Twist1 Function in Endosomal Formation and Cell Shape Changes During Neural Tube Closure

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Objective: Neural tube disorders result from improper development and closure of the neural folds during early embryogenesis. The two most common NTDs, spina bifida and anencephaly, affect approximately 1 in 1,500 babies and, oftentimes, are severely debilitating or fatal. Despite the severity and graveness associated with many neural tube disorders, the etiology of most cases remains unknown. Various genes are known to be involved in the neurulation process including cell adhesion proteins β/ δ-catenins and endosomal factors Rab11b and LRP2. Based on our previous work, it has been suggested that TWIST1 protein interacts with β/ δ-catenins, but nothing is known about its interaction with endocytic factors during the process of neural tube formation. The present study aims to uncover the underlying mechanism of Twist1 function in endosomal formation and cell shape changes during neural tube closure.

Experimental Methods: Histological and immunofluorescent staining of mouse embryonic tissues for *Twist1*$^{-/-}$ and *Twist1*$^{cko/-}$ mice compared to wild type (WT) littermates were imaged and analyzed.

Results: Our results demonstrated that in WT mice the neural tube forms without defects as expected. However, in *Twist1* knockout embryos, the neural tube forms improperly and exhibits blebbing and structural defects. TWIST1 expression in apical cells of neuroectoderm overlaps with endosomal and cell adhesion markers. Furthermore, digital analysis demonstrated that a knockout of the *Twist1* protein results in a change of the cell shape and size at neural fold edges.

Conclusion: This study proposes that TWIST1 interacts with adherens junction and endosomal markers to regulate the endocytic vesical formation and cell shape changes during neural tube closure. Our findings suggest that these factors might contribute to the etiology of neural tube disorders.