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NEW INSIGHT INTO THE MECHANISM OF VENTRAL MIDLINE CLOSURE USING A NOVEL CONDITIONAL KNOCKOUT MOUSE MODEL

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Aim: Mechanisms leading to the development of abdominal wall defects are still largely unknown. We aim to study the mechanism of ventral body wall (VBW) closure in mice.

Methods: We studied VBW development in a Tagln-cre mouse model. Expression of TAGLN, a cytoskeletal protein, was evaluated in the developing embryo between E11.5-E16.5 using immunofluorescence staining. Tagln^{+} cells were FACS (fluorescent activated cell sorting) sorted from non-Tagln midline cells and qPCR for TGFβR2 was performed. Furthermore, we created a novel mouse model of VBW closure defect by selective removal of TGFβR2 in Tagln expressing cells (Tagln-Cre:Tgfβr2 $^{flox/flox}$) and evaluated the expression pattern of the migrating cells.

Results: TAGLN^{+} cells are predominant in the primary VBW and the expression of TAGLN diminishes with midline closure. These cells are migratory myofibroblasts that continuously pull the secondary body wall elements towards the midline. These cells incorporated in the closing sternbrae, briefly expressed SOX9 (Sex determining region Y-box 9) chondrogenic marker and acted as site specific fusion cells. We found high expression of TGFβR2 in Tagln cells in the ventral midline. Using FACS, we demonstrated that Tagln^{+} cells only accounted for 15% of the total cell population and qPCR showed higher expression of TGFβR2 in Tagln cells (2.1 vs 1.0, P=0.01). Conditional knockout of TGFβR2 in the Tagln-Cre mouse lead to complete failure of VBW closure and a phenotype similar to Pentalogy of Cantrell (Fig. 1). The lateral body wall formed normally and distinct lateral abdominal muscle layers developed. The Tagln-Cre:Tgfβr2 $^{flox/flox}$ mouse model had multiple developmental anomalies that all contributed to late embryonic lethality.

Conclusion: We have identified a population of pioneer cells that orchestrate VBW closure. We have created the first conditional knockout model of VBW closure defect and shown that the selective elimination of TGFβR2 from a minority subset of VBW cells is sufficient to prevent the closure process.

Figure 1