THE DEVELOPMENT OF THE MOUSE INNER EAR

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Abstract:

Our experiment focused on the development of the mouse inner ear during embryonic development. We also observed the affects that Sonic Hedgehog (Shh) has on the inner ear of a mouse as it relates to cartilage formation and mouse malformations.

What is inner ear Morphogenesis:

Morphogenesis concerns itself with the formation and development of biological structures at adult as well as embryonic stages, as in our experiment. In general, “ “morphogenesis investigates how the regulation of cell fates contribute to form and structures of the organism and its component parts.”

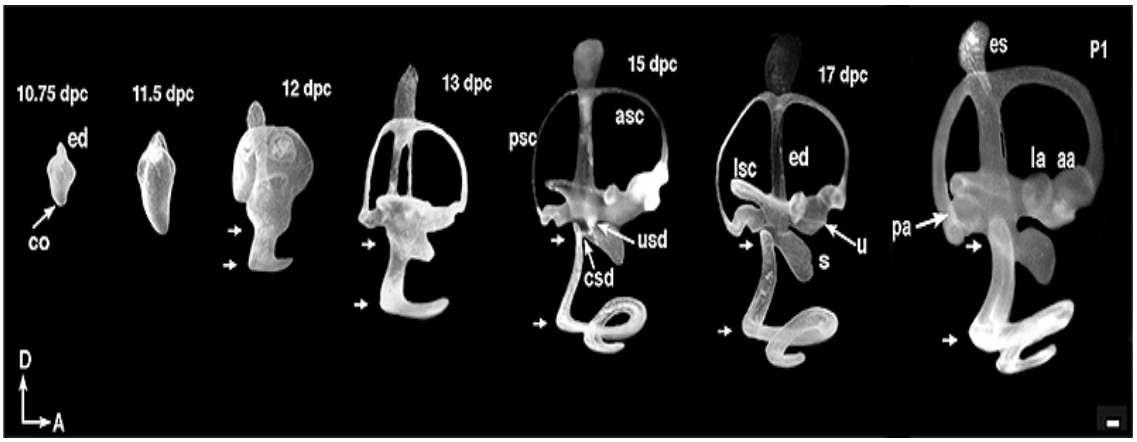


Figure 1: The Morphogenesis of the Mouse Inner Ear

The figure above displays how the inner ear of a mouse, similar to that of humans, develops over time. The ear starts as an otocyst and morphs into a more complex structure. The dorsal portions of the otocyst become committed to produce vestibular structures such as the semicircular duct and the associated cristae, and the ventral portion will differentiate into the coiled cochlear duct.

Sonic Hedgehog (Shh) in mouse inner ear development:

Sonic Hedgehog is one of three proteins in the mammalian hedgehog family. Of the three, Shh is the best and most frequently studied of the hedgehog signaling pathway. Sonic Hedgehog serves many purposes in the development. Shh plays a major role in regulating vertebrate organogenesis which is, “the process by which the ectoderm, endoderm and mesoderm develop into the internal organs of animals.”

In addition, Sonic Hedgehog is a growth factor that binds patched receptors on the cell membrane. In an embryo Shh can take on many jobs. In the nervous system, Shh is secreted by the notochord, ventralizes the neural tube, and inducing the floor plate and motor neurons. In the limbs, Shh is secreted by the zone polarizing activity (ZPA) organizing limb axis formation.

How Sonic Hedgehog works in inner ear Morphogenesis:

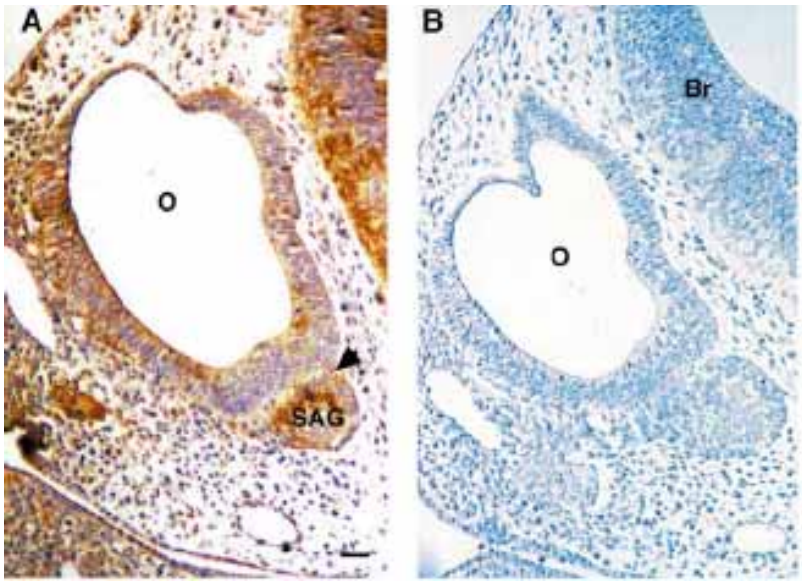


Figure 2: (A) E10.5 inner ear stained for Shh using an Shh specific antibody. Shh is present in the developing inner ear. (B) Negative control, showing absence of stain

In figure 2, the diagram showing the presence (A) and absence (B) of sonic hedgehog at ten and a half days of development embryonic development. The brownish are in diagram A is the location of the Shh. At 10.5 days embryonic development there is not a totally apparent distinction between an embryo with or without Shh. As a result we asked ourselves: **What consequences to inner ear cartilage formation are incurred if signaling by Sonic Hedgehog is inhibited?**

Proceedure:

1. Dissect mesenchyme and/or epithelium from mouse embryos' ears (E 10.5 – E 14)
2. Dissociate tissue into cells with 1ml Trypsin-EDTA for 1.5'. Stop reaction with 2ml culture media (F12 10%FBS+1%Penn-Strep) and dissociate by pipetting.
3. Adjust cell concentration to 2.5 X 107/ ml according to this formula: cell count X 3 X 1000/2.5 X 107
4. Place 10ul cell solution into each culture well. After 45' at 370C, add 1ml culture medium into each well.
5. Cultures stay in 370C 5% CO2 incubator for 7 days. Change medium every other day

or according to experiment needs. Cartilage should been seen after 5 days.

AFTER THE INCUBATION PERIOD

Procedure for staining

1. Fix the culture with 10% formalin plus 0.5% CPC for 1'
2. Stain the cultures with 0.5% Alcian blue in 0.1N HCL (pH 1.0) for a few hours.
3. Take the unbound stain out with rinsing in 3% acetic acid 2-3 times.
4. Take the bound stain out with 300ul 8M GuHCL. It will take 1-2 hours.
5. Measure the density of the bound staining with Optical Density Counter with 600um wavelength.

Control and Experimental Groups:

Effects of Shh oligonucleotides in cultured mescenchyme + epithelium

Treatment	Alcian blue stain
Control	**.243+/-.02**
Shh sense	**.298+/-.05**
Shh missense	**.286+/-.06**
Shh antisense	**.101+/-.02**

In the experiment we used four separate groups: The Control, Shh Sense, Shh missense, and Shh anti-sense. The one we focused on the most was the antisense. When

the antisence is introduced in to the mouse it inhibits the Sonic Hedgehog from expressing itself by binding the RNA, thus no cartilage forms. This finding shows an effect of Shh inhibition in vitro (in culture), leading us to question, is there an effect of Shh inactivation in vivo (in the inner ear itself)?

Shh Null mutant mice vs. Wild Type mice

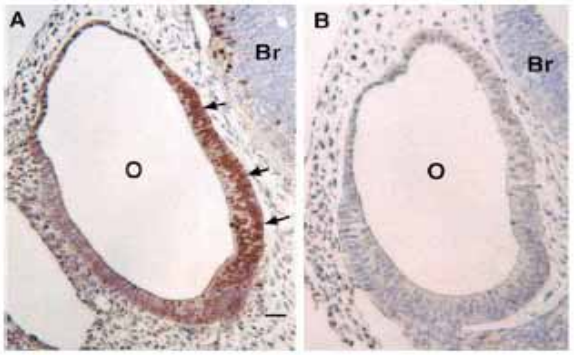


Figure 3: Normal morphology (overall shape) of the inner ear was observed at E10.5 days in both the wild-type (A) and mutant (B) embryos.

In figure three, picture (A) shows wild-type mouse in which the gene pax2 has been expressed (the brownish area), and figure (B) shows a mutant mouse in which pax2 has not been expressed. This decrease of pax2 in mutant mice suggest that the loss of Shh may impact inner ear development at later stages.

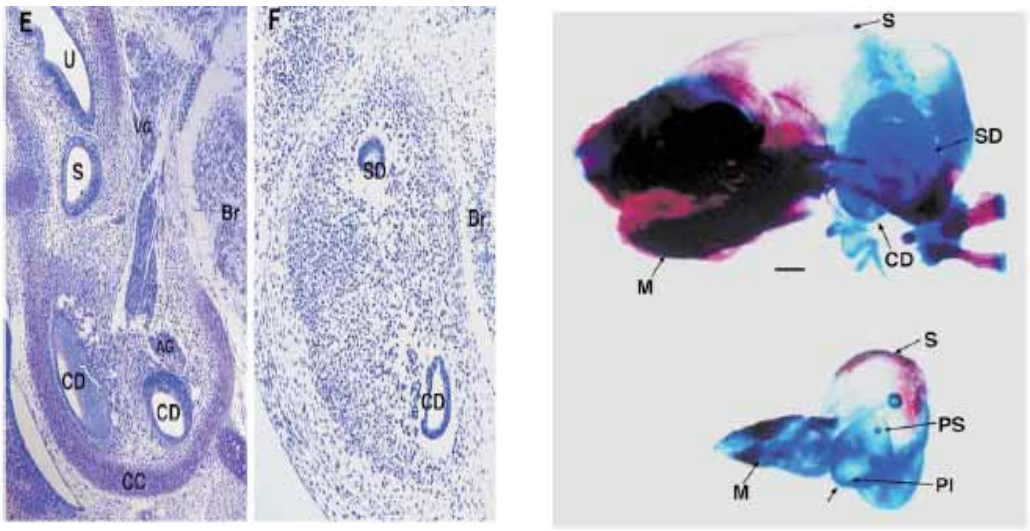


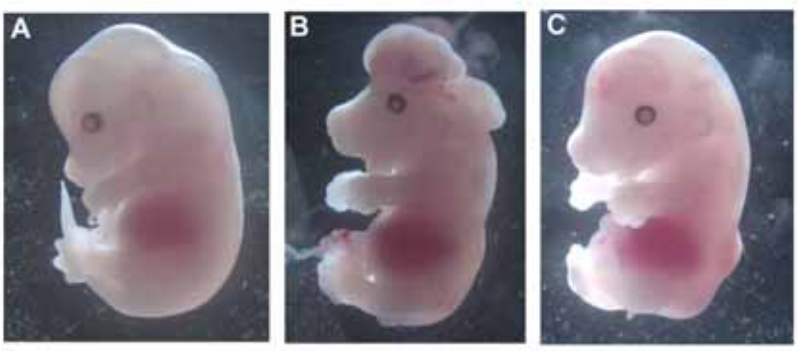
Figure 4: This is evidenced at E15.5 (top) and E17.5 (bottom), when the Shh null mutant inner ear is characterized by rudimentary cochlear duct and semicircular ducts, absent or disorganized ganglia, and a poorly defined capsule.

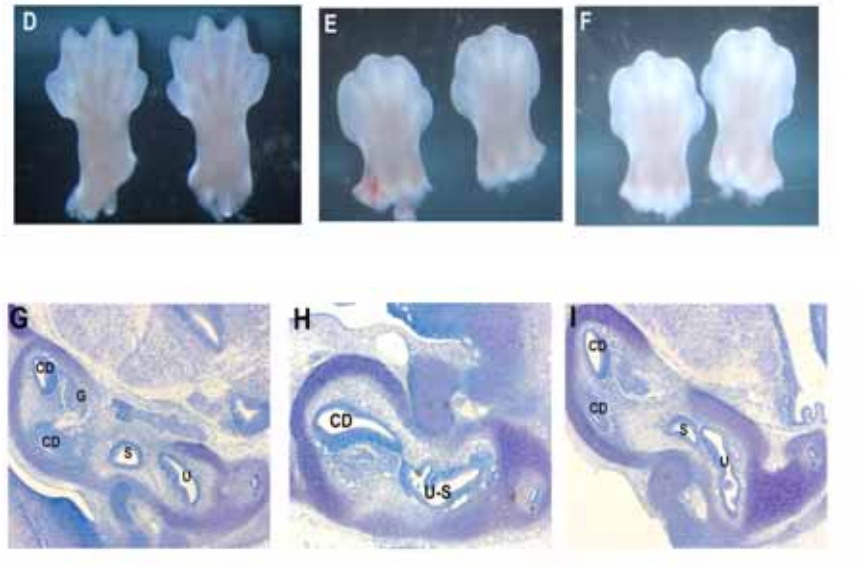
As conveyed by figure 4, the photos show how the inner ear malformations begin to show themselves as development progresses. The inner ear of the wild-type mice progress normally, however the mutant mice inner ear lacks major components of the ear and it is significantly smaller than a normal inner ear.

How Sonic Hedgehog works in the Inner ear and Malformations?

Sonic Hedgehog elicits its biological effects through a gene called Gli3, Gli3 is present in the inner ear and activates Shh target genes. Mice that are Gli3 negative have craniofacial defects such as server polydactyl, shortened skeletal elements and abnormal inner ears as well as abnormal forelimbs and hind limbs. In addition, Gli3's affects can be positive or negative depending or whether Shh is present. When Shh is absent, Gli3 only has negative effects, leading to malformations. However, if we remove the Gli3 gene we can reverse its negative activity and rescue the tissues.

Shh & Gli3 **No Shh & Gli3** **No Shh/ No Gli3**





Future Directions

In the future we want to determine if and when other hedgehog signaling molecules play a role in inner ear development (Indian Hedgehog and Desert Hedgehog), investigate the functional role of components of the Shh receptor complex in inner ear development , and determine how our findings relate to patients with mutations in hedgehog genes.

Work Cited

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ACKNOWLEDGEMENTS

Dr. Dorothy Frenz PhD

Wei Liu

Lijun Li

Dr. Sat Bhattacharya

Harlem Children Society

Albert Einstein School of Medicine