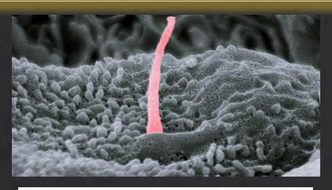
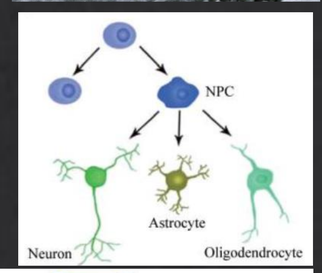
Preliminary evidence of primary cilia expression and function in the chick embryonic hindbrain

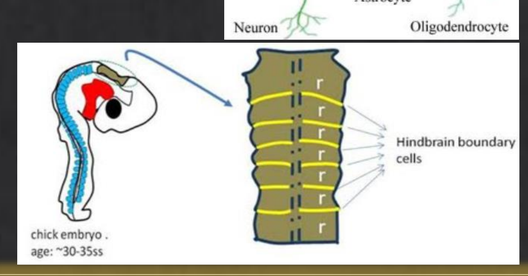
Lior Matatya, Dalit Sela-Donenfeld
Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agricultural, Food & Environment,
The Hebrew University of Jerusalem

Abstract:

- **The primary cilia** is an important organelle that has been discovered in a wide variety of tissues in vertebrates. It has an antenna like structure and various sensory functions has been attributed to it, such as chemical, mechanical and physical sensing. It is also known to have a regulatory role in the cell cycle.
- Neural stem cells (**NSCs**) are the earliest source for the entire nervous system. They can divide or proliferate and have a crucial part in the embryonic development. This has been shown in chick and mammal embryo models, in late stages of the embryonic development and in post natal as well.
- The hindbrain is a highly conserved area, that in early days of development is divided into several segments termed rhombomeres. Between the rhombomeres defined boundaries are formed, in which cells have different morphology and gene expression compared to the nearby rhombomeres. These hindbrain boundary (HB) cells are also dividing at a much slower pace than rhombomere cells.
- In our lab it has been shown that hindbrain boundary (HB) cells expresses Sox2, a key marker of NSCs.





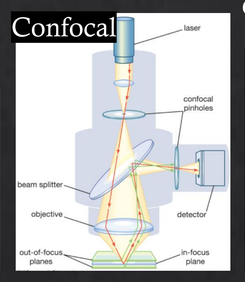


It appears that upon presenting of primary cilia, cells are prevented from dividing. Since it has been shown, using the Sox2 gene, that NSCs concentrate in HBs and divide slowly, we suggest that the primary cilia is a crucial factor in the cell cycle of those cells, enabling them to be quiescent.

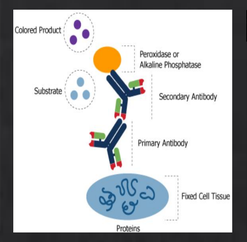
Methods:

Animal model

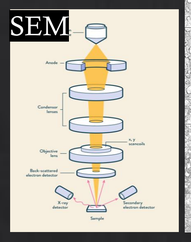
Confocal

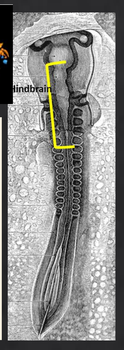


Immunohisto-chemistry



SEM

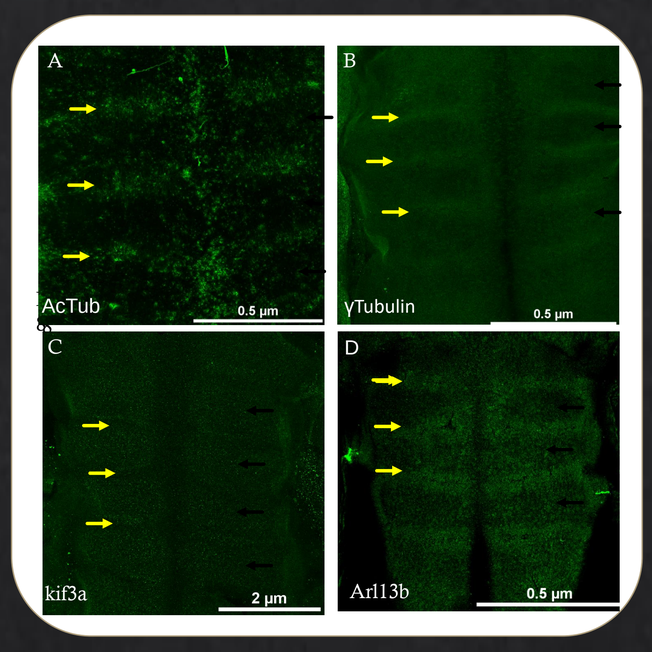




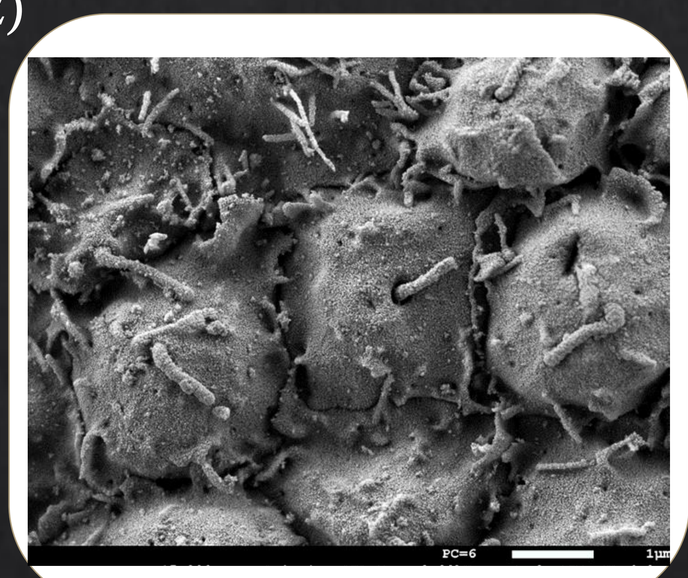
Results:

1. Characterization of several cilia markers in HB cells.
2. Validation of the cilia expression by SEM imaging of HBs.
3. Examination of cilia markers in a range of different stages.
4. Confocal analysis of cilia markers.
5. Quantification of cilia ratio between rhombomeres and HBs
6. First steps in inhibition assays, trying to prevent cilia activity in the hindbrain to assess effects on HB-NSCs.

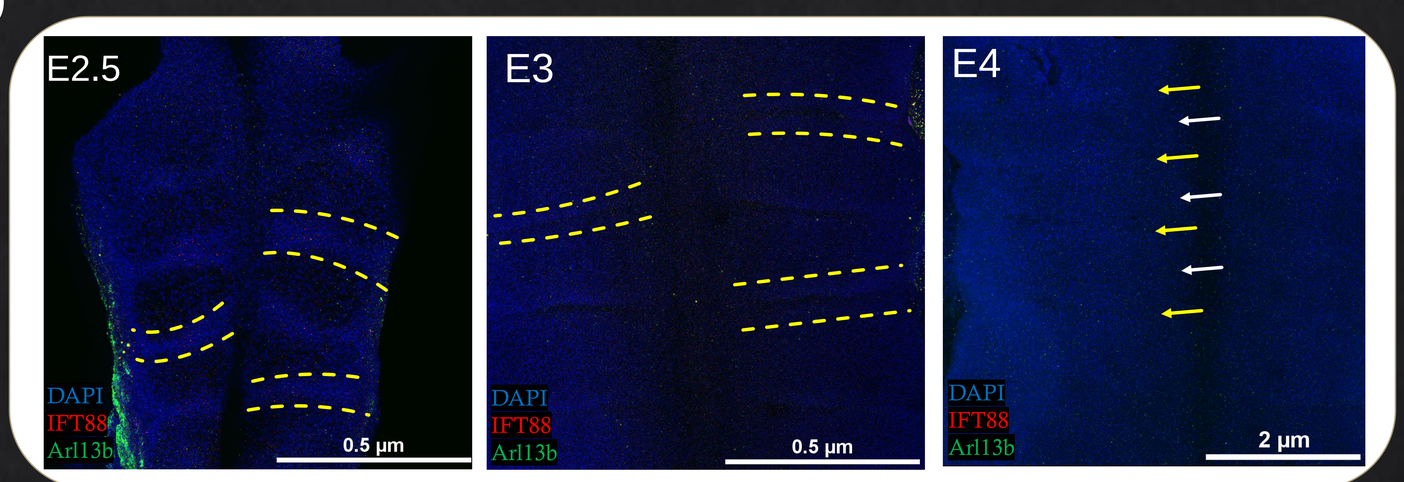
1)



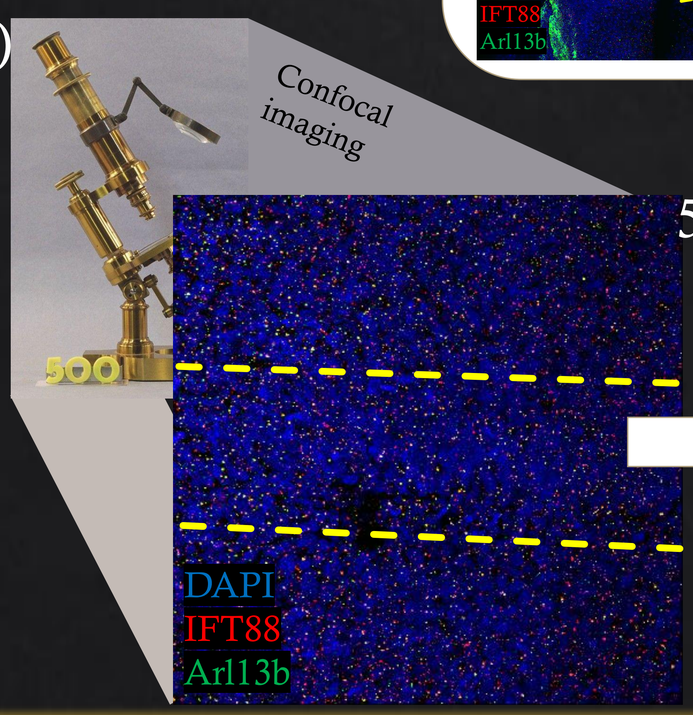
2)



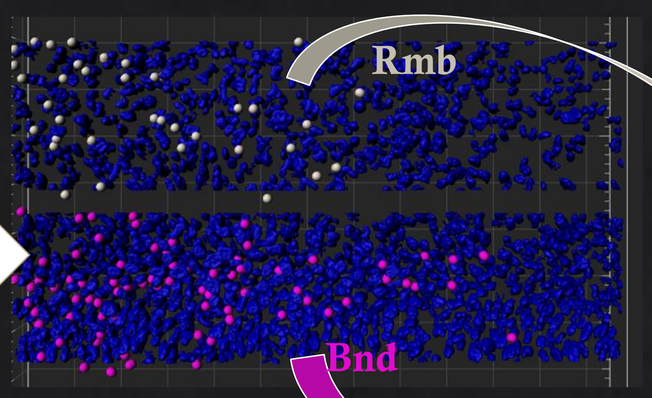
3)

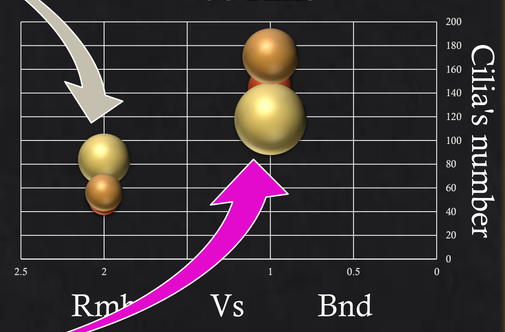


4)



5)





Conclusions:

- Using several markers validates that Primary cilia are expressed in HB-NSCs of the chick embryo.

- There is a dynamic expression of cilia at different stages of hindbrain development.

- IMARIS quantification of primary cilia distribution shows that primary cilia are elevated at HB cells compared to rhombomere cells, suggesting that they may regulate the quiescent NSCs behavior in HBs.