

Pathways to embryonic heart failure

David Sedmera

Charles University in Prague, First Faculty of Medicine, Institute of Anatomy, and Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic

HEART FAILURE IS A COMPLEX pathological process with increasing prevalence worldwide (10). Many recognized causes lead to several well-defined pathways in the adult, and correlations between functional status (right and left failure, systolic and diastolic dysfunction) and morphology (ventricular hypertrophy and/or dilatation) are well characterized. Considerably less is known about heart failure in the fetus (17), but in the second and third trimester, the symptoms and pathological changes are similar to the adult, although they develop much faster.

In contrast, our knowledge about the function of the embryonic heart, much less about its pathophysiology, is considerably more fragmented. Pioneering studies characterizing the function (and morphology) of the developing heart were, mostly for methodological reasons, performed in the chick embryo (1). While there are quantitative differences (e.g., contribution of atrial filling to cardiac output or reaction to heart rate variation or filling), the embryonic heart with formed, albeit nonseptated chambers, observes most laws first described in the adult heart (6). The special case is the early tubular heart (2, 12), which operates in a completely different mode (suction pump, with effectively 100% ejection fraction due to complete occlusion of the lumen).

Until recently, most of the information about embryonic heart function was inferred from its morphology. Explosion of different mouse mutants with “cardiac phenotype” is thus, with a few exceptions, characterized only from the structural point of view with main attention focused on the role of particular gene in cardiac (dys)morphogenesis (27). Functional analysis of embryonic mouse cardiac phenotypes has emerged relatively late, but it is possible, with current state-of-the-art equipment, to perform correlative structure-function studies that broaden our understanding of embryonic heart functioning.

Cardiac function in the developing mouse heart was first characterized, using the same technique initially employed in the chick, by Keller et al. (8). Although technologically relatively simple (requiring only use of a video camera and pulsed Doppler, plus setup for mouse anesthesia), the invasive, non-survival nature of their imaging protocol precluded longitudinal analysis of individual embryos. A major breakthrough was provided by adaptation of ultrasound technology, mainly increase in ultrasound frequency and thus spatial resolution, termed “ultrasound biomicroscopy” (3, 18). Soon, functional studies (19, 28) of mutant mouse phenotypes followed.

In this issue of *Am J Physiol Heart Circ Physiol*, a new study from the Phoon laboratory (15) provides us with new insights into embryonic heart function by meticulous structure-function analysis of mice with conditionally deleted bone morphogenetic protein (BMP) receptor type 1A (also known as ALK3) in the neural crest cells. While the role of BMP signaling in heart development is well established, most “traditional,” i.e., complete knockouts,

show early embryonic lethality precluding analysis of cardiac phenotype. This is the case of the BMPRIA knockout (14), which dies at embryonic day (E) 8.5 due to problems with gastrulation (mesoderm formation). Targeting the deletion specifically to neural crest cells, either through Wnt1-Cre mouse (25) or P0 (15), leads to mortality at E12.5, with the phenotype of persisting truncus arteriosus and signs of myocardial thinning.

A previous study performed in a very similar model (differing only in the choice of Cre line) showed a morphologically identical phenotype and suggested that embryonic demise was due to insufficient myocardial performance, since there was a decrease of ventricular myocyte proliferation. Indeed, neural crest ablation studies in the chick demonstrated both outflow tract (persistent truncus arteriosus) and ventricular (thin compact myocardium) phenotypes; what is missing is the causal link between the primary neural crest cell anomaly and myocardium. This is a sort of “chicken or egg” question, but paracrine signaling from these (however, few) cells in the epicardium is one plausible explanation, since the epicardium is a well-known source of growth factors stimulating myocyte proliferation (7) and mouse mutants with deficiency in this cross-talk often exhibit thin compact myocardium (reviewed in Refs. 24, 27). The present study offers another, potentially more intriguing, explanation through volume overload of the ventricles due to leakiness of the embryonic outflow tract. Outflow tract cushions are significantly populated by migrating cardiac neural crest, and cushion hypoplasia leads to deficient outflow tract septation manifesting as persistent truncus arteriosus. However, the distal outflow tract cushions also give rise to the semilunar valves, and before that, their apposition during contraction helps to assure prograde blood flow (12, 16). Findings of the present study based on ultrasound interrogation of >100 embryos, namely that hypoplastic outflow tract cushions cause significant regurgitation and blood flow reversal in major embryonic arteries, present the first proof of immediate functional consequence of cushion anomaly. Significantly, the flow reversal was convincingly shown to be a prelude to embryonic demise and was present 24 h before actual death.

There are further lessons to be learned from this study, apart from confirmation of the critical role of BMP signaling for growth of cardiac cushions and their role in preventing backflow. This is, in fact, the first description of an embryonic mammalian model of ventricular volume overload. Until now, the only similar condition was the experimental left ventricular hypoplasia in the chick (21, 23), which created volume (and later on, also pressure) overload in the right ventricle. Common in both cases is the thinning of the ventricular compact layer, suggesting some ventricular dilatation. Unlike increased pressure load, volume load is not a stimulus for ventricular myocardial growth, and the rates of myocyte proliferation are actually decreased not only in the volume underloaded hypoplastic left ventricle but also in the overloaded right ventricle (21, 22). This seeming paradox is likely due to significant functional reserve of the trabeculated ventricular myocardium (this is well before deployment of coronary circulation at stages where

Address for reprint requests and other correspondence: D. Sedmera, Institute of Anatomy, First Faculty of Medicine, U nemocnice 3, 12800 Prague 2, Czech Republic (e-mail: david.sedmera@lf1.cuni.cz).

spongy trabecular meshwork forms up to 80% of myocardial mass).

This study also expands our spectrum of known reasons for prenatal cardiac failure. Until now, if we exclude placentation problems, there were three periods of demise thought to be due to distinct sets of cardiac anomalies. The first is due to early (~E10.5) heart failure due to lack of formation of ventricular trabeculation, first described in mice deficient for neuregulin or its receptors (4, 9, 13) and later also in the Nkx2.5 knockout (11). The second, occurring around E14.5, is believed to be a result of deficient trabecular compaction (or establishment of coronar circulation). This transition is supposed to increase the ability of the ventricle to generate pressure, and deletion of variety of genes from various pathways (retinoid or adrenergic signaling, epicardial transcription factors, and vasculogenesis) leads to so-called "thin compact myocardium" phenotype (reviewed in Refs. 24, 27). The third period occurs immediately after birth and comprises all severe forms of congenital heart disease that are incompatible with extrauterine life (severe outflow tract obstruction, transposition of great arteries, and absence of pulmonary valves). Failure of temporary valve function of the outflow cushions leads to significant hemodynamic disturbance (up to 42% of the stroke volume regurgitating back) resulting in embryoletality at E12.5, in contrast to death at E14.5 in NFATc1 mutants, where the outflow tract septation initially appears normal and regurgitation develops at E13.5 due to lack of semilunar valve formation (of note, also 24 h before actual embryonic death).

What else could we learn from this mouse model? Cardiac neural crest cells also contribute to the development of conduction system, and in the chick ablation model, functional and morphological alterations were recently documented (5). Although the mouse conduction system is not fully developed at E12.5 (20, 26), both morphological and functional analysis should be possible and might detect anomalies of its formation. Clearly, there are many interesting mouse phenotypes that await physiological analysis, and with ongoing perfection of noninvasive imaging tools, we can look forward to full exploitation of these models.

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