Atrial Fibrillation: General Considerations

Atrial fibrillation (AF), a common adult cardiac arrhythmia, involves abnormal atrial contractions. As every medical student is taught, it is easily diagnosed as an irregularly irregular pulse and loss of organized atrial activity on an ECG. Clinical interest in AF is considerable because AF is the most common adult arrhythmia that increases in prevalence with age, with 5% of the over 65 population having AF. Moreover, patients with AF have a significantly increased risk of stroke.1

Electrical impulses, critical for a coordinated and physiologic heartbeat, are normally initiated in the sinoatrial (SA) node. However, in AF the disorganized atrial activity overrides normal SA node function resulting in irregular conduction of impulses to the ventricles. In the majority of cases, ectopic electrical activity originates in the pulmonary veins.1

In this issue of Circulation Research, Mommersteeg and colleagues2 make a significant advance in our understanding of pulmonary vein development by making a strong connection between AF and embryonic axis determination. Moreover, their work fits nicely with exciting new data from human genetic studies implicating the same genetic pathways in familial AF.3

Mommersteeg and colleagues make a number of fundamental observations regarding pulmonary vein development. Previous work suggested that pulmonary myocardium derives from atrial myocardium.4 Using lineage tracing, the authors convincingly rule out this possibility. Moreover, they show other data revealing that pulmonary myocardium is derived from Isll-positive second heart field and has a distinct origin from the systemic venous circulation.5 Together, these findings suggest that the pulmonary vein myocardium forms by differentiation of mesenchyme around the pulmonary vein.

To address this hypothesis, the authors use a Pitx2 mutant mouse model that had been shown to have defects in pulmonary vein development.6 The Pitx (Pituitary homeobox) family of homeobox genes, containing 3 genes, Pitx1, Pitx2, and Pitx3, is a Bicoid-related subfamily within the larger Paired-related superfamily of homeobox genes.7,8 The Pitx group, a remarkably important gene subfamily, has been implicated in human development, disease, and evolution.9–11 The most extensively studied of the 3 genes, Pitx2, was identified as the gene mutated in Rieger Syndrome I that includes ocular, tooth, and anterior body wall defects as cardinal features.10 Subsequent work on Pitx2 revealed an important function for the Pitx2c isoform of Pitx2 in left right asymmetry (LRA), a fundamental component of organ morphogenesis in vertebrates.

Left Right Asymmetry and the Pulmonary Vein

In vertebrates, internal organs show morphological differences between the left and right sides. For example, the number of pulmonary lobes is different between the left and right lungs and the intestines have a characteristic, asymmetric rotation. One critical consequence of LRA morphogenesis is the formation of left and right anatomic characteristics that have critical functions in separating the systemic and pulmonary circulation that is vital for efficient, physiologic organ function. The pulmonary vein, connecting directly to the left atrium, is a left-specific character.

Certain aspects of cardiac development have been clearly linked to LRA including septal and valve defects.12 Moreover, cardiac looping morphogenesis is also a well known characteristic of LRA. Clinical studies have also uncovered LRA defects that were part of a larger syndrome. Clinicians have long recognized the link between defects in cilia function or primary cilia dyskensia (PCD) and laterality defects, referred to as Kartegener syndrome.13 In addition to LRA abnormalities, Kartegener patients commonly present with respiratory infections, male infertility, and diminished smell that are secondary to abnormalities in the dynein arms of the microtubules. Thus, LRA, recognized to be a causative factor in syndromic congenital heart disease, has not been a high priority for adult cardiologists.

This perspective has recently changed with exciting recent work that has made the connection between LRA and AF. Human genetics studies identified sequence variants on chromosome 4q25 that were strongly associated with increased risk for AF in multiple populations. Notably, sequence variants with high correlation to AF were found in proximity to Pitx2 making Pitx2 the likely candidate AF locus in this region.3

The Nkx-Pitx2 Connection in Left Right Asymmetry

Left right asymmetric morphogenesis is initiated in the presomite mouse embryo and is mediated through the Nodal signaling molecule. A major downstream effector of the Nodal pathway is the Pitx2 homeobox gene.14 Studies performed in chick, mouse, zebrafish, and Xenopus embryos made a firm connection between Pitx2 and the Nodal-regulated left right asymmetry pathway. Experiments performed in transgenic mice indicated that although Nodal
signaling was required for induction of Pitx2 expression, Nkx factors were required for persistence of Pitx2 transcription on the left side of developing organs.15

Previous analysis of the Pitx2c expression pattern in mice and chick embryos revealed that Pitx2c was expressed on the left side of the anterior precardiac splanchnic mesoderm and expression persisted in the left heart tube before looping morphogenesis. This early expression domain of Pitx2c suggested a role for Pitx2c in looping morphogenesis,16–18 Gain-of-function studies in chick embryos demonstrated that Pitx2c, when overexpressed in right lateral plate mesoderm, resulted in hearts with reversed or ambiguous situs. Moreover, Nodal misexpression in the right lateral plate of chick embryos also resulted in induction of ectopic Pitx2 expression on the right.17–19 These data suggested the existence of a linear signaling cascade with Pitx2 serving as the final effector of the left right asymmetry pathway within each organ primordium.

In addition to a potential role for Pitx2 in cardiac looping morphogenesis, other work suggested a direct role for Pitx2 in patterning a broad range of left-sided cardiac and vascular structures. Pitx2c was expressed in the left atrium and atrioventricular canal, looped outflow tract, right ventricle, and interventricular myocardium. Moreover, Pitx2c was also expressed in the primary and secondary interatrial septum, left atrial appendage, left superior caval vein, and pulmonary vein myocardium.20,21 Pitx2-null embryos had severe defects in AV valve formation with complete AV canal. There were also defects in sinuatrial morphogenesis including failure of outgrowth of the primary interatrial septum and isomerized atrial appendages.21,22 Arterioventricular connections were abnormal with double outlet right ventricle (DORV) and great vessel transposition (TGA) commonly observed.21,23 Growth of the ventricular myocardium was also defective resulting in right ventricular hypoplasia.6,21,22 Thus, the expression and functional analysis pointed to a role for Pitx2 in cardiac looping morphogenesis and also in the morphogenesis of complex cardiac and vascular structures.

The findings of Mommersteeg and colleagues have now made the leap to pulmonary vein and AF. Expression studies by Mommersteeg et al indicated that Pitx2c is found in both undifferentiated pulmonary mesenchyme and pulmonary myocardium in the 10.5 dpc to 12.5 dpc time frame. Interestingly, after 12.5 dpc, Pitx2c was expressed in pulmonary myocardium but was excluded from the pulmonary mesenchyme suggesting that Pitx2c may have distinct, stage-specific functions in pulmonary vein development.

In Pitx2c-/- embryos, the authors discovered a major deficiency in pulmonary vein myocardium in all (n=24) Pitx2c mutants studied. Further investigation of Pitx2c mutants revealed complete absence of the first pulmonary myocardial cell population that normally forms around 12.5 dpc. This loss of pulmonary myocardium was attributable to a proliferative defect in the pulmonary mesenchyme. Importantly, Pitx2c has been shown to regulate proliferation in other types of muscle including outflow tract myocardium and cultured skeletal myoblasts.23–25

The authors next investigate the role of the Nkx2.5 homeobox gene in pulmonary vein formation. Previous work in human genetics revealed that Nkx2.5 was mutated in patients with atrial septal defect (ASD) associated with conduction defects.26 Moreover, mouse models revealed a central role for Nkx2.5 in conduction system development.27 The authors noted that Nkx2.5 and Pitx2c had a similar developmental expression patterns in pulmonary vein progenitors before myocardial differentiation. Because Nkx2.5-null embryos are lethal at stages before pulmonary vein development, the authors used an Nkx2.5 hypomorphic allelic combination to dissect Nkx2.5 function in pulmonary vein development. Interestingly, in embryos with reduced Nkx2.5 the pulmonary vein gene program reverts to that of the systemic venous myocardium uncovering a role for Nkx2.5 in specifying pulmonary vein myocardial phenotype. This also suggests that the systemic venous myocardial genetic program is the default program and that repression of this systemic program by Nkx2.5 is important to prevent AF.

Concluding Remarks
The unexpected connection of LRA to AF provides a number of basic research and clinical opportunities. One avenue that should be pursued is the molecular connection between Pitx2c and Nkx2.5 (Figure). There is conclusive evidence that these two transcription factors genetically interact, but the molecular details of this and other possible interactions need to be pursued in more detail. Although Mommersteeg’s data suggest that Pitx2c and Nkx2.5 have distinct functions in pulmonary vein development, it should be remembered that

Figure. Genetic relationship between Nkx2.5 and Pitx2c. Three potential genetic interactions between Pitx2c and Nkx2.5. A, Nkx2.5 directly regulates Pitx2c by binding to the asymmetric element in the Pitx2 gene. B, Pitx2c and Nkx2.5 directly interact to coordinately regulate target genes. C, Pitx2 is upstream of Nkx2.5 and directly regulates Nkx2.5 transcription.
an Nkx2.5 hypomorphic combination was used for their analysis. Analysis of a Nkx2.5 conditional null allele is needed to evaluate Nkx2.5 pulmonary vein function further. One potentially fruitful possibility would be that Pitx2c and Nkx2.5 share common target genes. In this case, a bioinformatics-based inquiry for common target genes would be made more approachable.

In this era of emphasis on translational research, it is important to remind ourselves of the critical role for basic research in disease-based research. The work of Mommersteeg, which sheds new light on the role of embryonic axis development and a common, adult cardiac defect atrial fibrillation, is a case in point.

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References

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