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RESEARCH NEWS

Elucidating the Molecular and Genetic Interactions Responsible for Congenital Heart Disease

A review of: Bruneau BG, Nemer G, Schmitt JP, *et al*. 2001 A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. Cell 106:709–721

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NEARLY TWICE AS many children die from congenital heart disease in the United States than from all forms of pediatric cancer combined. Ironically the molecular basis of the former is less well understood than the latter. Less than a decade ago linkage analyses revealed mutations of cardiac transcription factors–Tbx5 in Holt-Oram syndrome (1, 2) and Nkx2-5 in familial ASDs and atrioventricular conduction defects (3). Although the spectrum of cardiac defects in affected patients cannot be explained by the few known downstream targets of either transcription factor, recent papers show how Tbx5 and Nkx2-5 might orchestrate cardiac development via protein-DNA and protein-protein interactions. These interactions are demonstrated in a Tbx5 knockout mouse by Bruneau et al and in complementary *in vitro* studies (4–6).

Heterozygous Tbx5 knockout mice mimic the limb and cardiac defects of Holt-Oram syndrome (4). All heterozygous adult mice have large secundum ASDs. Heterozygous fetuses had muscular and membranous VSDs; one fetus had a malformed left ventricle. The mice also had first-degree atrioventricular block and episodes of higher-grade block and sinus pauses; diminished connexin 40 expression in Tbx5 mutant mice may cause the conduction defects. Of note, other cardiac defects seen in Holt-Oram such as tetralogy of Fallot or aortic stenosis were not observed in the mice possibly because of rarer occurrence or differences between mouse and human in the expression pattern of Tbx5 (7–9).

Bruneau et al analyze the promoters of two Tbx5 target genes. Both atrial natriuretic factor (ANF) and connexin 40 contain multiple Tbx5 binding sites in close proximity, each of which is important to drive high-level expression by Tbx5. This may be a general feature of target genes, as suggested by DNA-database analysis of cardiac-expressed genes (6). Thus, the number of transcription factor binding sites may regulate the level of gene expression. Some cardiac defects may result from mutations of these sites.

Combinations of cardiac transcription factors acting on a promoter are also likely to be important in regulating cardiac development. Within a 500 base pair interval of the ANF promoter there were three Tbx5 binding sites and an additional Nkx2-5 binding site. Tbx5 and Nkx2-5 synergistically activate a reporter gene when the ANF promoter construct contains binding sites for both transcription factors (4, 5). In addition, Tbx5 and Nkx2-5 physically interact with each other (5). Thus, one may hypothesize that intersecting expression patterns of transcription factors mediate the spatial regulation of cardiac gene expression. Mutations that alter protein-protein interactions, DNA-binding affinities, tandem transcription factor binding sites, or the normal level or pattern of expression of transcription factors would be expected to cause cardiac defects.

We are still a long way from a detailed molecular and genetic understanding of cardiac development despite its high relevance to pediatric morbidity and mortality. For example, which genes do Tbx5 and Nkx2-5 act on to effect atrial septation? The answer is unknown, but the work of Bruneau and others offers suggestions where we may look to elucidate the mechanisms.

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