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SJSU Undergraduate Research Grants

Probing the Cellular Mechanism of Tumorigenic β-Catenin Mutations

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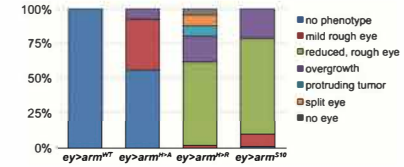
Abstract

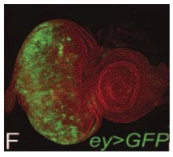
Beta-catenin is a dual function protein with roles in cell-cell adhesion and in the Wnt signaling pathway. Beta-catenin protein levels regulate Wnt signaling, and mutations that stabilize Beta-catenin are associated with >10% of human tumors. Preliminary data demonstrates that Beta-catenin is pH-sensitive, with a conserved histidine that titrates between positively charged (lower pH) and neutral (higher pH) to regulate binding to the E3-ubitquitin ligase Beta-TrCP; at lower pH ~7.3, Beta-catenin has decreased binding to Beta-TrCP and higher protein levels. We predicted that mutating this histidine to Arginine (H47R, positively charged) would decrease Beta-TrCP binding and increase protein levels. We expressed the *Drosophila* ortholog of Beta-catenin, Armadillo containing this mutation, arm^{H47R}, and found increased Wnt signaling and novel phenotypes including pronounced tumors in the *Drosophila* eye that are not reported with other Arm-stabilizing mutations. Here, we will investigate the cellular basis for these phenotypes. We hypothesize that these phenotypes are caused by higher proliferation in the developing *Drosophila* eye. We will dissect retinal tissues from developing *Drosophila*, and immunolabel against markers for proliferation and cell junctions. We will quantify the size of the retinal tissue, and the number of proliferating cells in transgenic flies that express either the arm^{H47R} tumorigenic mutation, the negative control (neutral alanine substitution) arm^{H47A} or wild type *arm*. Finally, we will determine relative expression levels of the transgenes to understand how protein levels affect our phenotypes.

What is the functional significance of Histidine 47 in *Drosophila* Armadillo?



- H>R mutation increases Wnt signaling
- H>R mutation causes tumors
- H>R acts as a neomorphic mutation

Experimental Approach

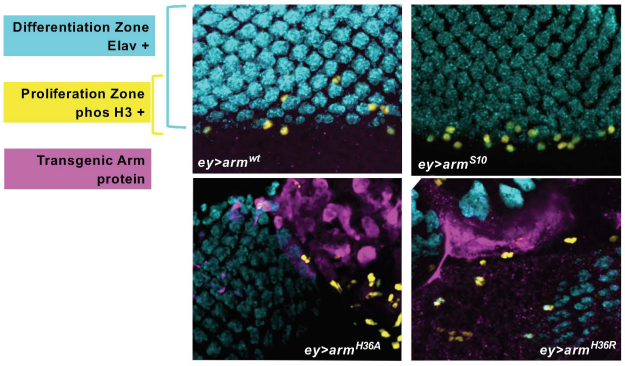


Oros, 1970

w;eyGal4

- Experimental: *w; UAS-arm^H47R* (High and Low)
- Negative Control: *w; UAS-arm^WT* (High and Low)
- Control-His Mutation: *w; UAS-arm^H47A* (High and Low)
- Control-Mutation (Increases Wnt, No Tumors): *w; UAS-arm^S10*

Results



Conclusions

1. Expression of Arm^{WT} and Arm^{S10} do not cause defects in third instar eye imaginal discs.
2. Expression of $Arm^{H>R}$ and $Arm^{H>A}$ proteins causes patchy accumulation of the transgene-encoded proteins. This suggests that the His residue may be important for protein degradation in both mutants.
3. Expression of $Arm^{H>R}$ and $Arm^{H>A}$ proteins inhibits neuronal differentiation. This fits with other papers suggesting that activation of Wnt signaling inhibits cell fate specification in the eye.
4. Because the patterning errors including by expression of $Arm^{H>R}$ and $Arm^{H>A}$ proteins are so severe, we cannot make conclusions about how these proteins regulate proliferation at this time.

Citations

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K.A. White1, B. K. Grillo-Hill1c, M. Esquivel*, J. Peralta*, V.N. Bui*, I. Chire*, D. L. Barberc. "β-catenin is a pH sensor with decreased stability at higher intracellular pH". 2018. Journal of Cell Biology 217(11):3965-3976.

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