Isil1 Expression at the Venous Pole Identifies a Novel Role for the Second Heart Field in Cardiac Development

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The right ventricle and outflow tract of the developing heart are derived from mesodermal progenitor cells from the second heart field (SHF). SHF cells have been characterized by expression of the transcription factor Islet1 (Isil1). Although Isil1 expression has also been reported in the venous pole, the specific contribution of the SHF to this part of the heart is unknown. Here we show that Isil1 is strongly expressed in the dorsal mesenchymal protrusion (DMP), a non–endocardially-derived mesenchymal structure involved in atrioventricular septation. We further demonstrate that abnormal development of the SHF-derived DMP is associated with the pathogenesis of atrioventricular septal defects. These results identify a novel role for the SHF. (Circ Res. 2007;101:971-974.)

The myocardium of the right ventricle (RV) and outflow tract (OFT) is derived from mesodermal cells in the second heart field (SHF).1-3 SHF cells have been shown to express the transcription factor Islet1 (Isil1).4 Isil1 is necessary for normal RV and OFT development.5-7 In Isil1-deficient mice, abnormalities are also found in structures at the venous pole.4-6 Although atrial Isil1 expression at the venous pole has been reported,4-5 a specific atrial role for Isil1 in development has not been established.

Materials and Methods
Tie2-Cre and Rosa26R mice, X-Gal staining, and 3-dimensional reconstruction techniques have been described.2 Immunohistochemistry was performed with antibodies recognizing Isil1 (39.4D5;DSHB), Nkx2.5 (H-114; Santa Cruz), sarcomeric actin (39.4D5;DSHB), Isl1 (A2172;Sigma), and MLC2a. Some experiments were performed on previously collected Ts16 specimens.8 Detailed information is available in the supplemental materials (available online at http://circres.ahajournals.org).

Results and Discussion
We studied Isil1 protein expression in the developing mouse between embryonic day (ED)11 and ED14.5. At the earliest stage, Isil1 expression was, as previously described,5 found in the OFT (Figure 1A and 1C), foregut endoderm (Figure 1C), and splanchnic mesoderm (Figure 1A, 1C, 1D, and 1F). Isil1 expression was also seen in cardiac tissues at the venous pole (Figure 1D and 1F). Three-dimensional reconstructions illustrate that these Isil1 expression domains are contiguous (Figure 1G through 1J), demonstrating the contribution of the SHF to both poles of the heart.

Isil1-expression at the venous pole was observed in distinct subsets of atrial myocytes (Figure 1D and 1F) and, most notably, in a discrete set of mesenchymal cells that was found to be associated with the dorsal mesocardium (Figure 1D through 1J). This particular mesenchyme was first mentioned by His, who referred to it as the “spina vestibule”,9,10 describing it as “ein besonderes bindegewebiges Gebilde” (a specialized connective tissue), and later by others using a variety of different names.11 In recent years, several studies have rekindled interest in the role of this mesenchyme in cardiovascular development.7,10-12 While studying its role in the development of the human heart, we came to the conclusion that the term “dorsal mesenchymal protrusion (DMP)”, most accurately described the anatomical features of this intracardiac extension of the splanchnic mesenchyme into the atrial cavities.11 Thus, as we continued to gain more insight into the matter we opted to use this terminology.7,12 We acknowledge, however, that “atrial spine”, “vestibular spine”, and “spina vestibuli” are also commonly, and legitimately, used in other articles to describe this mesenchymal population.

We previously showed that the DMP is a non–endocardially-derived mesenchymal structure that forms an integral component of the AV mesenchymal complex, thereby playing an important role in cardiac septation.7,10,13 Historically, septal defects in the AV canal region have been associated with abnormal development of the AV cushions, hence the term “endocardial cushion defects”, a term still frequently used.14 Recent studies suggest that impaired development of the DMP is involved in the pathogenesis of atrial and AV septal defects,7,12,13 particularly in Downs Syndrome patients and in the Trisomy 16 (Ts16) mouse model for this condition.15,16 The identification of this structure as an Isil1-expressing SHF derivative prompted us to revisit its involvement in AV canal defects of the Ts16 mouse.16 Examination of ED12 Ts16 mouse embryos with AV canal defects confirmed that the DMP was severely underdeveloped. Virtually no Isil1-positive cells were found in the region where it normally develops (Figure 2D, asterisk). Although we still know little about the molecular mechanisms that lead to normal DMP development, we believe that these results provide the first evidence for a specific role for the SHF in AV septation.
After completion of septation, the DMP largely becomes myocardial, forming the inferior muscular rim at the base of the atrial septum. To clarify whether muscularization of the DMP results from ingrowth of flanking myocardium or from a mesenchymal-to-myocardial transition, we examined whether Isl1-expressing SHF cells are undergoing myocardial differentiation, using Nkx2.5, a transcription factor commonly associated with this event.

In the developing mouse heart, Nkx2.5 is widely expressed in the atrial and ventricular myocardium (Figure 3A through 3C). Most, if not all, cells in the DMP at ED11.0 express Isl1 (Figure 3E). Nkx2.5 expression, however, is limited to the flanking atrial myocardium (Figure 3F). By ED13.0, as the DMP is undergoing its myocardial transition (Figure 3G), many cells still express Isl1 (Figure 3H and 3J). A significant number of cells now also express Nkx2.5 (Figure 3I through 3J). After completion of muscularization (ED14.5), few, if any, DMP derivatives still expressed Isl1. The myocardium in these tissues was, however, expressing Nkx2.5 (Figure 3K through 3M). The expression profiles of Isl1 and Nkx2.5 in the DMP throughout muscularization demonstrate that this event occurs by a mesenchymal-to-myocardial differentiation of SHF-derived DMP cells.
In conclusion, our studies confirm previous notions that the SHF contributes to the cardiac venous pole and shows that within this region, the DMP is an Isl1-expressing derivative of the SHF, playing a crucial role in AV development. This new insight into the significance of the SHF may provide an explanation for the atrial and atrioventricular septation defects observed in Isl1 mutant mice and in mice carrying perturbations in other SHF-associated genes. Furthermore, it will form the base for further studies on the development of the DMP and its involvement in the etiology of congenital heart disease.

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Disclosures
None.

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Expanded Material and Methods:

Immunohistochemistry:

Staging and isolation of embryonic tissues were carried out following established protocols. Immunohistochemistry was performed on 5μm thick paraffin sections of embryos fixed in 4% paraformaldehyde or Amsterdam Fixative. Antigen retrieval consisted of incubation of slides in Vector Unmasking Solution (H-3300, Vector Laboratories, Burlingame, CA, USA) for 5 minutes at high temperature in pressure cooker. Vectastain Elite ABC Kit (PK-6200, Vector Laboratories, Burlingame, CA, USA) was used for detection of primary antibody binding following the manufacturer’s Rapid Staining protocol. The following antibodies were used for the respective experiments: 39.4D5 (obtained from the Developmental Studies Hybridoma Bank under the auspices of the NICHD and maintained by the University of Iowa); A2172 (Sigma) recognizing sarcomeric actin; H-114 (Santa Cruz) recognizing Nkx2.5; and a non-commercial polyclonal antibody recognizing MLC2a. Immunofluorescence co-labeling was performed by sequential detection of the two antigens using the above mentioned protocol with streptavidin Alexa Fluor 488 and 568 conjugated secondary antibodies (Molecular Probes). Tissue sections were blocked for 30 min in secondary antibody before proceeding with detection of the second antigen.