Unexpected role of apicobasal cell polarity genes in the salivary gland.

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Introduction

- Recent studies have identified a tumor hotspot in the salivary gland of *Drosophila*, located in the transition zone between the imaginal ring cells and the giant cells of the salivary gland. When the Notch intracellular domain, NICD, is upregulated; the transition zone undergoes tumorigenesis through neoplastic growth, while the imaginal ring of the salivary gland will only undergo hyperplasic growth with the ring cells over-proliferating.
- As this transition zone serves as a novel tumor model, we wanted to investigate how apicobasal cell polarity genes function in the salivary gland compared to the imaginal discs in *Drosophila*.
- We focused on the Scribble polarity complex that consists of discs large (*dlg*), lethal giant larvae (*lgl*), and scribble (*scrib*). Previous studies have found that these genes function as tumor suppressors in the imaginal discs.
- We performed experiments to test if these apicobasal cell polarity genes would maintain their function as tumor suppressors in the salivary gland.

Methods and Experimental Approach

- To begin our investigation of the cell polarity genes, we performed an RNAi knockdown of *lgl* in the transition zone and analyzed the results.
- We then wanted to check if NICD upregulation along with the knockdown of either *lgl* or *scrib* would still be able to induce tumorigenesis in the transition zone.
- In these two experiments, the salivary gland was dissected from animals containing the genetic knockdown. The salivary gland was then stained with primary and secondary antibodies before being mounted. All images were taken using a confocal microscope.
- To further investigate the differences between the salivary gland and imaginal discs, experiments were performed involving lgl^4, a loss of function mutation of the *lgl* gene. Both the wing discs and salivary glands were dissected from animals that contained the homozygous mutant allele.
- For the next experiment, we used the CoinFLP transgene that produces RFP clones through flip-out. Genetic crosses were made to produce offspring that contained *CoinF>RFP* and specific knockdown/overexpression of the cell polarity genes at 25 °C. Once the offspring reached the first larval stage, they were heat shocked for 1 hour each day and moved to 29 °C until the third larval stage. The third-stage larvae were then dissected, stained, and mounted.

Salivary Gland Transition Zone Tumor Model

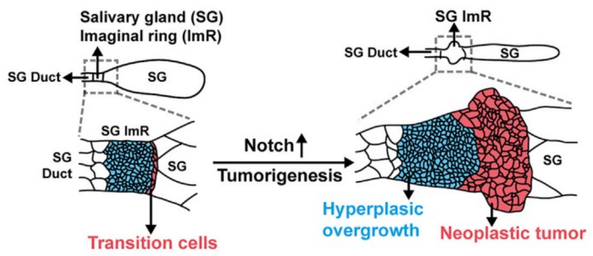

Figure 1: Graphical representation of tumorigenesis in the transition zone and hyperplasic growth in the imaginal ring of the salivary gland.

Yang, Sheng-An et al. "Oncogenic Notch Triggers Neoplastic Tumorigenesis in a Transition-Zone-like Tissue Microenvironment." *Developmental cell* vol. 49,3 (2019): 461-472.e5. doi:10.1016/j.devcel.2019.03.015

Knockdown of *Lgl* in the Salivary Gland Transition Zone

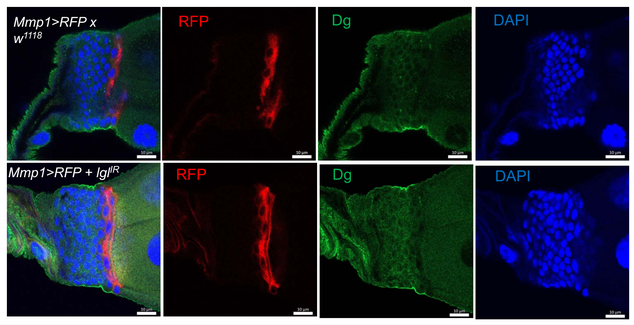

Figure 2: Knockdown of *lgl* was unable to induce tumorigenesis in the transition zone.

NICD Overexpression Paired with *Lgl* and *Scrib* Knockdown

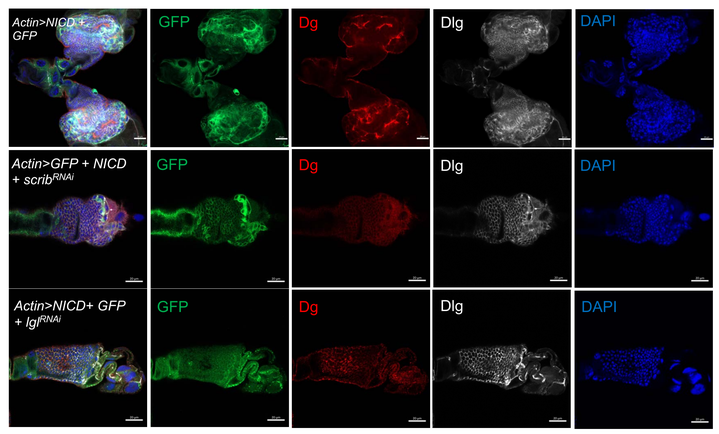

Figure 3: NICD overexpression by itself was able to induce tumorigenesis in the transition zone. However, when NICD overexpression was paired with *lgl* or *scrib* knockdown, the transition zone showed signs of suppressed tumor growth.

Effects of *Lgl⁴ in the Wing Disc Compared to the Salivary Gland*

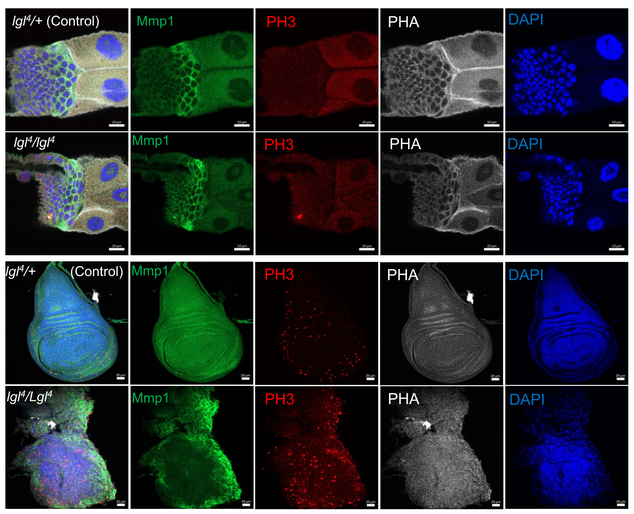

Figure 4: The loss of function of *lgl* induced tumor formation in the wing disc but was unable to induce tumorigenesis in the salivary gland.

Overexpression of *Scrib* in the Salivary Gland

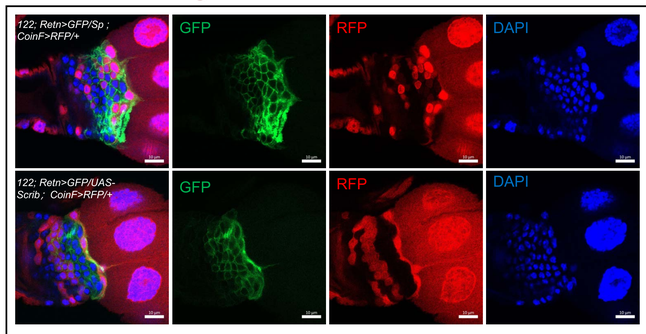

Figure 5: Using the CoinFLP system, we were able to investigate how the overexpression of *scrib* affected the RFP clones produced from flip-out. We quantified the average number of RFP clones by counting only the RFP clones of cells that expressed GFP.

Data Table: RFP Clone Density

Sample	Total Volume of GFP Expression (μm)	Average Clone Density of Imaginal Ring	Average Clone Density of Transition Zone	P Value (Two-Tailed)
Hs-122; Retn>GFP/Sp; CoinF>RFP/+	17294.03	0.001475	0.0003373	0.76
Hs-122; Retn>GFP/ UAS-Scrib ; CoinF>RFP/+	14273.93	0.001857	0.0003678	

Figure 6: Sample Size: n=8. The sample of animals overexpressing *scrib* displayed a 25.9% increase in RFP clone density in the salivary gland imaginal ring and a 9.04% increase in the transition zone compared to the control sample. However, the P value was calculated to be 0.76 so we cannot conclude from the data that there was a significant difference.

Conclusion

- **Knockdown of *lgl* by itself was insufficient to induce tumorigenesis in the salivary gland transition zone.**
- **When NICD was overexpressed along with either *lgl* or *scrib* knockdown, the transition zone showed signs of suppressed tumor growth.**
- **Animals containing the homozygous lgl^4 allele displayed that although tumor formation occurred in their wing discs, there were no signs of tumorigenesis in the salivary gland and transition zone.**
- **This data conveys that the cell polarity genes of the Scribble complex may be necessary for tumor growth in the salivary gland transition zone.**
- **We will need more samples and to perform more experiments using the CoinFLP system to be able to assess if there is a significant increase or decrease in RFP clone number when certain genes are knockdown or overexpressed.**

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