

The ventral body wall closure in amniote development

Kengo Buma

Introduction

The vertebrate body wall is composed of skin, muscle, skeleton, etc. During development in amniotes, the body wall components including the sternum and pectoral muscles, which are located in the most ventral portion of the definitive body wall, initially arise in the dorso-lateral portion of the early body wall. Through the process known as ventral body wall closure, these components change their positions toward the mid-ventral portion to establish the definitive body wall. Failure in the ventral body wall closure during human development leads to severe congenital abnormalities accompanying herniation of the visceral organs. Previous studies using gene knockout mice have revealed various genes required for the ventral body wall closure. However, the cellular and tissue-level mechanisms to achieve the positional change of the musculoskeletal components in the course of closure has poorly been explored.

The body wall prior to the ventral body wall closure is divided into two regions: the primary body wall (PBW), which occupies the mid-ventral region and lacks any musculoskeletal components, and the secondary body wall (SBW), which represents the remaining dorso-lateral region and bears the musculoskeletal components including the sternum and pectoral muscles. Two opposing models for the closure process are possible; one is the invading closure model, in which the musculoskeletal components originally located in the SBW infiltrate into the PBW, and the other is the replacing closure model, in which the entire PBW degenerates to be replaced by the SBW. The present study, using chicken embryos, has verified which of the closure models is appropriate, and has further investigated the detailed course and basic mechanisms of ventral body wall closure.

Materials and Methods

The study was conducted using chicken embryos. Tissue movements of the PBW and SBW during the ventral body wall closure were examined by *in ovo* cell labeling with PKH fluorescent dyes followed by tracing of the post-closure destination of labeled cells. For the labeling of late stage embryos, which is usually difficult to access for experimental manipulations, a newly developed method to lift an embryo up *in ovo* was introduced. Cell death in the closing body wall was observed by the vital staining with LysoTracker Red.

To explore the role of the PBW in the ventral body wall closure, defective closure was experimentally induced by incising the avascular domain of the PBW. Growth of the SBW in normal and defective closure embryos was evaluated by measuring rib elongation after preparing skeletal specimens with Alcian blue staining and clearing.

Results and Discussion

To test which of the two closure models is appropriate, PBW cells were PKH-labeled at the pre-closure stage. After the closure, both mesodermal and ectodermal cells of the PBW are confined to the ventral midline, the results contradicting the invading closure model, according to which PBW cells should remain laterally. It is thus suggested that the PBW degenerates to be replaced by the SBW in accordance with the replacing closure model. This is also supported by the observation that the PBW undergoes massive cell death during the closure. PKH-labeling of SBW cells showed that the dermis and connective tissue in the mid-ventral region of the post-closure body wall as well originate from the SBW originally located dorso-laterally as the musculoskeletal components, further corroborating the replacing closure model.

It was previously described that the mid-ventral body wall of chicken embryos initially lacks blood vessels, and subsequently, but before the SBW replaces the PBW, the vascularized domain expands from either lateral side toward the midline. PKH-labeling

in the present study showed that the avascular domain is replaced by the vascularized domain prior to the SBW closure. Combined with the results described above, it is likely that ventral body wall closure is achieved by two-step tissue replacements.

Based on the replacing closure model, both PBW degeneration and SBW growth are essential. To examine the role of PBW in the SBW closure, defective closure was experimentally induced by impairing the PBW. Defective closure embryos exhibit a large ventral opening of the unclosed SBW ; Nevertheless, the SBW growth is little affected. These results suggest that the intact PBW is not necessary for SBW growth, but essential for midline closure of the growing SBW.

Conclusion

Ventral body wall closure is achieved by two-step tissue replacements; the avascular domain of the PBW is replaced by the vascularized domain, which is, in turn, replaced by the SBW. The SBW form the definitive body wall, while the PBW including both the avascular and vascularized domains is just a transient structure to cover the ventral surface of the early embryo. Sequential tissue replacements lead the SBW to midline closure. This study provides a refined view on the course of ventral body wall closure and its basic mechanism for future studies.