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RESEARCH ARTICLE

Current advances and concepts of Embryological and Genetic basis of the developing human Heart

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Abstract

The current comprehension regarding embryologic and genetic basis of heart has changed with fate mapping and lineage studies of the developing hearts of chicken and mice. Genetic studies with knockout animals give practical insights into developing culture techniques of stem cell derived cardiomyocytes. Intracellular calcium cycling plays a major role in the generation of automaticity in embryonic cardiomyocytes and so could be used to generate stem cell derived spontaneously beating myoblast cells. An in depth theoretical biological framework will go a long way in clarifying many uncertainties and itself can serve as a powerful instrument in the hands of the theoretician as well as the practical researcher. We hereby propose certain theoretical postulates that could throw light on structure, function and development of myocardium. We propose to understand the vertebrate circulatory system as a network of smooth muscle, the centre of which is transformed into cardiac muscle. When increased circulatory load is imposed on the system, smooth muscle underwent some emergent properties that led to the attainment of automaticity. Excitability of the smooth muscle is utilized to the maximum extent in vertebrate circulatory system, but it is now subordinated to cardiac muscle. Smooth muscle too has pacemaker activity but it has undergone a phase transition in the myocardium and has got further stabilized in the form of a structural pacemaker.

Introduction

For more than a century, the mechanisms of the development of a four chambered heart and its valves from a straight primary heart tube are a matter of hot debate and speculation. Classical embryologists were forced to confine to observations based on histological sections and three dimensional reconstruction of the developing heart because of the limitations of time. They drew their conclusions on the basis of morphogenetic mechanisms that could possibly explain the pattern of growth and differentiation as they did not possess the necessary molecular markers and tools to study genetic lineage[1].

Development of the vertebrate heart and formation of its chambers constitutes an intricate process in which the growth and differentiation of its different parts is regulated in a spatiotemporal pattern. Various forms of congenital heart disease are a result of defects in these mechanisms that could lead to underdevelopment of the walls or valves of the chambers, or misalignment of the ventricles with their outflow tracts and great vessels. An insight into embryological basis of the malformations of the human heart is limited, in spite of the fact that the recent advances of molecular embryology have improved our understanding of the normal cardiac development. Most of our current

knowledge is based on extrapolation of experimental data from animal studies as direct experimentation on human cardiac development faces insurmountable problems.

All authoritative textbooks on human embryology depict the development of the human heart as starting from a straight tube that is compartmented in a sequential way. This segmental concept is disproved by fate mapping studies in chicken previously and by molecular lineage analysis in mice more recently[2,3]. Chicken experiments revealed that the primary heart tube proliferates minimally and the growth and differentiation occurs mainly by addition of differentiating visceral mesoderm at the arterial and venous poles.

However, fate mapping and lineage studies of developing hearts of chicken and mice have changed the scenario. Genetic studies with knockout animals will give practical insights into developing culture techniques of stem cell derived cardiomyocytes. We hereby propose certain theoretical postulates that could throw light on structure, function and development of myocardium.

The changing scenario

As the heart tube elongates it is forced to loop inside the restricted pericardial tube producing its internal and external curvatures, thus laying down the general plan for the building of the cardiac chambers (Figure 1) and its conduction system[4,5].

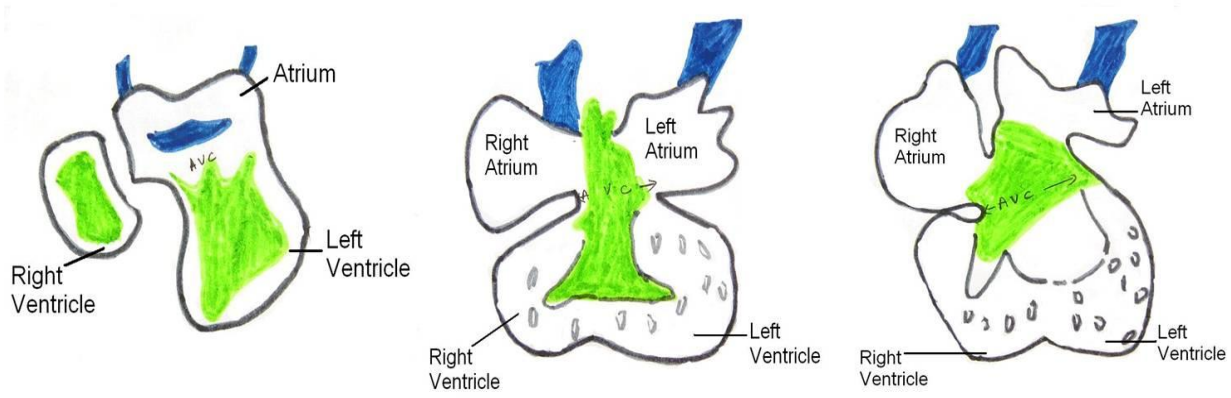

Figure1. Chamber formation in embryo heart during stages 12, 14 and 16(From left to right).The ballooning of walls of outer curvature of looped heart tube causes expansion of chamber cavities. Figure drawn by Dr Gaurav Agnihotri.

The chambers themselves are formed by differentiation of the visceral mesoderm into primary myocardium. This is now covered by another layer of differentiating myocardium; the previous layer is suppressed from further differentiation by transcription repressors. These repressors called TBX2 and TBX3 pave the way for formation of the cardiac conduction system[6, 7].

The primary myocardium differentiates into secondary working myocardium, but this process is suppressed in some parts like sinus venosus, atrioventricular canal and outflow tract. The suppression of this differentiation is regulated by two transcription repressors called TBX2 and TBX3[8]. Expression of TBX2 decreases in the ventricular direction, a process that is observed in many species and from this we can infer that the mechanisms of chamber formation and those of conduction system are fairly conserved in the course of evolution[9,10]. We now introduce the readers to some of the markers that are found useful by several investigators for the study of the developing heart.

Expression of certain markers in the primary heart tube

In stage 9 embryos, the heart tube is as yet not identified and the domains of expression of NKX2 and ISL1 are overlapping from the heart primordium over to the adjacent mesoderm. In stages 10 and 11, the non-differentiated (troponin I –negative) mesodermal cells at the venous and arterial poles expressed NKX2 and ISL 1 suggesting that they are precursors to cardiomyocytes. Most of the myocardial tube is negative for ISL1, whereas all myocardial cells express NKX2 indicating that myocardial tube as such does not give rise to myocardium. When the expression of Ki67 was quantified[11,12], it was found that only 33% of cardiomyocytes but 84% of cells within the adjacent ceolomic wall were positive for Ki67 in stage 10. Later 50% of cardiomyocytes became positive for Ki67 in late stage 10 and only cardiomyocytes are positive in stage 11.

Growth and differentiation of cardiac chambers

In stage 12, the proportion of proliferating cardiomyocytes increases rapidly. Myocardium of atrial and ventricular chambers shows high levels of expression of PCNA from stage 12 through 16[13, 14]. The morphological changes depicting the chamber formation is accompanied by expression of several molecular markers like ANF[15] (atrial natriuretic factor) and fast-conducting Connexins[16] 40 and 43 in the developing myocardium (Figure 2).

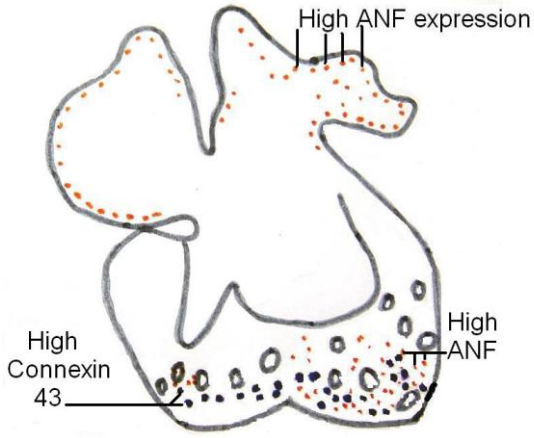

Figure2. Embryo heart Stage 16.Atrial chamber formation is marked by high expression of Atrial Natriuretic Factor(ANF).The ventricular chamber formation is characterized by gradual thickening of the compact layer and by appearance of extensive trabeculae which are positive for ANF and Connexin 43.The inner curvature ,atrioventricular canal and outflow tract are devoid of these markers. Figure drawn by Dr Gaurav Agnihotri.

In contrast, the non-working myocardium of atrioventricular canal, and outflow tract was shown to be lacking the expression of ANF, connexin 40 and 43 and of PCNA[17]. At these locations that are flanking the ballooning atrial and ventricular chambers, PCNA and chamber marker expression is absent giving credence to the hypothesis that these areas show a persistence of primary myocardium. The transcriptional repressors and conduction system marker TBX3 were found to be first expressed in the floor of the common atrium as well as the atrioventricular canal in stage 12[18]. TBX3 expression became asymmetrical in the atrioventricular canal in stage 14, being stronger in the right side[19, 20].

In the process of generating myocardium from stem cells

We also would like to throw some light on how genetic studies with knockout animals will give practical insights into developing culture techniques of stem cell derived cardiomyocytes. Going into the details of latest physiological concepts of automaticity and impulse generation of myocardium is out of scope of this paper and will be discussed in a separate one. We just give an introduction of the scope of using genetic studies in the culture of spontaneously beating cardiomyocytes.[21]

Intracellular calcium cycling plays a major role in the generation of automaticity in embryonic cardiomyocytes and so could be used to generate stem cell derived spontaneously beating myoblast cells.[22] As SR calcium cycling is crucial to their function, calcium cycling proteins are expressed very early in developing cardiomyocytes. Stem cell derived cardiomyocytes are shown to express ICaL even when they start beating, the density of which increases three fold in the process of differentiation[23]. Ryanodine receptors[24] are tightly coupled with L-type calcium channels in these stem cell derived cardiomyocytes. In contrast to adult myocardial cells, calcium induced calcium release synchronizes local SR Ca^{++} oscillators leading to spontaneous generation of rhythmic action potentials in developing cardiomyocytes.

Death of embryos in RYR2 knock out mice is known to be due to disturbances in calcium metabolism, but studies of cardiomyocyte function derived from mouse embryonic stem cells revealed that the knockout leads to suppression of spontaneous contractions due to failure of spontaneous release of calcium into the cytosol.[25, 26]

Discussion

Our understanding of the embryological and genetic basis of the human heart is undergoing a sea change with the introduction of the monoclonal antibodies into the study the embryological markers and other molecular biological techniques. But our understanding is still lagging at the theoretical plane. An in-depth theoretical biological

framework will go a long way in clarifying many uncertainties and itself can serve as a powerful instrument in the hands of the theoretician as well as the practical researcher. We hereby propose certain theoretical postulates that could throw light on the structure, function and development of myocardium.

We propose to understand the vertebrate circulatory system as a network of smooth muscle, the centre of which is transformed into cardiac muscle. When increased circulatory load is imposed on the system, smooth muscle underwent some emergent properties that led to the attainment of automaticity. Excitability of the smooth muscle is utilized to the maximum extent in vertebrate circulatory system, but it is now subordinated to cardiac muscle. Myocardial excitability is coupled to that of the smooth muscle through the pressure wave generated in the heart.

The increase in mechanical load could have been managed by an increase in thickness of vessel musculature, but at a certain point, it leads to instability that could be addressed only through a phase transition. Smooth muscle too has pacemaker activity but it has undergone a phase transition in the myocardium and has got further stabilized in the form of a structural pacemaker.

Most of the researchers are studying the embryology and genetics of the heart with help of monoclonal antibodies that identify specific cell surface markers. But these markers are not expressed in an isolated fashion but are expressed as networks of proteins. The expression of these networks of proteins proceeds in a synchronized way and in particular spatiotemporal patterns. It is the disruption of these patterns that lead to pathology rather than just the presence or absence of a particular protein marker.

A sound theoretical summation coupled with the extensive use of the latest molecular approaches will go a long way in elucidating the underlying patho-embryology of congenital heart disease and their genetic treatments.

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