Endocardial-mesenchymal transition underlies fusion of the conotruncal ridges during embryonic cardiac outflow tract septation

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The embryonic cardiac outflow tract (conotruncus) is a single tubular chamber that connects the right ventricle with the aortic arch arteries. It contains two opposite, long and helical mesenchymal cushions covered by endocardial cells (conotruncal ridges). Conotruncal division (septation) gives rise to the adult right and left outflows together with the aortic and pulmonary valves. It takes place by fusion of the two opposite ridges and formation of the conotruncal septum. Although the participation of neural crest cells in septation is well established, the mechanism of fusion of the conotruncal ridges remains unknown. Defects in fusion have been shown to produce bicuspid aortic valve, the most prevalent human congenital cardiac malformation, in a hamster model.

Three fusion mechanisms have been proposed to operate during embryonic development: epithelial adhesion, epithelial apoptosis and epithelial-mesenchymal transition (EMT). The first mechanism entails the expression of adhesion molecules and the maintenance of the identity of cells in contact, whereas in the other two, epithelial cells covering the fusing structures disappear by apoptosis or by transforming into mesenchymal cells.

The objective of this study is to elucidate the mechanism involved in the fusion of the conotruncal ridges. With this aim, ED 11-12 hamster embryos were used. Endocardial cell adhesion was assessed by immunofluorecence with VE-CAD and CD34 antibodies. Apoptosis was detected by inmmunofluorescence for active caspase 3 and the TUNEL method. For EMT detection two methods were used: inmmunofluorescence colocalization of the above mentioned endocardial markers with the migration marker α-actin, and mesenchymal localization of fluorescence after 12 hours *ex vivo* incubation of embryos with the membrane impermeable fluorescent molecule carboxifluorescein (CFSE).

During septation, endocardial cells covering the surface of the conotruncal ridges were VE-CAD^{+}, CD34^{+}, and α-actin^{-}, whereas those in the area of fusion, at the point of contact between opposite ridges were positive for the three markers. Distal to the area of fusion, only VE-CAD^{-}/CD34^{-} mesenchymal cells were found, many of them positive for α-actin. Apoptosis was detected in mesenchymal cells of the ridges, but not in endocardial cells. It was abundant during cushion remodeling at late stages, scarce during septation, and absent in the area of fusion. Embryos incubated with CFSE showed fluorescent endocardial cells as well as scattered fluorescent mesenchymal cells, usually positive for α-actin, in the area of fusion closed to the endocardium.

The results indicate that the mechanism of EMT, but not epithelial adhesion or apoptosis, is involved in the process of fusion of the conotruncal ridges. The EMT mechanism associated with conotruncal septation seems to be uncoupled from the process of formation of the endocardial cushions, which takes place at early stages. With these results, we can raise the hypothesis that defects in the EMT process may lead to different morphological types of bicuspid aortic valve.

Support or Funding Information
This study was supported by P10-CTS-6068 (Junta de Andalucía).