Metabolic Modulation of Hippo–Yorkie Signaling Drives Tumor-Like Eye Overgrowth in *Drosophila melanogaster*

Cintya Medina
Texas A&M International University
cintyamedinarangel@dusty.tamiu.edu

Keywords: Hippo signaling, Yorkie, insulin signaling, cancer metabolism, tumor progression

Background

One characteristic of tumor development is unchecked tissue growth, which frequently arises from the deregulation of conserved growth-control mechanisms. By limiting the activity of the transcriptional coactivator Yorkie (Yki), the Drosophila homolog of mammalian YAP/TAZ, the Hippo signaling pathway—a crucial tumor suppressor pathway—controls organ size. Yorkie is phosphorylated at serine 168 by the kinase Warts when Hippo signaling is active, which stops it from entering the nucleus and inhibits genes that encourage cell division and stop apoptosis. On the other hand, Yorkie builds up in the nucleus and promotes excessive tissue growth when Hippo signaling is interfered with.

On the other hand, insulin signaling regulates metabolism and cellular growth and has been linked to cancer development. Although recent studies using cancer cell line model systems have shown crosstalk between insulin and YAP/TAZ signaling, the role of these two pathways in an *in vivo* tumor model system remains unexplored.

Methods

Drosophila melanogaster eye is an excellent *in vivo* model system for investigating gene function in tumor growth. Driving the expression of constitutively active Yorkie (Yki^{S168A}) specifically in the developing eye using GMR-Gal4 driver induces massive overgrowth. To investigate the role of insulin signaling in hyperactive Yorkie (Yki^{S168A})-mediated abnormal eye growth, we altered the expression of *InR* and *Akt*, key components of insulin signaling, in Yki^{S168A}, GMR-Gal4 genetic background.

Virgin female flies with Yki^{S168A}, GMR-Gal4 genetic background were mated with male insulin receptor RNAi lines (BDSC 35251), insulin receptor (InR) constitutively active line (BDSC 8263), Akt RNAi lines (BDSC 31701). Female InR tyrosine kinase domain mutant (BDSC 8251) virgin were mated with male Yki^{S168A}, GMR-Gal4 flies. Following eclosion, F1 progeny from these crosses, were mildly sedated with CO2 collected and sorted by genotype. Eye phenotypes were assessed under an Olympus stereo microscope and stored at -75°C to preserve morphology until imaging.

Adult eye size and shape were qualitatively compared among the control, Yki^S168A overexpression, insulin receptor-reduction, and insulin receptor-overexpression groups to determine the extent of the tumor-like overgrowth.

Results

In our preliminary findings, compare to Yorkie (Yki^{S168A}) expression alone, we observed a dramatic reduction in Yki^{S168A}-mediated overgrowth when the InR expression level was reduced. Conversely, the eye overgrowth caused by the hyperactive Yki^{S168A} were significantly enhanced when constitutively active *InR* was expressed. Loss of Tyrosine kinase domain

function in InR as well as reduction of Akt, also notably decreased Yki^{S168}-mediated eye overgrowth, suggesting that insulin signaling regulates Yki-driven unchecked tissue growth.

These results demonstrate that severity of Yorkie-induced tumor-like eye growth is modulated by insulin signaling. The ability of insulin receptor levels to either suppress or enhance the tumor phenotype provides functional evidence of crosstalk between metabolic signaling and the Hippo pathway in controlling tissue expansion.

Conclusion

The results of this study demonstrate that insulin signaling plays a direct modulatory role in Yorkie-driven tumor-like eye overgrowth in *Drosophila melanogaster*. Reducing *InR* or *Akt* activity significantly suppressed Yki^S168A-mediated tissue expansion, while constitutive activation of *InR* greatly enhanced the hyperplastic phenotype. These findings show that insulin signaling is not simply supportive of growth but actively drives the severity of Yorkie-mediated over-proliferation, providing unambiguous evidence of functional crosstalk between metabolic pathways and the Hippo–Yorkie growth-control system.

More broadly, this work highlights how metabolic state influences oncogenic signaling, reinforcing the view that tumorigenesis arises from the integration of both growth-control pathways and metabolic conditions. Because Yorkie is homologous to mammalian YAP/TAZ—frequently dysregulated in human cancers—these findings have important implications for understanding metabolic contributions to cancer progression. The sensitivity of Yorkie-driven overgrowth to insulin pathway activity suggests that factors such as nutrient availability or systemic insulin levels could significantly modulate tumor severity. Future studies exploring upstream Hippo components and downstream metabolic effectors will help clarify how these pathways converge to regulate tissue overgrowth.

Through the Science Influencers REEU program, this project strengthened my skills in genetic cross design, phenotype analysis, experimental control, and scientific communication. This experience reinforced my interest in cancer biology and deepened my understanding of how growth and metabolic pathways cooperate to drive disease progression.

References

Chen, J., & Verheyen, E. M. (2012). Homeodomain-interacting protein kinase regulates Yorkie activity to promote tissue growth. *Current Biology, 22*(17), 1582–1586. https://doi.org/10.1016/j.cub.2012.06.047

Hwang, J.-H., Kim, A. R., Kim, K. M., Park, J. I., Oh, H. T., Moon, S. A., Byun, M. R., Jeong, H., Kim, H. K., Yaffe, M. B., Hwang, E. S., & Hong, J.-H. (2019). TAZ couples Hippo/Wnt signaling and insulin sensitivity through Irs1 expression. *Nature Communications, 10*, 421. https://doi.org/10.1038/s41467-019-08287-x