Getting to the Heart of Cardiac Morphogenesis

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One of the most difficult concepts in heart development is to convert a looped tube with the presumptive right and left chambers in series to a 4-chambered heart with blood flowing from right atrium to right ventricle and left atrium to left ventricle. Try to explain how it happens to a room full of typical first-year medical students (“just tell me what I need to know”) if you are into self-laceration.

The key to shifting from serially connected presumptive chambers to chambers connected in parallel is the atrioventricular (AV) canal. Because this little canal originally connects only the part of the tube that will become the atria with the part that becomes the left ventricle, it is critical that it moves or expands to gain access to the more distal part of the tube that will become the right ventricle. It is only possible because the tube is looped. It may be obvious that the myocardium at the inner part of the loop must be remodeled along with a change in position or expansion of the AV canal.

My awakening to the complexity of this region occurred several years ago with the publication of a study by Webb et al1 in Circulation Research. Until then, my rather naive view of the AV canal was fairly simplistic and involved some shifting and fusion of the AV cushions. Webb et al presented a coherent exposition of the central mesenchymal mass (septum intermedium) in developing mouse embryos. This central mass comprises the AV endocardial cushions, the valves of the sinus venosus and the sinus septum, and mesenchyme of the right pulmonary ridge. This ridge is additionally complicated because it has a core (called the spina vestibuli) originating from body mesenchyme, which is covered by mesenchyme that is continuous with the leading edge of the primary atrial septum. If you have trouble visualizing how these structures get together to form a central mass of mesenchyme, this study can help. During later development, according to the authors, some areas of this central mass become fibrous, but the larger part of the mass is muscularized to form the inferior edge of the fossa ovalis (oval fossa). After delamination of the septal leaflet of the tricuspid valve, the central mass becomes the AV component of the membranous septum and the fibrous skeleton of the heart. Webb et al did not address what happened to the myocardium of the AV canal, which is really a separate issue.

The fate of the AV myocardium is very important, because it is actively involved in changing the connection of atria to ventricles from being serial to parallel. Another important result of this remodeling is to restrict the electrical continuity of atria and ventricles to the AV node, which is specialized for electrical conduction. If nonspecialized (working) myocardium remains at the AV junction, propagation of the electrical current through the heart is not normal and results in premature ventricular contractions, as seen in Wolff-Parkinson-White syndrome.2-5

In this issue of Circulation Research, Kim et al6 present a detailed study using molecular markers to show the reorganization of the AV myocardium in formation of the AV junction in developing human heart. They have been able to follow certain portions of the AV canal myocardium using the spatial expression patterns of 2 markers, which this group has studied in heart development for many years. The GIN2 antibody was used to track parts of a ring of positive myocardium at the midpoint of the looped tubular heart that separates the presumptive left and right ventricles (called the interventricular foramen). This antibody was developed against a carbohydrate epitope in nodose ganglion by Barbu et al7 in Dr Nicole Le Douarin’s laboratory. Because so many neural antigens are expressed by myocardial cells that ultimately become specialized conduction myocardium, it should come as no surprise that this ring of tissue is thought to become the AV bundle and bundle branches.8 The second marker used in this study was creatine kinase M, which is expressed by the ventricular myocardium but not the AV canal myocardium. So the story of AV myocardial fate is very cleverly based on the region where GIN2 expression overlaps with absent expression of creatine kinase M. Using the dynamic patterns of positive and negative staining and the areas where they overlap, Kim et al6 confirm the results of previous studies by their group that the myocardium of the AV canal does not contribute to ventricular myocardium but is mostly incorporated into the smooth walls of the atria just above the AV valves. Although this was known earlier,9,10 the exact location of the AV myocardium in the definitive atria was not determined. The present study shows definitively that the continuity of the right side of the AV canal with the ascending (right ventricular) portion of the cardiac loop forms by local expansion of the inner curvature of the heart and, finally, that the AV node that connects the atria and ventricles electrically develops as a persisting portion of this myocardium.

At issue was how the AV canal repositions and remodels itself to allow blood to flow from an incipient right atrium through the right side of the canal into the ascending (right ventricular) portion of the cardiac loop. It has been acknowledged for years that the AV canal must expand to the right.11 Several studies proposed that the AV endocardial cushions,
when they first developed, were located to the left of the primitive interventricular septum and that the right ventricle acquired its inlet when these 2 cushions became aligned with the primitive interventricular septum, that is, the whole AV canal moved toward the right. In contrast, others thought that the 2 endocardial cushions are in continuity with the primitive interventricular septum from their first appearance, so that the presumptive right atrium communicates with the right ventricle and the left atrium with the left ventricle. In this model, differential growth would have to occur on the right. de la Cruz actually describes the figure 8 formed by the septum primum, endocardial cushions, and primitive interventricular septum as a unit, which she calls the first cardiac septum. The present study shows that the position of the cushions is less important than the AV myocardium that blends into the myocardium of the inner curvature (ie, on the right side). The myocardium at this site, which is GIN-positive and creatine kinase M-negative, expands, allowing for a right-sided channel. This expansion is accompanied or followed by a rightward expansion of the right ventricle, which is quite small before the expansion. This is the first demonstration of the extent and position of the myocardium involved in the rightward expansion of the AV canal. Of course, it does not preclude a shift to the right in the position of the canal relative to the ventricles, but it is really not necessary given a rightward shift by myocardial expansion.

The other significant finding of the study by Kim et al is that the bulk of the AV canal myocardium is incorporated into the atria just above the AV valves, whereas the myocardium of the AV node and bundle is set aside for electrical conduction. To get proper insulation of the AV node and separation of the atrial from the ventricular myocardium at the AV junction, nonmuscle mesenchyme is vitally important, as mentioned previously. In an elegant study of human hearts based on differential expression of myocardial and mesenchymal markers, Wessels et al showed the progressive incorporation of the AV myocardium into the atria accompanied by fusion of (nonmyocardial) mesenchyme just above the ventricular myocardium. This leaves the AV node and bundle (of His) as the only site of myocardial, and thus electrical, continuity. However, the study left unresolved the site of origin of the AV node and bundle myocardium, which is now shown in detail by Kim et al.

Unfortunately, the definition of the vestibular spine, an extension of mesenchyme from the splanchnic mesoderm ventral to the foregut, in this study is at variance with that of Webb et al. Whereas the primary atrial septum is comprised of muscle, its leading edge is covered by mesenchyme. Webb et al considered the mesenchymal cap on the primary atrial septum as distinct from the mesenchyme of the vestibular spine, whereas Kim et al consider it a cranial extension of the spine. Puerta Fonolla and Orts Llorca describe closure of the AV canal by introduction of the spina vestibuli of the septum primum between the 2 endocardial cushions. In contrast, Arrechedera et al propose that it arises in the chick from epithelial-to-mesenchymal transformation of the endocardium similar to that seen in cushion formation. Although this could be attributable to species differences, it seems unlikely, because the formation and closure of the primary atrial septum is quite similar in all 3 species. The point is an important one, because failure of the mesenchyme at the leading edge of the primary atrial septum to fuse with the AV cushions results in AV canal defects. If the mesenchyme involved in atrial/AV canal septation is reminiscent of that involved in outflow septation, as suggested by Kim et al in their discussion, the true situation may be that endocardially derived and extracardiac mesenchyme both play a role.

Most readers will find the study by Kim et al difficult to read, partly because of the variance in vocabulary used by different groups to name structures and partly because the orientation of the developing heart changes with respect to the body and the relationships are difficult to follow, but the struggle is ultimately worth the effort. It represents a significant step in our evolving understanding of heart development. It is truly regrettable that the legacy of heart development has been one of contentions in naming structures as well as in conceptualization. To study heart development, one must learn a new language. The situation is not simplified when even the native speakers argue over what each word means (or if it means anything, or if the structure even exists).

Keep in mind that our forebears had only histological sections of developing and adult, normal, or malformed hearts along with good imaginations to link them. For better or worse, creative ideas often inspire strong opinions. Now that we begin to have the tools to sort through some of the major issues of cardiac morphogenesis, those of us struggling to understand are fortunate to have a small cadre of investigators who recognize the problems and the heritage and are capable of leading us through the unanswered questions.

References

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