

Septation of the Intrapericardial Arterial Trunks in the Early Human Embryonic Heart

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Abstract

Background: Outflow tract (OFT) septation defects are a common cause of congenital heart disease. Numerous studies have focused on the septation mechanism of the OFT, but have reported inconsistent conclusions. This study, therefore, aimed to investigate the septation of the aortic sac and the OFT in the early embryonic human heart.

Methods: Serial sections of 27 human embryonic hearts from Carnegie stage (CS) 10 to CS19 were immunohistochemically stained with antibodies against α -smooth muscle actin (α -SMA) and myosin heavy chain.

Results: At CS10–CS11, the OFT wall was an exclusively myocardial structure that was continuous with the aortic sac at the margin of the pericardial cavity. From CS13 onward, the OFT was divided into nonmyocardial and myocardial portions. The cushion formed gradually, and its distal border with the OFT myocardium was consistently maintained. The aortic sac between the fourth and sixth aortic arch arteries was degenerated. At CS16, the α -SMA-positive aortopulmonary septum formed and fused with the two OFT cushions, thus septating the nonmyocardial portion of the OFT into two arteries. At this stage, the cushions were not fused. At CS19, the bilateral cushions were fused to septate the myocardial portion of the OFT.

Conclusions: Data suggest that the OFT cushion is formed before the aortopulmonary septum is formed. Thus, the OFT cushion is not derived from the aortopulmonary septum. In addition, the nonmyocardial part of the OFT is septated into the aorta and pulmonary trunk by the aortopulmonary septum, while the main part of the cushion fuses and septates the myocardial portion of the OFT.

Key words: Aortopulmonary Septum; Human Embryonic Heart; Immunohistochemistry; Outflow Tract; Outflow Tract Cushion

INTRODUCTION

Abnormal development of the cardiac outflow tract (OFT) constitutes approximately 30% of congenital heart defects.^[1] Therefore, investigating the mechanism of the OFT septation and remodeling is important. At present, many reports regarding this process have been published, revealing that, during the early developmental stage of the embryo, the OFT is a single tube positioned between the aortic sac and the right ventricle.^[1-4] According to Anderson's report, the OFT contains nonmyocardial and myocardial portions, and the nonmyocardial portion of the OFT is septated into the aorta and pulmonary trunk.^[5,6] However, a different report has shown that the aorta and pulmonary trunk are septated from the aortic sac.^[2] Therefore, aortopulmonary septation should be further investigated in the human embryonic heart.

It is well known that the aortopulmonary septum and the OFT cushion contribute to aortopulmonary septation.^[3,4] According to Anderson, given that the aortopulmonary septum

is a transient structure and does not persist in the postnatal heart, the term "protrusion" is more accurate than "septum."^[7] Henceforth, we will use the term "protrusion" in this study. The protrusion forms in the dorsal wall of the aortic sac, which has a core derived from the second heart field, with only its outer layer derived from the cardiac neural crest,^[7] which is defined as the neural crest residing between the otic placode and the third somite. Cardiac neural crest cells migrate not only to the protrusion, but also to the cardiac jelly of the OFT to participate in the formation of the OFT cushion.^[3,7-10] In the cushion, cardiac neural crest cells accumulate and form

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a condensed column. In a study, the cushion containing the condensed column has been named the aortopulmonary septal complex.^[11] Recently, investigators have argued whether the protrusion in the dorsal wall of the aortic sac divides the distal OFT.^[5,6] However, the term aortopulmonary septal complex is still used in some studies regarding OFT septation.^[12] The inconsistent opinions regarding OFT septation have partly resulted from historical inconsistencies in terminology. In this study, the remodeling and septation of the aortic sac and OFT were observed in human embryos from Carnegie stage (CS) 10 to CS19 to explore the relationship between the OFT cushion and the protrusion and the contribution of these structures to the septation of the aortic sac and OFT.

METHODS

Ethical approval

The human embryos were collected from patients who provided informed consent using procedures approved by the Medical Ethics Committee of Shanxi Medical University.

Human embryos

Twenty-seven human embryos were obtained from Shanxi Children's Hospital. The collected embryos were graded according to the Carnegie developmental stages.^[13] Three embryos at each stage from CS10 through CS13 and CS15 were collected. In addition, five CS14 embryos, three CS16 embryos, two CS17 embryos, and two CS19 embryos were obtained. Embryos were fixed overnight in an ice-cold mixture of methanol: acetone: water (2:2:1 v/v), dehydrated through a graded ethanol series, and embedded in paraffin. Front and transverse sections were cut at 7 μ m and used for hematoxylin and eosin (H and E) staining or immunostaining.

Immunohistochemical staining

Peroxidase anti-peroxidase immunohistochemical staining

Sections were pretreated sequentially with 3% (v/v) H₂O₂ for 30 min and TENG-T (10 mmol/L Tris, 5 mmol/L ethylenediaminetetraacetic acid, 150 mmol/L NaCl, 0.25% gelatin, and 0.05% Tween-20, pH 8.0) for 30 min and then incubated overnight with primary antibodies against α -smooth muscle actin (α -SMA; 1:1000, IMM-2, α -SMA, Sigma, USA) or myosin heavy chain (MHC; 1:1000, A4.1025, MHC, Upstate, USA). Then, the sections were incubated sequentially with rabbit anti-mouse IgG (1:7500, noncommercial, a gift from Professor Lamers WH, Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, the Netherlands), goat anti-rabbit IgG (1:250, noncommercial, a gift from Professor Lamers WH), and rabbit peroxidase-antiperoxidase complex (1:750, Nordic, the Netherlands). Then, 3,3'-diaminobenzidine tetrahydrochloride supplemented with 3 mmol/L H₂O₂ was added to the sections to develop the color at the binding site of the primary antibody. Stained sections were rapidly passed through graded ethanol solutions, cleared in xylene, and mounted in resin.

Alkaline phosphatase immunohistochemical staining

Sections were pretreated with TENG-T for 30 min and

then incubated overnight with primary antibodies against α -SMA (1:1000, IMM-2, α -SMA, Sigma, USA) or MHC (1:1000, A4.1025, MHC, Upstate, USA). Then, the sections were incubated with goat anti-mouse IgG-alkaline phosphatase (1:100, A3562, Sigma, USA). Antibody binding was demonstrated by staining with the NBT/BCIP complex (1:50, 1681451, Roche, Swiss). All antibodies were diluted with TENG-T. Stained sections were rapidly dehydrated through graded ethanol solutions, cleared in xylene, and mounted in resin.

Three-dimensional reconstruction

Three-dimensional (3D) reconstructions of serial sections of three embryos from CS14 to CS16 were performed using AMIRA software version 5.2 (AMIRA International Limited, Australia) to display the morphological differences in the embryonic hearts. One embryo was chosen at each stage. Serial sections of every embryonic heart were stained and photographed. All the images of one embryonic heart constituted one group. Thus, we obtained three groups of images. The images from each group were loaded onto the AMIRA software to perform 3D reconstructions.

RESULTS

Outflow tract and aortic sac before septation

At CS10–CS11, the aortic sac was located at the ventral pharyngeal mesenchyme. The aortic sac wall was composed of endothelium and mesenchyme and was α -SMA and MHC negative [Figure 1a and 1b]. In the OFT wall, α -SMA and MHC were expressed from the proximal pole to the distal pole, and the myocardial OFT was continuous with the aortic sac at the margin of the pericardial cavity [Figure 1a and 1b, arrows]. The cardiac jelly of the OFT consisted of acellular matrix between the myocardium and endothelium [Figure 1a and 1b].

At CS12, the aortic sac was still situated in the pharyngeal mesenchyme [Figure 2a]. From CS13 onward, the distal pole of the OFT wall was α -SMA negative [Figure 2b, arrowheads] and composed the nonmyocardial portion of OFT wall. From CS12 to CS13, in the distal portion of the OFT, mesenchymal cells were initially detected in the cardiac jelly and partly were α -SMA positive to contribute to OFT cushion formation [Figure 2a–2c, arrows]. In the proximal portion of the OFT, α -SMA-positive mesenchymal cells were rarely observed [Figure 2d].

At CS14, the distal pole of the OFT wall still was α -SMA and MHC negative [Figure 3a and 3b, arrows]. Meanwhile, extending to the proximal pole, α -SMA and MHC expression was initiated [Figure 3c and 3d, arrows]. Thus, the OFT could be divided into nonmyocardial and myocardial portions [Figure 3e]. Serial sections and 3D reconstruction revealed that the fourth and sixth aortic arch arteries were connected to the aortic sac [Figure 3a, 3c and 3e]. By CS14, the number of mesenchymal cells has increased in the septal and parietal OFT cushion, and α -SMA-positive cells have accumulated and formed columns of condensed mesenchyme

throughout the cushion [Figure 3f–3h]. Front sections revealed that two α -SMA-negative mesenchymal cell masses were located between the OFT cushions [Figure 3h, asterisks].

From CS15, the aortic sac between the fourth and sixth aortic arch arteries was degenerated [Figure 4a]. In the ventral wall of the distal OFT, MHC expression was first observed in the left portion [Figure 4b]. The cushion formation was accompanied by the myocardialization of the left wall of the OFT distal pole. The cushion contained abundant α -SMA-positive cells [Figure 4c, arrow]. However, at the same level in the right wall, the myocardium did not form [Figure 4b, arrow] and only a few α -SMA-positive cells were observed in the neighboring cardiac jelly [Figure 4c, asterisk]. When extending caudally, the right wall gradually became MHC positive, but the myocardium was thin [Figure 4d, arrow], and the neighboring cardiac jelly contained a few α -SMA-positive cells [Figure 4e, asterisk]. Serial sections showed that by CS15, in bilateral cushions, the number of α -SMA-positive cells had increased [Figure 4f]. In the dorsal wall, the MHC-negative

area was again observed in the left portion [Figure 4g, asterisk]. 3D reconstruction further confirmed that parts of the ventral and dorsal walls were composed of the nonmyocardial component of the OFT at this stage [Figure 4a and 4h]. Compared with CS14, the nonmyocardial portion was markedly extended at CS15 [Figures 3e, 4a and 4h].

Septation of the outflow tract

At CS16, septation of the OFT was initiated [Figure 5a–5e]. An α -SMA-positive protrusion formed in the dorsal wall of the aortic sac between the fourth and sixth aortic arch arteries and extended into the OFT. After its formation, the protrusion fused incompletely with the distal pole of the septal and parietal cushions [Figure 5a–5d]. Due to the fusion of the protrusion and the bilateral OFT cushions, the nonmyocardial part of the OFT was septated into the aorta and pulmonary trunk at CS16 [Figure 5a–5d]. The arterial walls were MHC negative [Figure 5b, arrows]. Hence, the nonmyocardial portion of the OFT formed the free wall of the aorta and pulmonary trunk at this stage [Figure 5b], containing several α -SMA-positive cells [Figure 5a, arrowhead]. In several sections, a narrow space was observed between the protrusion and the cushion, which was referred to as the aortopulmonary foramen [Figure 5c, arrow]. At the root of the artery, the valvar primordium was initially observed and was contiguous with the OFT cushion [Figure 5c]. Intercalated cushions were observed in the distal part of the OFT wall [Figure 5d]. At this stage, the myocardial OFT and its cushions became shorter than those at CS15 [Figures 4f and 5b–5e], and their distal margin was positioned at the valvar primordium level [Figure 5b and 5e]. Below the valve level, the two cushions were not fused [Figure 5c–5e]. At CS17, the protrusion fused completely with the cushions and the aortopulmonary foramen was not observed [Figure 5f and 5g, asterisks].

At CS19, in the aorta and the pulmonary trunk wall, more α -SMA-positive cells were observed than at

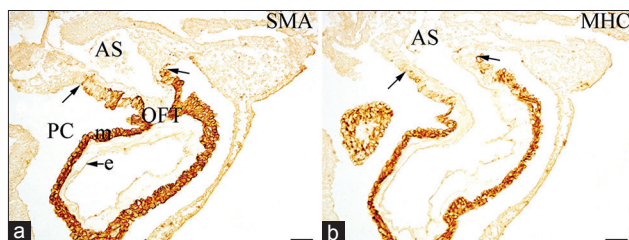


Figure 1: The aortic sac and OFT of the human embryonic heart at CS11. The aortic sac was located at the ventral pharyngeal mesenchyme, the α -SMA-positive (a) and MHC-positive (b) OFT myocardium extended to the reflection of the OFT and the pericardial dorsal wall (arrows). No mesenchymal cells were observed in the cardiac jelly of the OFT. Immunohistochemical staining. Scale bar: 50 μ m. e: Endothelium; m: Myocardium; SMA: Smooth muscle actin; MHC: Myosin heavy chain; AS: Aortic sac; OFT: Outflow tract; PC: Pericardial cavity; CS: Carnegie stage.

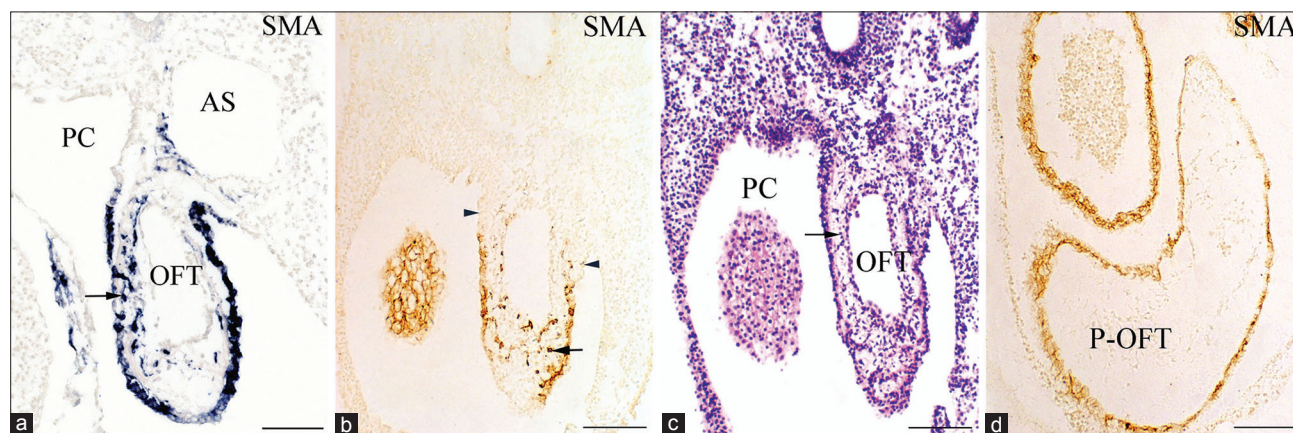


Figure 2: The aortic sac and OFT of the human embryonic heart at CS12 (a) and CS13 (b–d). The aortic sac was situated in the pharyngeal mesenchyme (a). An α -SMA-negative portion was observed at the distal pole of the OFT wall (b, arrowheads). The cardiac jelly of the OFT was filled with mesenchymal cells (c). In the distal portion of the OFT, some of the mesenchymal cells were α -SMA positive (a and b, arrows). In the proximal portion of the OFT, the mesenchymal cells were α -SMA negative (d). (a, b and d) immunohistochemical staining with α -SMA; and (c) H and E staining. (a) Scale bar: 100 μ m; and (b–d) scale bar: 200 μ m. SMA: Smooth muscle actin; PC: Pericardial cavity; AS: Aortic sac; OFT: Outflow tract; P-OFT: The proximal part of the outflow tract; CS: Carnegie stage.

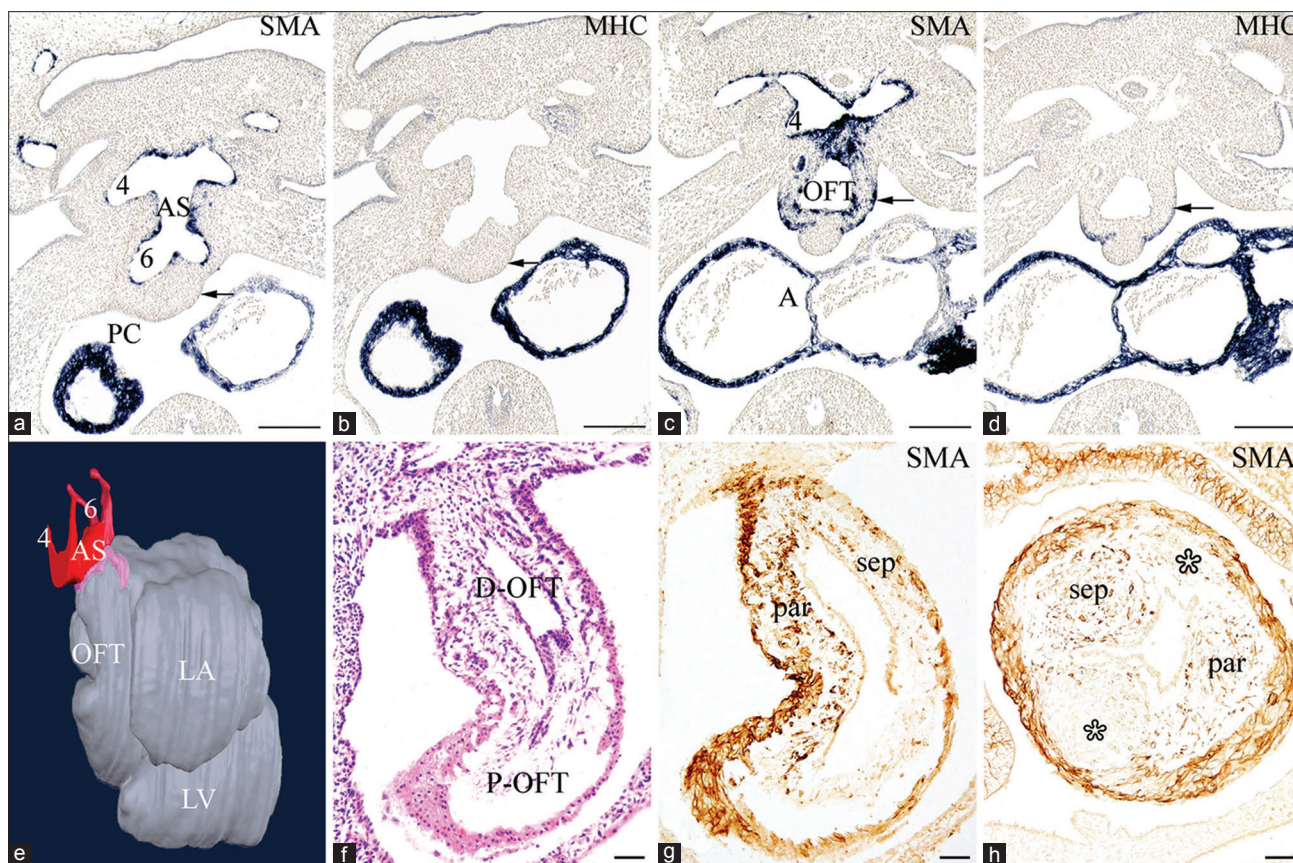


Figure 3: The aortic sac and OFT of the human embryonic heart at CS14. (a–e) The OFT contained α -SMA- and MHC-negative portion and α -SMA- and MHC-positive portion. (f–h) The number of mesenchymal cells increased in the OFT cushion and α -SMA-positive cells were observed throughout the cushion. (h) Two α -SMA-negative cell masses were observed between the OFT cushions (asterisks). In left view of the heart (e), the myocardium is labeled in gray. The nonmyocardial OFT is labeled in pink. The fourth aortic arch arteries and the aortic sac are noted in red. The sixth aortic arch arteries are noted in brown. (a, c, g, and h) IHC staining with α -SMA; (b and d) IHC staining with MHC; and (f) H and E staining. (a–d) Scale bar: 200 μ m; and (f–h) scale bar: 50 μ m. The number 4 and 6 indicate the fourth and sixth aortic arch arteries, respectively. sep: Septal cushion; par: Parietal cushion; SMA: Smooth muscle actin; MHC: Myosin heavy chain; IHC: Immunohistochemical; H and E: Hematoxylin and eosin; PC: Pericardial cavity; AS: Aortic sac; A: Atrium; OFT: Outflow tract; LA: Left atrium; LV: Left ventricle; D-OFT: The distal part of the outflow tract; P-OFT: The proximal part of the outflow tract; CS: Carnegie stage.

CS16 [Figures 5b, 5d and 6a–6c]. The distal margin of MHC expression in the OFT wall was used to detect the border of the OFT and the two arteries [Figure 6d–6f], where the arterial valves were clearly observed [Figure 6a–6f]. Below the valve level, fusion of the two cushions was initiated, thus forming the mesenchymal septum that divided the myocardial OFT. The α -SMA-positive cells in the two cushions mixed and formed the α -SMA-positive whorl [Figure 6b, arrow]. Even after extending to the right ventricle, the proximal portions of the OFT cushions were not yet fused [Figure 6c and 6f, arrows].

DISCUSSION

It has been confirmed that the protrusion in the dorsal wall of the aortic sac and the OFT cushion play crucial roles in the aortopulmonary septation.^[3,4] Therefore, in this study, we observed the formation of the protrusion and the OFT cushion in human embryonic hearts. Our previous work and that of other studies have indicated that α -SMA is expressed in migrating mesenchymal cells, which are derived from the

cardiac neural crest or endocardium.^[14,15] Hence, in this study, the α -SMA expression pattern in the OFT cushion and the protrusion revealed the distribution of mesenchymal cells, which contributed to the formation of the cushion and the protrusion. Our data revealed that at CS12–CS14, the number of α -SMA-positive and α -SMA-negative mesenchymal cells gradually increased in the cardiac jelly of the OFT and contributed to the formation of the bilateral OFT cushions. In addition, from CS14 to CS15, in human embryos, the swelling of the α -SMA-positive cushions was observed concurrently with the α -SMA-negative column-like structures, which were adjacent to the cushions. The latter structures were positive for *isl1*, which may be involved in forming precursors of the intercalated cushions.^[6] The findings in our previous work and that in other studies also prove that the two cushion structures play different roles in OFT septation.^[5,16] Moreover, the OFT cushions were lengthened from CS14 to CS15. It has been shown that the formation of the cushions depends on the contributions from the OFT endocardium and myocardium.^[17–19] Our data showed that at the distal pole of the myocardial OFT, the myocardium in the left wall is thicker than that in

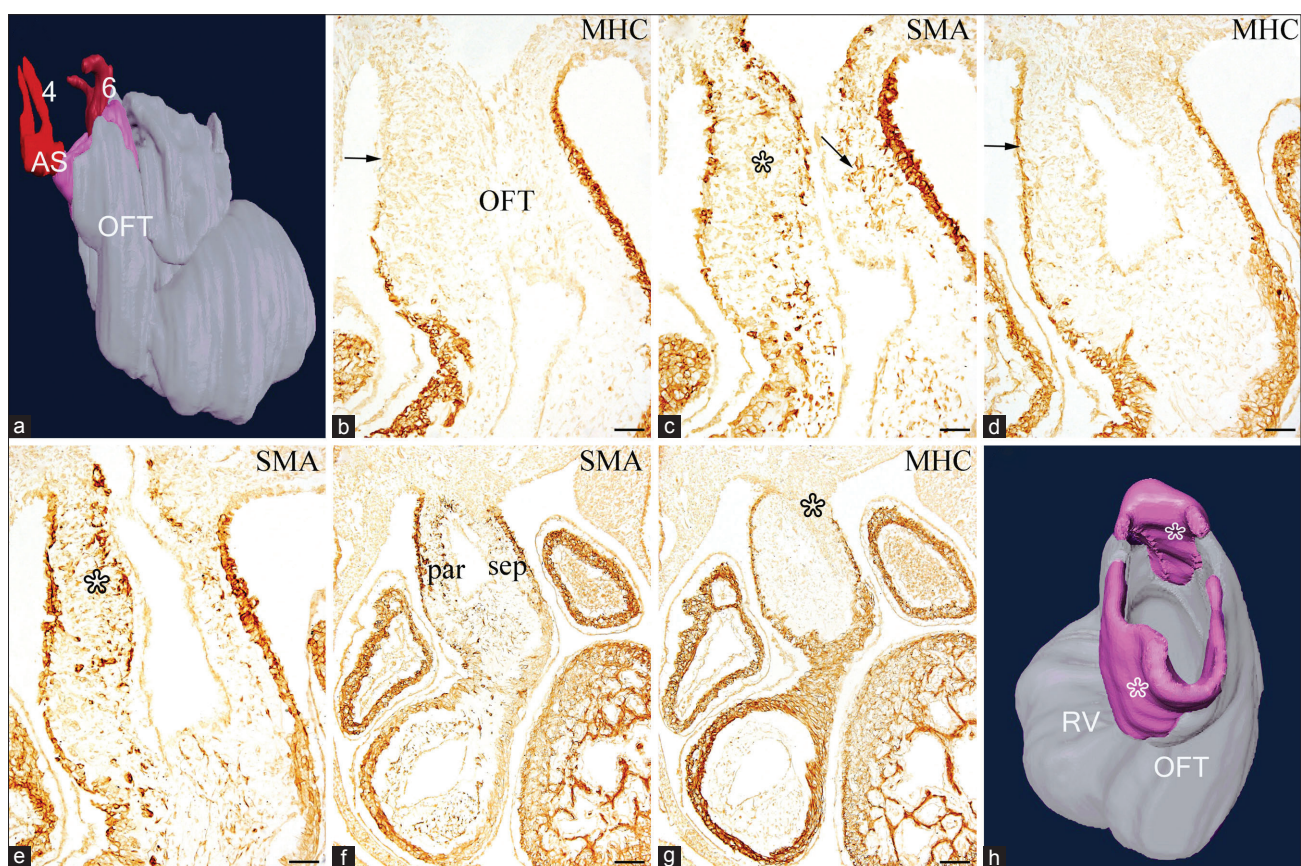


Figure 4: The aortic sac and OFT of the human embryonic heart at CS15. The aortic sac between the fourth and sixth aortic arch arteries degenerated (a). The MHC-negative area was located in the ventral and dorsal walls of the distal portion of the OFT (a, b, g and h). In the ventral wall, MHC expression was negative in the right wall (b, arrow). When the OFT extended dorsally and caudally, the right wall became positive for MHC (d, arrow). α -SMA-positive cells increased in the cardiac jelly of the right wall (c and e, asterisks). The cushions contained numerous α -SMA-positive cells (f). In a and h, the myocardium is labeled in gray; the nonmyocardial OFT is in pink; the fourth aortic arch arteries and the aortic sac are in red; and the sixth aortic arch arteries are in brown. A shows the left view of the heart. H shows the ventral view. (b, d, and g) IHC staining with MHC; (c, e, and f) IHC staining with α -SMA. (b–e) Scale bar: 50 μ m; and (f and g) scale bar: 100 μ m. The number 4 and 6 indicate the fourth and sixth aortic arch arteries, respectively. sep: Septal cushion; par: Parietal cushion; SMA: Smooth muscle actin; MHC: Myosin heavy chain; IHC: Immunohistochemical; AS: Aortic sac; OFT: Outflow tract; RV: Right ventricle; CS: Carnegie stage.

the right wall. In addition, more α -SMA-positive cells were observed in the left portion of the cardiac jelly than those in the right portion, thereby contributing to cushion formation. Our data suggest that cushion formation is accompanied by myocardial cell differentiation of the OFT. Bartman *et al.*^[17] confirmed that the myocardium secretes various factors, such as BMP, that are involved in cushion formation. Thus, at the distal pole of the myocardial OFT wall, the thicker myocardium may induce neighboring cardiac jelly to form the cushion by releasing some factors, thus resulting in the lengthening of the OFT cushion. From CS16, the cushion is shortened as the distal margin of the OFT myocardium regresses compared to that at CS15. Therefore, the distal margin of the cushion is always positioned at the same level as that of the OFT myocardium. After cushion formation, the protrusion in the dorsal wall of the aortic sac can be detected at CS16. Hence, protrusion formation occurs after cushion formation, indicating that the mesenchymal cells of the cushion are not derived from the protrusion in human embryos. This finding is consistent with the conclusions of a previous study.^[7]

The OFT septation defects are a common cause of congenital heart disease. Hence, numerous studies have focused on the septation mechanism of the OFT; however, these studies have reported inconsistent conclusions. First, studies have reported that the aorta and pulmonary trunk are derived from the aortic sac or the distal portion of the OFT. Although some researchers have argued that the distal portion of the OFT is septated into the aorta and pulmonary trunk,^[4-6,20] others have revealed that the aorta and pulmonary trunk are derived from the septated aortic sac.^[2] Second, studies have reported that the aorta and pulmonary trunk are septated by the protrusion or the distal portion of the fused OFT cushion. Some investigators have argued that the protrusion divides the distal OFT.^[5,6] However, other studies have suggested that the aortopulmonary septal complex is the structure that partitions the OFT.^[3,12] To investigate the septation mechanism of the aortic sac and OFT in human embryos, we observed the development of the aortic sac and OFT from CS10 to CS19.

It is crucial to clarify the definition of the term OFT before investigating its septation. In Anderson's study, the distal

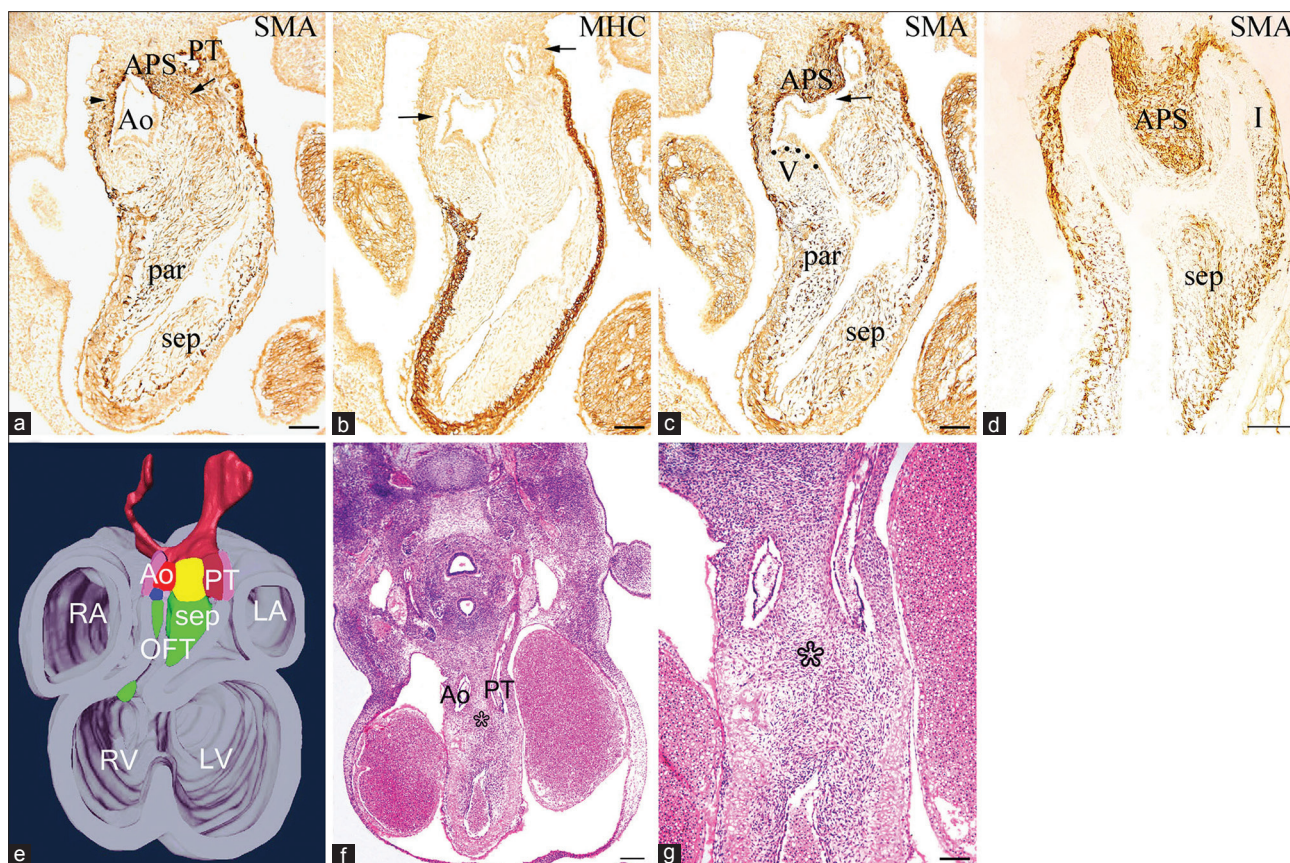


Figure 5: The OFT of the human embryonic heart at CS16 (a–e) and CS17 (f and g). The α -SMA-positive aortopulmonary septum formed and fused incompletely with the two cushions, separately (a–e). As a result, the nonmyocardial portion of the OFT was divided into the aorta and pulmonary trunk (a–e). The aortopulmonary foramen was observed at CS16 (c, arrow). At the root of the artery, the valve anlage began to emerge and was contiguous with the OFT cushion (c and e). The myocardial OFT and its cushion became shortened (b–e). The two cushions were not fused (c–e). At CS17, the aortopulmonary foramen was not observed (f and g, asterisks). In ventral view of the heart (e), the myocardium is labeled in gray; the nonmyocardial OFT is in pink; the aorta is in red; the sixth aortic arch arteries are in brown; the aortopulmonary septum is in yellow; the valve anlage is in blue; and the cushion is in green. (a, c, and d) IHC staining with α -SMA; (b) IHC staining with MHC; (f and g) H and E staining. (a–c and g) Scale bar: 100 μ m; and (d and f) Scale bar: 200 μ m. Ao: Ascending aorta; sep: Septal cushion; par: Parietal cushion; SMA: Smooth muscle actin; MHC: Myosin heavy chain; IHC: Immunohistochemical; H and E: Hematoxylin and eosin; PT: Pulmonary trunk; APS: Aortopulmonary septum; V: Valvar primordium; I: Intercalated cushion; RV: Right ventricle; LV: Left ventricle; RA: Right atrium; LA: Left atrium; OFT: Outflow tract; CS: Carnegie stage.

margin of the OFT is located at the border of the pericardial cavity.^[5] We adopted this definition in this study. In addition, the aortic sac is located in the pharyngeal mesenchyme. Our data indicated that from CS10 to CS12, the OFT is a completely myocardial structure, and its distal margin is located at the pericardial border. From CS13 onward, the distal portion of the OFT becomes nonmyocardial. Hence, at these stages, the OFT consists of nonmyocardial and myocardial portions. At CS16, the OFT is shortened and is still composed of nonmyocardial and myocardial portions. Therefore, in this study, we divided the OFT into these two parts.

Anderson demonstrated that in human embryos, the protrusion forms in the dorsal wall of the aortic sac between aortic arch arteries 4 and 6 and partitions the distal portion of the OFT.^[5] In this study, at CS16, the protrusion extended into the nonmyocardial portion of the OFT and septated it into the aorta and pulmonary trunk. At the border of the nonmyocardial and myocardial portions of the OFT, the cushions were continuous with the protrusion and were not

fused at this stage. Hence, our data further confirmed that the protrusion divides the two arteries. During septation of the aorta and pulmonary trunk, the aortopulmonary foramen, which is the space between the protrusion and the OFT cushion, transiently forms between the developing intrapericardial aorta and pulmonary trunk; the aortopulmonary foramen closes as the protrusion fuses with the distal pole of the cushion. In this study, the foramen was observed at CS16 and was closed at CS17. At the time of its closure, the two arteries are completely septated. However, the bilateral cushions are not fused. Failure of foramen closure leads to an aortopulmonary window, which may coincide with the normal fusion of the outflow cushions.^[7,21] According to our data, this malformation may result from failure of the abnormal protrusion and the cushion to fuse. Therefore, our data provide an explanation for the formation of aortopulmonary window.

After septation, the wall of the aorta and pulmonary trunk was MHC negative and contained several α -SMA-positive cells

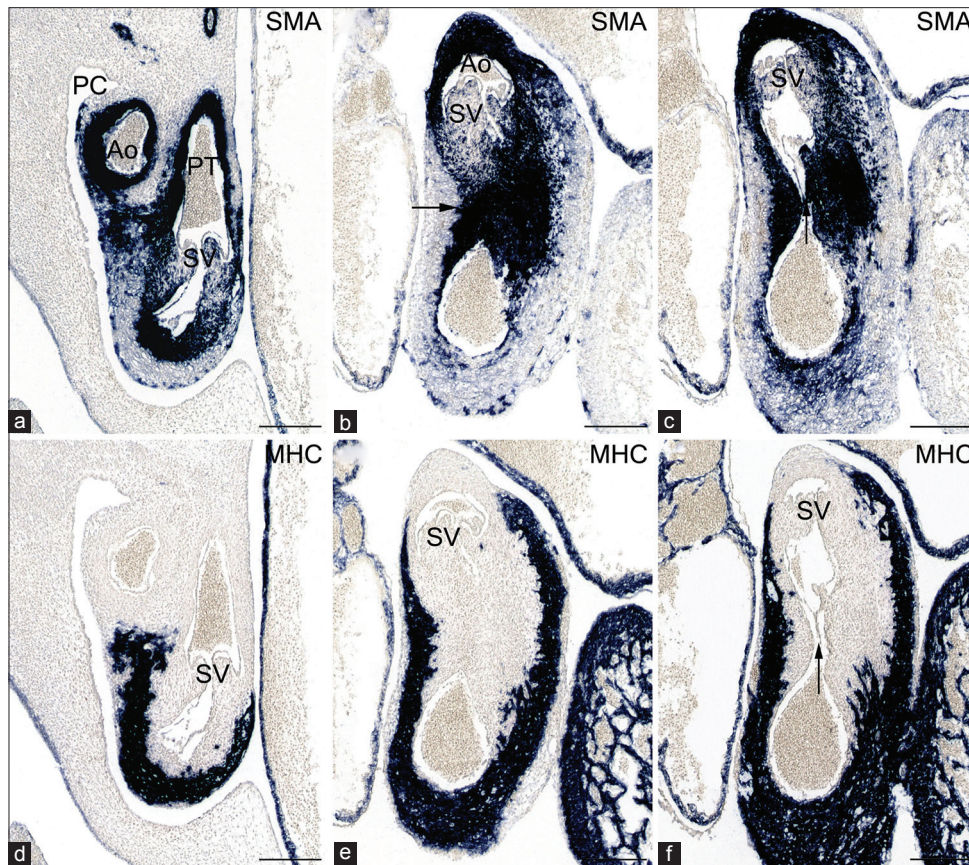


Figure 6: The OFT of the human embryonic heart at CS19. In the aorta and the pulmonary trunk wall, α -SMA-positive cells surrounded the endothelium (a–c). MHC expression was positive in the OFT wall (d–f). The arterial valves were clearly observed at the roots of the aorta and pulmonary trunk (a–e). Below the valve level, the two cushions began to undergo fusion (b, arrow). Even after the OFT extended to the right ventricle, the cushions were not fused yet (c and f, arrows). (a–c) IHC staining with α -SMA; (d–f) IHC staining with MHC. (a–f) Scale bar: 200 μ m. Ao: Aorta; SMA: Smooth muscle actin; MHC: Myosin heavy chain; IHC: Immunohistochemical; H and E: Hematoxylin and eosin; PC: Pericardial cavity; PT: Pulmonary trunk; SV: Semilunar valve; OFT: Outflow tract; CS: Carnegie stage.

surrounding the endothelium. This means that the arterial wall consists of endothelium and mesenchyme at CS16. Waldo confirmed that the second heart field progenitor cells migrate and differentiate into the α -SMA-positive smooth muscle cells of the two arteries.^[22] Our data indicate that after aortopulmonary septation, the number of α -SMA-positive cells surrounding the endothelium increases gradually in the arterial wall, thus supporting smooth muscle formation in the arterial tunica media.

Concurrent with the nonmyocardial OFT septation, the valvar primordia form at the distal pole of the cushion. Thus, the distal pole of the cushion may contribute only to valve formation at the aorta and pulmonary trunk. Intercalated cushions, which are derived from α -SMA-negative column-like structures and are two smaller elevations between the OFT cushions,^[6,23] were also observed in the septated OFT wall. According to Kramer, fused OFT cushions participate in the formation of the dextral and sinistral cusps of the arterial valves. The dorsal cusp of the aortic valve and ventral cusp of the pulmonary valve are derived from the intercalated cushions.^[23]

It is confirmed that below the arterial valve level, the proximal part of the OFT is septated into the aortic vestibule

and pulmonary cone due to the fusion of the proximal part of the OFT cushion.^[5] In this study, at CS16, the OFT cushion was clearly shortened, and it was hard to divide this structure into the distal and proximal portions. Thus, after the distal pole of the cushion contributes to the formation of the arterial valve, the main part of the bilateral cushions begins to fuse from the valve level to the proximal pole, thereby forming the mesenchymal OFT septum at CS19, which partitions the myocardial OFT into the aortic vestibule and pulmonary cone.

There were some limitations in this study. First, it is hard to collect the human embryo after CS19; thus, the complete septation of the myocardial OFT could not be shown. Second, some methods such as conditional gene knockout were unable to be performed in human embryo, which limited the molecular mechanism investigation of the OFT septation.

In conclusion, our findings suggest that the formation of the OFT cushion occurs before that of the aortopulmonary septum. Thus, the OFT cushion is not derived from the aortopulmonary septum. In addition, the nonmyocardial part of the OFT is septated into the aorta and pulmonary trunk by the aortopulmonary septum, while the main part

of the cushion fuses and septates the myocardial portion of the OFT.

Acknowledgments

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Conflicts of interest

There are no conflicts of interest.

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早期人胚心脏心包内动脉干的分隔

摘要

背景: 流出道分隔缺陷是先天性心脏病的一个常见原因。因此,大量研究聚焦于探讨流出道分隔机制,但结论尚不一致。所以本研究旨在探讨早期人胚心脏动脉囊与流出道的分隔过程。

方法: 选用抗 α -平滑肌肌动蛋白(α -smooth muscle actin, α -SMA)、抗肌球蛋白重链(myosin heavy chain, MHC)抗体对27例Carnegie stage (CS) 10–CS19人胚心脏连续切片进行免疫组织化学染色。

结果: CS10–CS11, 流出道壁为心肌性结构,与动脉囊在心包腔边缘相连。CS13后,流出道分为非心肌部和心肌部。流出道心内膜垫逐渐形成,其远端界限与流出道壁心肌界限保持一致。第4、6弓动脉之间的动脉囊退化消失。在CS16, α -SMA阳性主肺动脉隔形成且与流出道心内膜垫融合,将流出道非心肌部分隔为主、肺动脉。此期,流出道心内膜垫未融合。至CS19,两侧流出道心内膜垫融合分隔流出道心肌部。

结论: 流出道心内膜垫的形成早于主肺动脉隔。因此,前者并非来源于主肺动脉隔。流出道非心肌部由主肺动脉隔分隔为主动脉与肺动脉干;而流出道心内膜垫的大部融合将流出道心肌部进行分隔。