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Heart Tube Formation

Introduction

The human heart is a vital organ because it controls fundamental body processes, including the blood flow and oxygen regulation. When there is insufficient oxygenation in the body, it becomes challenging for the body to attain optimum operations levels. This underlines why there is need to understand the heart tube formation. This research paper outlines some of the intricate processes as understood by researchers about the formation of the heart tube. In this paper, we summarize some of the key scientific methods behind heart tube formation.

Background Information

The heart is usually the first organ to form and become functional, and in the process reaffirming the role of transporting materials to and from the infant. It is produced within 18 days from the mesoderm, after which it starts beating and pumping blood. The heart forms from the cardiogenic region found close to the heard and are visible as a heart bulge found on the embryo's surface. Initially, it is made up of cardiogenic cords that create hollow lumen (Merks et al. 2018). Various scholars have tried to identify with the concept of heart tube formation, and in the process producing varied results. This paper is about a literature review of the causes of heart tube formation and some of the defining studies that have helped to explain these occurrences.

Analysis

Many scholars have researched and presented information about heart tube formation. For example, Hosseini and colleagues (2017) questioned the credibility of the conventional scientific principles and the biological processes and models. In this study, they explained the physical mechanisms behind heart tube (HT) and the foregut (FG) morphogenesis, during the human embryonic development stage, including mammals and chicken. They proposed a new hypothesis to explain the complex process in three different phases: splanchnic mesoderm/endoderm differential growth in FG formation and presumptive heart field (HF) reorientation, actomyosin contraction along anterior intestinal portal (AIP) pulling the splanchnopleure (SLP) downwards in AIP descension stage and possible migration of cells to HF from neighboring cells causing the expansion of HF to form HT due to the resulting cardiac jelly pressure and circumferential growth. They were inspired by different fate-mapping studies in the past two decades, which postulated that HT and FG morphogenesis was more complex than the lateral-to-medial SPL folding in the same cranial-caudal polarity.

Studies have confirmed that altered proliferation patterns with the addition of aphidicolin (mitotic inhibitor) concentration during Phase 1 of HT and FG morphogenesis [Fig. 2]. HH5 embryos cultured in the media containing the inhibitor demonstrated reduced folding or completely no folding [Fig. 1 S] when stained with 5-ethynyl-2′-deoxyuridine. Controlled setup showed standard folding and AIP descension, confirming the effectiveness of aphidicolin. The observation was further validated by removing aphidicolin from some of the positive test samples to observe any possible effect. The experiment demonstrated normal development a day after washing the inhibitor out, creating a partially looped HT. Part of the positive test samples where the inhibitors were not washed out did not show any folding or AIP descension. Besides, the same test was repeated on the Phases 2 proliferation; HH8 embryos were cultured in the same

concentration of aphidicolin. The experiment showed slow AIP descension (relative oSPL length, Fig. 2C), 8 hours after the administration of the treatment, and beating HT, 21 hours after the inhibitor was administered. Cell proliferation initiates and aids the initial development of inner SPL folding (Phase 1). Ramakrishnan et al. (2016) argue that cell proliferation inhibition hinders the second development stage (Phase 2) but does not stop the growth of the inner SPL and AIP descension. The result confirmed the hypothesis that FG morphogenesis is a function of cell proliferation, but this affects only the first two phases of development.

iSPL folding pattern

Another observation was the typical iSPL folding pattern under myosin inhibitor. Despite reduced AIP descension rates, the study concluded that actomyosin contraction does not play a role in iSPL in Phase 1 and HT formation in Phase 3 of the development. AIP resumed normal descension after washing the inhibitor out, proving that the complex is required for the descension and FG and HF elongation during the second phase of the development. this supports the hypothesis for Phases 1 and 2. However, the test failed to the third phase theory as blebbistatin inhibits cytokinesis that is required for HT formation. But a study on the growth strains indicated that blebbistatin concentration lowered the FG; this suggested that the differential growth and subsequent folding would not be affected if SPL layers are affected equally. The simulation results agreed with the experimental laboratory results. Internal SPL folded diagonally and produced morphology, as observed in the experiment. Negative growth was used to represent contraction and observed at tangential to AIP within the thin region of endoderm. The region represents the AIP border; the motion observed, therefore, represents the contraction near the arch-shaped AIP that generates tension responsible for pulling the AIP downward during the folding in the second Phase. The same simulation demonstrated positive axial growth in the iSPL, proving the initial

assumption that growth occurs on the floor of the foregut as the AIP descends. It also shown that HF mesoderm and endoderm separates as the differential growth simulation was ran.

From the laboratory experiments, the study concluded that proliferation is responsible for the initial development of inner SPL folding and the general FG morphogenesis during Phase 1 and Phase 2. This was confirmed by three more experimental runs proving the conclusion. However, the observations did not prove the hypothesis in a third of Phase of growth (blebbistatin-cytokinesis inhibition), but there was a potential indication that differential growth and subsequent folding would be possible if the inhibition affected both SPL layers. Despite the demonstrated concept by the model simulation, I would conclude this as an exclusive proof of the hypothesis. The model formation might have formed with a previous conception of the expected simulation outcome causing study bias. It would, however, be a perfect presentation demonstration of the principle. Other scholars have discussed about heart tube formation by looking at the cardiogenesis problem. Cardiogenesis is the postnatal heart development of an embryo through the formation of new heart tissues. During the initial stages, the heart primordia are transformed to an elongated midline tube from bilateral sheets of mesoderm. A study done by AbuIssa and Kirby, (2008), Kelly et al., (2001), shows that the long anteroposterior dimension of the elongated heart tube forms from the narrow mediolateral dimension of the oblong heart primordia, thus creating an hypothesis, as to what mechanism drives the dramatic morphological change resulting in rapid anteroposterior elongation of the heart tube in this analysis. The midline foregut (tubular), is formed by the folding of the endoderm ventral to the heart primordia when the heart tube forms. Through the analysis of previous differing research projects done explaining the elongation of heart tube and foregut, raised questions on what exactly drives the morphological changes thus leading to rapid anteroposterior elongation of the heart tube , the research explains while objectifies the hypothesis

how the anterior intestinal portal (AIP), which is the posterior opening of the foregut, descends posteriorly, the foregut and primitive heart tube elongates together posteriorly.

Although the extents of the movements of the heart tube and the foregut are unknown, the elongation of the heart tube and the foregut are assisted by the contraction of the endodermal myosin around the AIP (Hosseini et al., 2017). This paper analyses a hypothesis that the heart undergoes convergent extension (CE) to form the heart tube and how the test for the hypothesis was carried out. In order to achieve on the hypothesis the researcher used cell cluster labeling and imaging to demonstrate and show how Cardiogenesis, Cell rearrangement, Convergent extension, Heart fields, Heart tube formation, Morphogenesis occurs. Although there exist large gaps of knowledge on the mechanisms behind the shaping of the early heart tube, fate-mapping studies have by a large part advanced knowledge on heart tube formation. Fate-mapping is a technique used to comprehend embryo development, by labelling the embryo using a chemical dye to determine how structures form. Fate-mapping was used as an experiment to discover how the movements of the AIP and the heart primordial affect the development of the heart. In the figure below both the lateral and medial parts of the bilateral heart primordial (LHP and MHP) were labelled so as to visualize the tissue-scale dynamics during heart tube formation.

Summary of Observations

The summary of the observations taken from the experiments are summarized into three. The first step is through the ventral folding of paired heart primordia diagonally toward the medial-posterior direction in concert with AIP convergence and descent, realigning the initial medial-lateral polarity along the anterior-posterior axis of the embryo. Secondly, the anterior edges of the paired MHP seam up due to the progressive fusion of the paired primordia at the midline. Lastly, as the paired heart converge towards the midline, the flat heart primordia transform into a narrow

midline tube. This is due to the converging of the primordia along the original anterior-posterior axis during folding (1) and fusion (2).

The second experiment was done using the epifluorescence time-lapse microscopy. The medial parts of the bilateral heart primordia (MHP) were labeled with DiO (green) and DiL (magenta) for LHP and MHP respectively. Selected images from the movies' ventral view shows images of the upper left and bottom left taken during the recording during the different stage. According to figure 3, the red and blue dots in A'-F',1' show the original medial and lateral edges. Along with the convergence and descendent of the AIP, each heart extended medioposteriorly. The other observation from the images is that distance reduced between the labeled cell stripes. In the other image taken from the time-lapse recording the medial was labeled by DiL (magenta) while lateral was labeled using DiO (green). The distance between adjacent stripes in the same primordium reduced during heart tube formation. This was particularly evident with the newly formed heart tube. Comparing the distances indicated by the arrow and asterisk. The hypothesis that the heart mesoderm undergoes CE to form the heart tube. In order to describe complexity and achieve accuracy in the range of experiments done the research utilized primary qualitative research to analyze and formulate possible hypothesis in relation to the previous studies. To justify the result and finding a cooperation of case studies and experiments were used to prove the formulated hypothesis.

In order to ensure accuracy hypothetical question was developed; whether heart mesodermal cells undergo cell-cell intercalation so as to test our hypothesis. Using magenta and green the cells were labeled at cellular resolution with confocal microscopy. At the time of labeling the two stripes were separate from one another, but after 24 hours the two stripes abutted one another. This data was collected four times (n=4). The myocardium at this stage had two cell

layers. Cells in the both layers were preferentially elongated along the convergence axis. Thus the data provide strong evidence that the heart mesoderm undergoes CE driven by cell-cell intercalation. An examination of the distribution of active/phosphorylated myosin regulatory light chain (PMLC) and immune histochemically was performed so as to determine if actomyosin drives directional cell rearrangement in the heart mesoderm. Out of seven embryos treated with, a Rho-associated protein kinase (ROCK), five cell clusters failed to form the heart tube. The sample size was seven in this experiment. The observation in this experiment was that ROCK-dependent myosin is important for driving directional rearrangement of heart cells/CE to lengthen the heart tube.

The results in the experiments prove conclusively how the hypothesis was tackled and proven to be right. Another experiment that was done was the confocal imaging of labeled cells. Cell clusters in the heart mesoderm labeled with DiO and DiL using a Leica SP5 laser-scanning microscope with a 20 times objective. The paper answers the main hypothesis by the experiments indicated in figure 3. It also answers how the heart tube is formed and how the midline foregut is formed. Through compiling of data the research shows that convergence occurs on the anterior-posterior component of the bilateral heart primordial to establish a system of narrow tubules as the anteroposterior axis converts to mediolateral axis . Hence CE, Not only does it extend the heart tube but it also restructures the flat primordial Converging toward the midline in a midline tube. The analysis further shows that the dorsal heart mesoderm together with the other parts of the heart converges towards the midline hence developing into the anterior pole of the heart tube. In summary, this research shows apparatus that stir dynamic and quick lengthening of morphological changes in coordination with neighboring foregut, early core primordial morphogenesis ,Morphologies .Which is more practical and experimental and related to research based on

previous studies as they both show that both endodermal forces and autonomous heart mesodermal forces has an important roles in the extension of heart tube. However additional experimental studies are required to solve the exact function of this tissues in the process of tube formation and elongation. This report is vital in understanding the process of heart formation as it draws concern on the function and role of various tissues that play a major role in the process of heart formation.

Heart Tube Formation in Drosophila

Vanderploeg & Jacobs (2017) explore heart tube formation in *Drosophila*, in an attempt to understand the roles of the Src42A. While a few genes have been linked to CHDs in large and small animals, experimental studies have proven useful in mice. Information from genetic proof in human implies a connection between integrin-related factors and cardiovascular illnesses. At the same time, the authors acknowledge that vertebrate models have been used to understand CHDs, simpler genetic models and cell culture are important tools for mapping the sophisticated networks that surround the genes. In this paper, the authors focus on the fruit fly that is well-used for the development of cardiovascular diseases and for aging experiments. The development of the organs need heart cells' specifications, and the medial alignment of the two parallel cells rows. The authors addressed this question because of the existing gap in the study.

Despite increased understanding of the heritable factors, full comprehension of genetic etiology is affected by the multifactorial and complex nature of the illnesses. While the vertebrate models have linked specific elements that are connected to CHDs, simpler genetic models and cell culture are useful tools for mapping the sophisticated signaling networks that surround the genes. During cardiogenesis, the heart primordia changes from the bilateral flat sheets to the elongated midline tube. The authors seek to understand and identify the rapid changes, and the way they are

driven by the cell rearrangement activated by actomyosin that causes the reshaping of the dynamic tissues. By using the live imaging and cluster labelling approaches, the authors demonstrate that the originality that is shown by the heart primordia directionally and rapidly extends to produce the whole lengthy of the heart tube. Kidokoro et al. (2018) further show that convergent extension is driven by the oriented rearrangement of the cells that depends on the contraction of the actomyosin. These are some of the reasons that drive the question formulated by the authors. The main finding is that Src42A is necessary for *Drosophila* heart development. The requirement for particular factors for development can be experimentally tested through the removal of factors and observation of whether or not development can proceed normally.

In *Drosophila*, target protein is removed through the introduction of loss-of-function mutation in the gene responsible for protein encoding. The authors identified that extensive heart mesoderm labeling shows cell movements and tissue dynamics in the formation of heart tubes. To reveal this, the authors used tissue-scale dynamics in the heart tube formation, where they labeled the cell clusters in both medial and lateral parts in the bilateral heart primordia. Another finding was that the narrow mediolateral heart primordia dimension provides the whole length in the early heart tube. The authors labelled anteroposterior parts of cells in the heart primordium to understand the changes to the heart primordia.

The Roles of Src42A

Careful assessment of the mutant heart disease and genetic interaction information show that the role of Src42A functions independently of Integrin and the Integrin-linked Focal Adhesion Kinase. To test their hypothesis, the authors inhibited cell contraction and proliferation in cultured embryos. The authors also used computational modeling towards the determination of the physical viability of the hypothesis. Finally, the authors tested the model when they used extra experimental

information. Genetic analysis is useful in mapping protein networks that underlie the heart's development. This study shows that the *Drosophila* heart is an established and helpful model organism for testing the roles and requirements of particular genes. For most genes found in the fly genome, the multiple mutant alleles are available from the stock centers or related experimental research laboratories. This study shows that the loss or reduction of Src42A causes the inability of the heart to properly form because the cells fail to fully migrate to their required positions. The ability of testing independently produced mutations provides hop that the defect in the heart is as a direct consequence of the Src42A loss and no other unknown genetic issues. Because a defective gene affects the entire organism, Src42A mutant assessment fails to show whether or not the defect in the heart is because of the need for Src42A in the heart or whether it is a secondary defect. Both the rodent and *Drosophila* models have shown that the integrin adhesion receptor is necessary in the heart formation.

The next step in the study is to understand the roles of the Src42A and whether or not it applies for human beings, like it does for animals. The authors identified the mechanisms that drive the rapid lengthening of early heart primordia and the dynamic morphological transformations in coordination with the foregut morphogenesis. Relying on the past published information and the present results, the authors have not solved the autonomous heart mesodermal forces and the endodermal forces that are critical in the extension of heart tube. The next step of study is to carry out more experiments to resolve the key roles played by the tissues in the formation and extension of hear tube. A properly functioning heart is connected to the structures. It shows that possible defects in the structure triggers later or immediate onset of heart disease. The congenital heart defects (CHDs) including the atrial or ventricular septal defect and patent ductus arteriosus are found in both animals and humans.

Conclusion

These studies contribute towards better understanding of the topic because they show the way the heart's development can be researched on to show the genes that determine heart cells identity and dictate the response to the signals. Besides, the main information covered in this literature review explains the concept of heart tube formation. A newly formed heart tube is linked to the dorsal wall by a tissue fold called the dorsal mesoderm. This article contributes to the topic of study as it shows the way convergence leads to the formation of the midline heart tube. It helps readers to understand that CE does not only extend to the heart tube but remodels the flat primordia through converging them at the midline.

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