The development of the venous pole of the heart
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Chapter 7

Two distinct pools of mesenchyme contribute to the development of the atrial septum


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The development of the venous pole of the heart
Abstract

Closure of the primary atrial foramen is achieved by fusion of the atroventricular cushions with the mesenchymal cap on the leading edge of the muscular primary atrial septum. A fourth component involved is the vestibular spine, originally described by His in 1880 as an intra-cardiac continuation of the extra-cardiac mesenchyme of the dorsal mesocardium. The morphogenesis of this area is of great clinical interest, because of the high incidence of atrial and atroventricular septal defects. Nonetheless, the origin of the participating components is largely unknown. Here we report that the primary atrial foramen is surrounded in its entirety by mesenchyme derived from endocardium. A second population of mesenchyme not derived from endocardium was observed at the caudal margin of the mesenchymal atrial cap, entirely embedded within the mesenchyme derived from endocardium and contiguous with the mesenchyme of the dorsal mesocardium. Our reconstructions show this second population does indeed take the form of a short spine, albeit that it is the right pulmonary ridge, rather than this spine, that protrudes into the atrial lumen. From the stance of morphologic description, therefore, there is little thus far to substantiate the existence of an atrial spine.
Introduction

It is currently thought that the atrioventricular (AV) cushions, along with the primary atrial septum and the mesenchymal cap carried on its leading edge, are the main contributors to the process of atrial septation. A growing body of evidence points to the involvement of a fourth component, namely the vestibular spine (“spina vestibuli”). The vestibular spine was initially nominated as playing such a role in 1880, by Wilhelm His the elder. He described the spine as a triangular mesenchymal wedge, which protruded into the lumen of the atrium from a non-muscular area, which he called the “area interposita”, in the dorsal wall of the common atrium (Fig. 1, stippled circle). The spine then disappeared from view for more than a century, eventually being retrieved by several workers, albeit with disagreements concerning the form and origin of this tissue. To clarify this, we have performed a lineage study using Tie2-Cre mice to label endocardium and endocardium-derived mesenchyme, in combination with three-dimensional reconstructions to permit independent evaluation of the structures involved in atrial septation.

Materials and Methods

The Tie2-Cre and R26R transgenic mouse lines have been described previously. Detection of β-galactosidase activity and immunohistochemistry were performed on 20 µm thick cryostat sections. Non-radioactive in situ hybridization analysis was performed on 12 µm thick paraffin sections. Three-dimensional visualization and geometry reconstruction of patterns of gene expression was then achieved. Movie clips of these reconstructions are available upon request.
Results

At embryonic day (E) 9.5, it is possible to recognize two ridges, of equal size, at the connection of the dorsal mesocardium to the atrium, the right and left pulmonary ridges (R/LPR; Figure 2A1 through 2A5). The area nominated by His as the “area interposita” is seen in our E9.5 reconstructions as the mesenchymal area between these ridges, contiguous with the dorsal mesocardium. It is made up of mesenchyme, which is not derived from endocardium, as it is negative for β-galactosidase, in contrast to all the mesenchyme within the AV canal and atrium, which is at this stage derived from the endocardium by epithelial-to-mesenchymal transformation (Figure 2A2). Differentiation of the mesenchyme of the dorsal mesocardium into atrial myocardium at the pulmonary ridges is indicated by the difference in expression of Mlc2a mRNA, a marker for early myocardium formation (Figure 2A4) and Serca2a protein, which identifies overt differentiated myocardium only (Figure 2A3). The expression of the transcription factor Islet1 (Figure 2A5), a marker for the second heart field, underscores the notion that this region is a cardiogenic area.

At E10.5 (Figure 2B1 through 2B5), β-galactosidase-negative mesenchyme, hence not derived from the endocardium, develops in the inter-positioned area between the pulmonary ridges, and also on top of the right pulmonary ridge (Figure 2B3-4). This area connects caudally with the inferior AV cushion and cranially with the mesenchymal cap crowning the tip of the muscular primary atrial septum, which both are composed of mesenchyme, which is derived from the endocardium (Figure 2B5). By E10.5, the cap itself connects cranially with the superior AV cushion (Figure 2B1-2). This finding is confirmed by analysis of sections of the embryos from E11.5 (Figure 2C1 through 2C6). Thus, the mesenchyme derived from the endocardium forms a ring around the primary foramen, albeit sparsely at its most dorsal side adjacent to the mesocardium. The mesenchyme that is not derived from endocardium develops at the exact side at which His described formation of the vestibular spine, albeit that we did not observe any mesenchymal protrusion into the atrial lumen other than the right pulmonary ridge, which contributes to the primary atrial septum. Unlike the cap on the primary atrial septum, the mesenchyme not derived from the endocardium expresses levels of collagen comparable with those found in the mesenchyme of the body (Figure 2C2). After E11.5, the mesenchymal cap on the atrial septum, which is derived from endocardium, along with the non-endocardium-derived mesenchyme forming the inter-positioned area, fuse with the inferior and superior AV cushions (Figure 2C5-6), thus closing the primary atrial foramen.
Discussion

We have shown that two distinct populations of mesenchymal cells are found within the developing atrial chambers. In Tie2-Cre/R26R double transgenic embryos, which show the lineage of tissues derived from the endocardium, the AV cushions and the cap on the leading edge of the primary muscular atrial septum are marked, as previously suggested.\(^{19,20}\) Sections from these embryos also show that the mesenchymal cells in the dorsal part of the heart, contiguous with the dorsal mesocardium, are not derived from the endocardium. Because the only known source of cardiac mesenchyme at the venous pole of the heart is that derived by transformation from endocardium, we presume that these additional mesenchymal cells take their origin from outside the heart. Such an extra-cardiac origin has been suggested previously\(^{2,5-7,11}\) but, as far as we are aware, has not been proven and the extent of this region of mesenchyme has not previously been charted. When seen in section, the mesenchyme takes the form of a spine (Figure 2C1 and 2D2), but the spine is not visible from the atrial lumen, since it is embedded within the mesenchyme that is derived...
Figure 2. Characterization of the mesenchymal tissues surrounding the primary atrial foramen at E9.5 (A1-5), E10.5 (B1-5) and E11.5 (C1-6). 3D reconstruction (A1); *Tie2-Cre/R26R* transverse sister cryosections stained for β-galactosidase (A2) and Serca2a (A3); Wild-type transverse sister sections stained for *Mlc2a* (A4) and *Islet1* (A5). 3D reconstruction, frontal view of dorsal atrial wall (B1) and left lateral view (B2); *Tie2-Cre/R26R* whole mount β-galactosidase stained transverse sections, double-stained for Serca2a (B3-5). *Tie2-Cre/R26R* whole mount β-galactosidase stained sagittal sister sections double-stained for cardiac Troponin I (C1), and collagen III (C2); Sister sagittal cryosections stained for β-galactosidase (C3) and Serca2a (C4). Sister transverse cryosections stained for β-galactosidase (C5) and Serca2a (C6). The diagrams (D1 and 2) depict schematic midsagittal sections of the atria; morphology (D1); lineage (D2); grey, myocardium; yellow, mesenchyme; blue, endocardium-derived mesenchyme (EDM); red, non-endocardium-derived mesenchyme (NEDM). Abbreviations: DM, dorsal mesocardium; L/RSI, left/right systemic inflow; L/RPR, left/right pulmonary ridge; AI, area interposita; (L/R)A, (left/right) atrium; (s/i)AVC, (superior/inferior) atrioventricular cushions; V, ventricle; L/RVV, left/right venous valve; SAJ, sinoatrial junction; PV, pulmonary vein; PAS, primary atrial septum; PF, primary foramen; OFT, outflow tract; CAP, mesenchymal cap on the leading edge of the primary atrial septum; BM, body mesenchyme. Bars represent 100 μm.
from the endocardium. From the stance of morphologic description, apart from acknowledging the importance of the original description by His, there is little thus far to substantiate the existence of an atrial spine.

Previous divergent opinions, therefore, have reflected the difficulties involved in unequivocal morphological delineation of the spine, along with the unproven assumption that the spine has an extra-cardiac origin\(^2,5\text{-}7,11\) as opposed to an endocardial origin.\(^19,21\) Given these ambiguities in the description of the spine by the different authors it is difficult to assess whether the atrial septal defects observed in the trisomy 16 mouse model\(^3\) and human fetuses with Down’s syndrome\(^7\) are due to impairment of the formation of extra-cardiac mesenchyme or of endocardium-derived mesenchyme.

We believe that our current investigation has reconciled these problems, suggesting that the mesenchyme that forms the antero-inferior rim of the primary atrial septum takes origin from both intra-cardiac and extra-cardiac sources. The non-endocardium-derived mesenchyme is located in an area that is highly active in myocardium formation\(^22\) as apparent from the expression of \textit{Islet1}, a marker for the second heart field.\(^18\) Most likely, therefore, this mesenchyme muscularises to form the buttressed inferior rim of the primary atrial septum.

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