The Role of Beta-Tubulin-III in Ectodermal Cell Proliferation, Migration, and Differentiation

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INTRODUCTION

Neural crest (NC) cells produce a plethora of different cell types including the peripheral nervous system and craniofacial cartilage and bone. In order for NC cells to produce their derivatives, they need to undergo an epithelial to mesenchymal transition (EMT) and migrate to distant locations in the embryo. The process of EMT involves the downregulation of epithelial cadherins (such as CDH1) and the upregulation of migratory cadherins (such as CDH11).

The expression of TUBB3, a microtubule subunit and component of the cytoskeleton, has recently been identified in pre-migratory NC[1]. Mutations in TUBB3 are linked to neurocristopathies including congenital bletharoptosis, extraoculomotor muscle fibrosis[2] and peripheral neuropathy[3]. The aim of this project is to investigate the role of TUBB3 in early formation and patterning of the ectodermally-derived tissues including the central nervous system (CNS), NC cells, and epidermis.

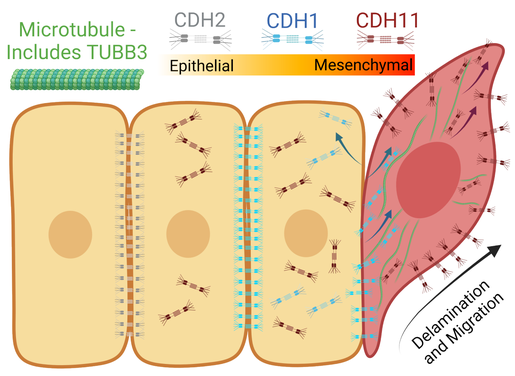

Figure 1: Cytoskeletal elements and adhesion proteins are associated with NC EMT. We hypothesize that TUBB3-dependent microtubules are necessary to traffic CDH1 internally (away from membrane) and migratory cadherins (CDH11, CDH7) towards the membrane during EMT.

METHODS

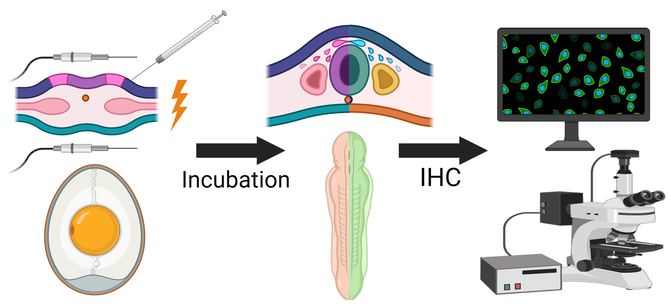

Figure 2. Flowchart of methods. Chicken embryos are injected and electroporated at gastrula stage ex ovo. These embryos are then incubated and collected for immunohistochemistry (IHC) at later developmental stages. Fluorescence microscopy is used to visualize changes in protein expression and localization.

CONCLUSION

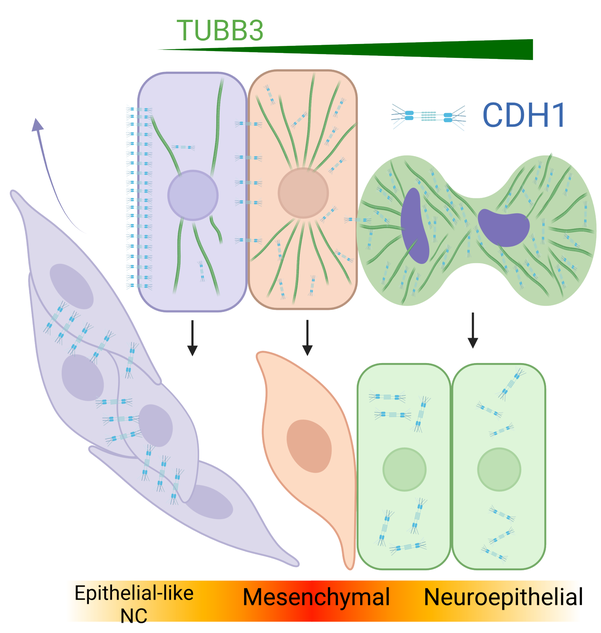

Figure 7: Graphical Illustration of conclusions. The "Goldilocks" hypothesis: Ectodermal cells require a specific amount of TUBB3 to regulate transport of cadherin proteins to and from the membrane. Our results show that reductions in TUBB3 lead to a maintenance of CDH1 on the membrane causing an epithelial NC cell phenotype and premature cell migration. In contrast, excess TUBB3 reduces membrane-bound, and increases cytoplasmic, CDH1 leading to excess cell proliferation.

RESULTS

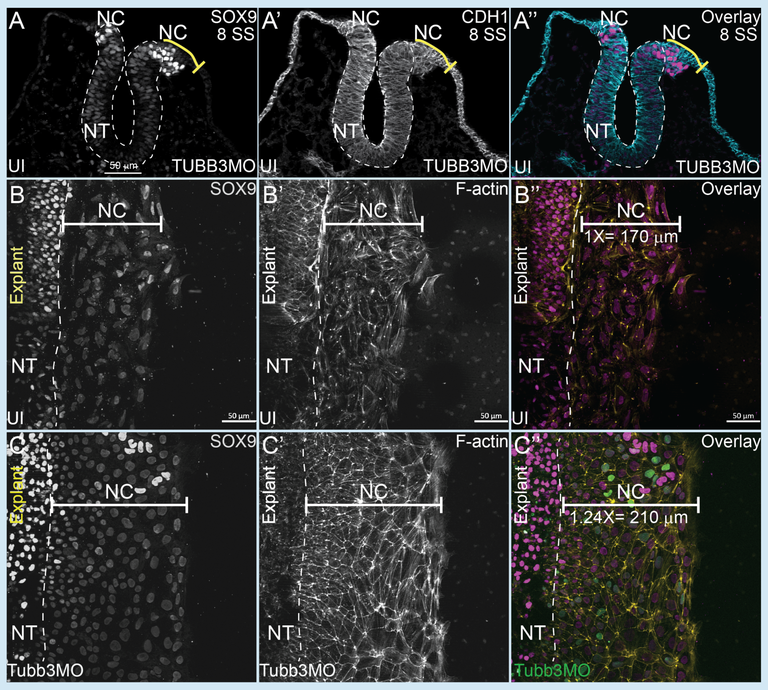

Figure 3: TUBB3 knock down expands the NC population. Morpholino-injected (TUBB3MO) sides were compared to uninjected side as an internal control. (A-A") Cross-section of stage 8-9 chick embryo shows that TUBB3MO increases the number of SOX9 positive cells. (B-B") Neural tubes were extracted from embryos and the explants were cultured. Explant imaging of uninjected side showing WT SOX9 positive cells and mesenchymal nature of NC, as seen through F-actin staining. (C-C") TUBB3MO explant demonstrates expansion of SOX9 population and increased membrane-associated F-actin staining indicating higher epithelialization of NC.

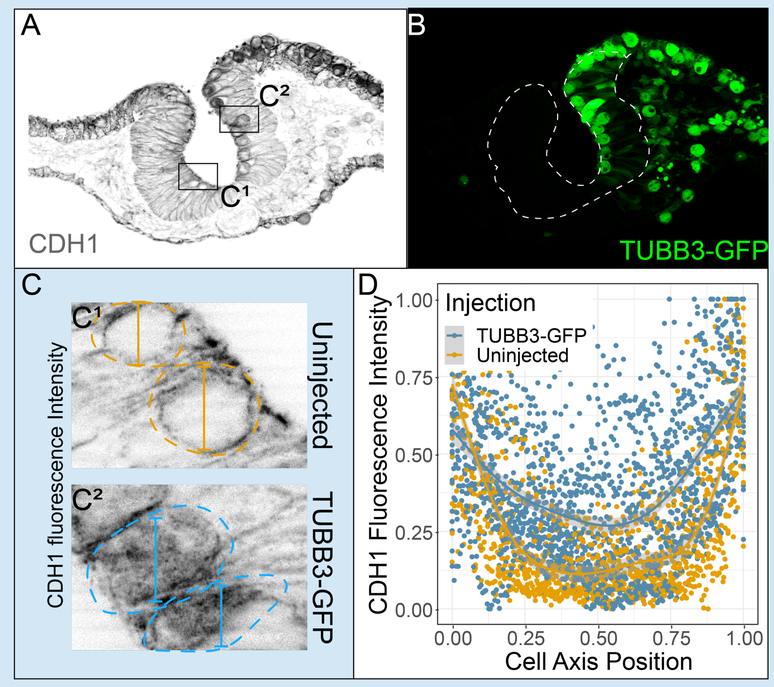

Figure 4: TUBB3 overexpression induces CDH1 internalization. (A-B) Cross section of stage 8 embryo following unilateral injection of TUBB3-GFP (OE), CDH1 fluorescence was increased on the injected side. Line profile of normalized CDH1 fluorescence was determined across the cell axis of dividing cells from both the injected and uninjected side. (C) High magnification zoom in of line profile sections in cells on the uninjected (C^1) versus the injected (C^2) side. (D) Scatterplot of CDH1 fluorescence across cell axis in cells on injected (TUBB3-GFP) and uninjected side.

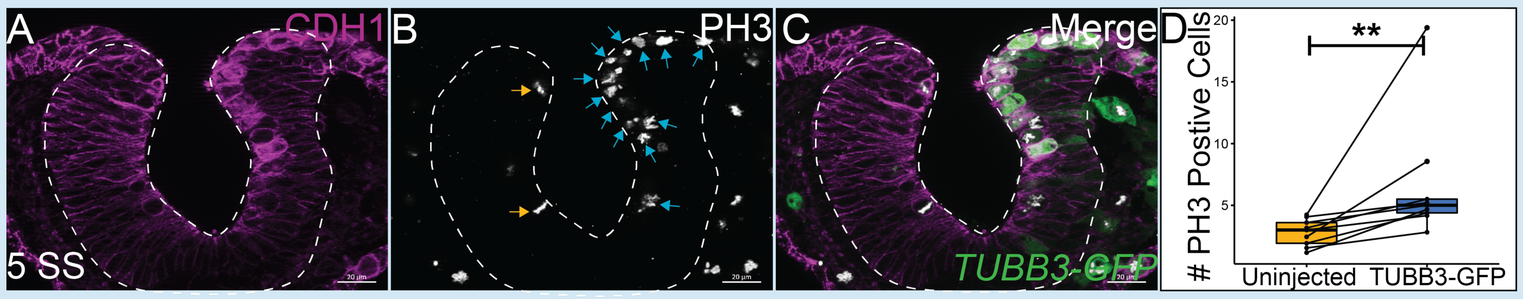

Figure 5: TUBB3 overexpression induces ectopic cell proliferation. The number of PH3 positive cells (cell proliferation marker) was quantified in cross sections from stage 8-9 chick embryos following unilateral TUBB3-GFP injections. (A-C) In cells with cytoplasmic CDH1, TUBB3OE increased number of PH3 positive cells. (D) PH3 was significantly increased (p= 0.0039) in TUBB3OE sides. Orange arrows are PH3-positive cells on uninjected side and blue arrows are PH3 on injected side. Outline indicates the neural tube.

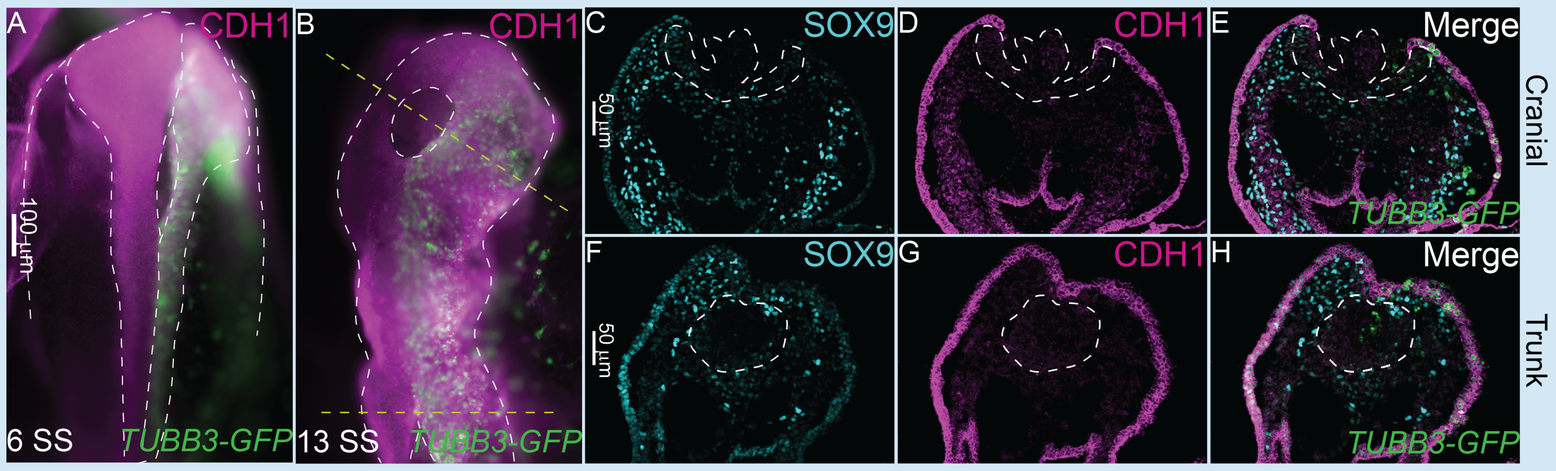

Figure 6: TUBB3 Overexpression induces "hole in the head" phenotype. (A-B) TUBB3OE causes progressively worse hole in the developing embryonic head from early to later staged embryos. (C-E) Cross sections of later staged embryos demonstrate loss of neural tube structure in cranial regions. (F-H) Posterior sections of the developing spinal cord indicate the absence of a healthy hollow neural tube and the presence of tissue detritus and dying cells. Outline indicates neural tube or expected location of neural tube.

FUTURE DIRECTIONS

- Define the cell and tissue phenotypes caused by different amounts of excess TUBB3.
- Determine if TUBB3 perturbations lead to alterations in other cadherins such as CDH2 and CDH11.
- Identify the mechanism downstream of TUBB3 in proliferation as it has been found to be upregulated in multiple types of cancers[4].

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