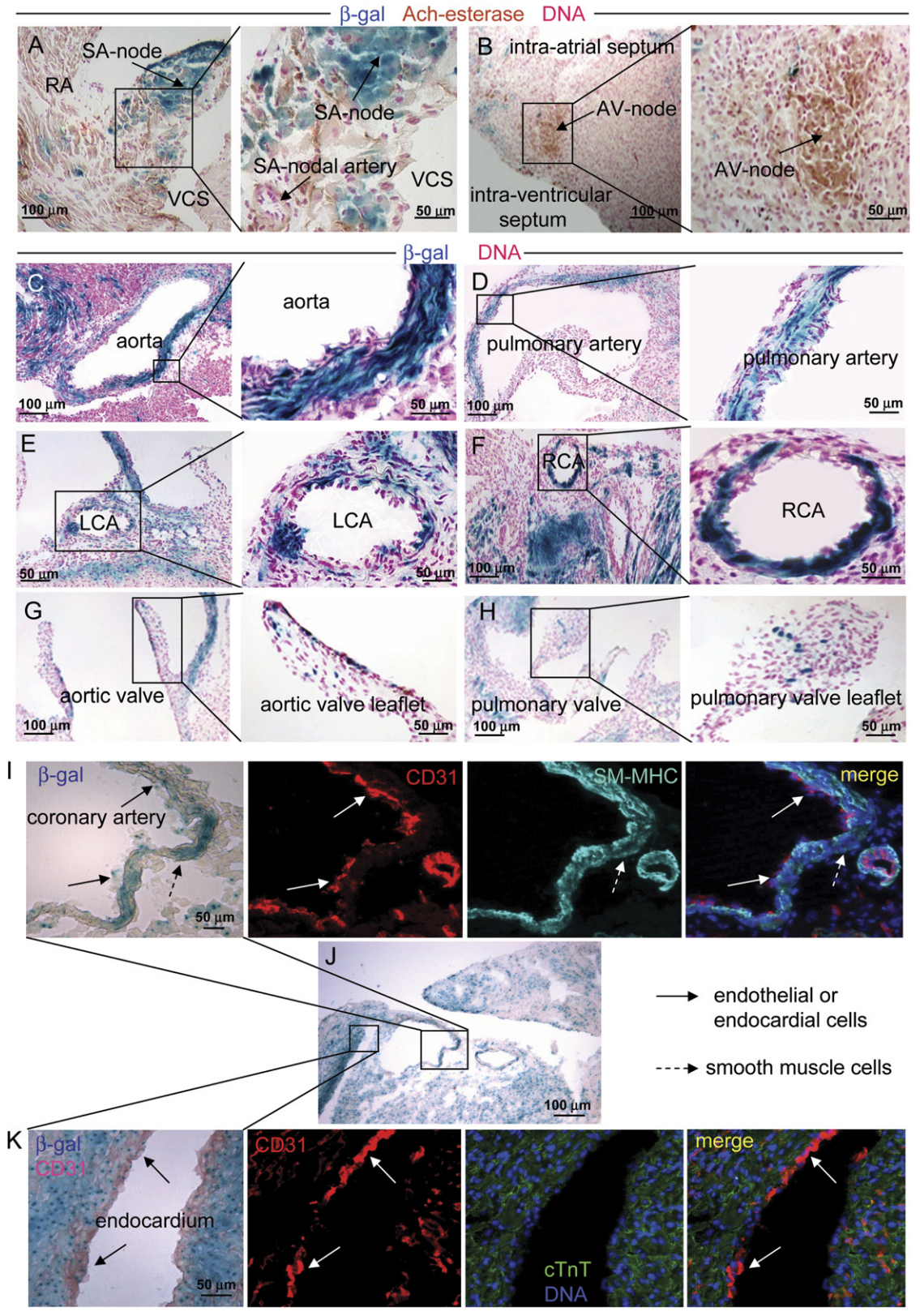
SUMMARY

Cardiogenesis requires the generation of endothelial, cardiac, and smooth muscle cells, thought to arise from distinct embryonic precursors. We use genetic fate-mapping studies to document that *isl1*$^+$ precursors from the second heart field can generate each of these diverse cardiovascular cell types in vivo. Utilizing embryonic stem (ES) cells, we clonally amplified a cellular hierarchy of *isl1*$^+$ cardiovascular progenitors, which resemble the developmental precursors in the embryonic heart. The transcriptional signature of *isl1*$^+$/*Nkx2.5*$^+$/*flk1*$^+$ defines a multipotent cardiovascular progenitor, which can give rise to cells of all three lineages. These studies document a developmental paradigm for cardiogenesis, where muscle and endothelial lineage diversification arises from a single cell-level decision of a multipotent *isl1*$^+$ cardiovascular progenitor cell (MICP). The discovery of ES cell-derived MICPs suggests a strategy for cardiovascular tissue regeneration via their isolation, renewal, and directed differentiation into specific mature cardiac, pacemaker, smooth muscle, and endothelial cell types.

INTRODUCTION

The formation of cardiac, smooth muscle, and endothelial cell lineages in the heart has largely been ascribed to a set of nonoverlapping embryonic precursors derived from distinct origins. Cardiac neural crest, the proepicardium, and the cardiac progenitors of the two heart fields are thought to follow separate parallel pathways for sequential lineage maturation (Mikawa and Gourdie, 1996; Manner et al., 2001; Waldo et al., 2001; Kelly and Buckingham, 2002; Stoller and Epstein, 2005). The discovery of several heart lineage-restricted genes suggests that the generation of different cardiac cell types might be driven by a unique combinatorial subset of transcriptional networks operating within distinct cardiovascular precursors (for review, see Srivastava and Olson, 2000). Nevertheless, an alternative possibility exists that diverse muscle and nonmuscle lineages arise from a single cell-level decision of multipotent, primordial cardiovascular stem cells, which in turn give rise to a hierarchy of downstream cellular intermediates representing tissue-restricted precursors for the fully differentiated heart cells. This clonal model of heart-lineage diversification would be analogous to hematopoiesis, in which a single hematopoietic stem cell can generate all of the blood-cell lineages (Morrison and Weissman, 1994; Weissman, 2000).

The recent identification of a second source of embryonic myocardial precursors has begun to modify the classical view of heart formation (Mjaatvedt et al., 2001; Waldo et al., 2001). The LIM-homeobox transcription factor islet-1 (*isl1*) delineates this second cardiogenic progenitor field (Cai et al., 2003; Laugwitz et al., 2005). In this regard, we have recently reported that after birth the mammalian heart harbors a rare subset of *isl1*$^+$ precursors in the atria, outflow tract, and right ventricle. The postnatal *isl1*$^+$ murine cells can be renewed on cardiac mesenchymal feeder layers and triggered into fully differentiated muscle cells, thereby fulfilling the criteria for endogenous


β-gal Ach-esterase DNA
A
SA-node
RA
VCS
100 μm
SA-node
SA-nodal artery
VCS
50 μm
B intra-atrial septum
AV-node
intra-ventricular septum
100 μm
AV-node
50 μm
β-gal DNA
C
aorta
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aorta
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D
pulmonary artery
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F
RCA
100 μm
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G
aortic valve
100 μm
aortic valve leaflet
50 μm
H
pulmonary valve
100 μm
pulmonary valve leaflet
50 μm
I
β-gal
coronary artery
50 μm
CD31
SM-MHC
merge
J
100 μm
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