The effect of Hedgehog signaling on *in vivo* neuronal morphogenesis

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Abstract: Neurons exhibit complex and diverse morphologies. The shape of a neuron has important functional implications, as it determines what signals a neuron receives and how these signals are integrated into neuronal circuits. To understand how the Hedgehog signaling pathway regulates neuronal morphogenesis *in vivo*, we are comparing dendritic arborization (branching) in *Drosophila melanogaster* (fruit fly) larvae with reduced, increased, and control (wild type) levels of Hedgehog signaling. Preliminary data suggests both increases and decreases in Hedgehog signaling affect the number of branches produced by epidermal sensory neurons.

Key words: *neuronal morphogenesis, dendrite, dendritic arborization, Hedgehog, Patched, Drosophila*

Introduction

The Hedgehog (Hh) pathway is an evolutionarily conserved signal transduction cascade which mediates how cells sense and process external information. While it is repeatedly used throughout development, our research specifically focuses on its role in neuronal outgrowth and circuit formation.

The proteins involved in the Hh pathway determine whether signaling occurs through a canonical or noncanonical mechanism [1, 2]. In the absence of the extracellular protein ligand Hedgehog (Hh), the pathway is inactive. During canonical signaling, the Hh receptor, Patched (Ptc) prevents Smoothened (Smo) from membrane localization and activation. However, in the presence of Hh, Hh binding to Ptc causes internalization of the Hh-Ptc complex, and translocation of Smo to the plasma membrane of the cell or primary cilia. This frees Cubitus interruptus (Ci)/Gli from a microtubule-associated complex containing the Fused (Fu) protein. As a result, Ci/Gli is converted from a transcriptional repressor to a transcriptional activator, which binds DNA and triggers gene expression.

In contrast, during Type II noncanonical Hh signaling, the presence of Hh stimulates Smo to regulate the actin cytoskeleton through multiple pathways, which do not involve Ci/Gli. Src family kinase members, the Tiam1 protein, and RhoA and Rac1 GTPase proteins are activated instead. Data suggests this noncanonical pathway regulates embryonic axon-guidance and *in vitro* dendritic spine formation [1].

Our goal is to understand how Hh signaling regulates neuronal morphogenesis *in vivo*. To accomplish this, we are studying how changes in Hh signaling activity affect the multi-dendritic epidermal sensory neurons of the fruit fly *Drosophila melanogaster*, a model organism, which is amenable to genetic analysis.

Methods

GAL4/UAS system: The GAL4-upstream activating sequence (UAS) system was used to regulate gene expression in *Drosophila* [3]. To perform this technique, flies are crossed to produce progeny that carry both a GAL4 gene and a UAS-gene of interest. The GAL4 protein is a transcriptional activator which binds DNA at UAS sites and activates transcription. A genomic enhancer sequence is positioned upstream of the GAL4 gene so that GAL4 is expressed in a tissue specific manner. In our study, the *pickpocket* (*ppk*) enhancer sequence was used to express GAL4 in class IV multidendritic neurons. UAS sites were located upstream of DNA sequences encoding Patched; Dicer, which is a protein that mediates the cellular short interfering (siRNA) response;

and siRNAs targeting Hh and Ptc mRNA.

siRNA gene silencing: siRNAs, are short RNA sequences, ranging from 20-25 base pairs in length, which are used to reduce or silence gene expression [4]. siRNAs are designed to bind to complementary mRNA sequences of interest, such as *Hh* and *Ptc* mRNA in our study. When the siRNA binds its target mRNA, a protein complex containing the Dicer protein binds and cleaves the double-stranded siRNA-mRNA molecule. Hence, the targeted mRNA is not translated into protein.

Visualization of neurons and quantitative analysis of dendrite branching: The GAL4/UAS system was used to drive Green fluorescent protein (GFP) expression in class IV multidendritic neurons. Fly larvae were bred to carry *ppk*GAL4, UAS-tandem GFP, UAS-Dicer 2, and either a UAS-siRNA molecule targeting Hh or Ptc, or UAS-*ptc*, which encodes for the Ptc protein. Neurons were examined in live larvae 140 hours after egg laying (AEL) using a Zeiss fluorescence microscope and camera, and Axiovision software. Dendrites were traced, and Sholl analyses were performed with Image J software.

Results and Discussion

Preliminary data suggests Hedgehog signaling regulates dendritic arborization in class IV multidendritic sensory neurons (Fig. 1). An increase in dendritic branching was observed in larvae with reduced Hh signaling: *hh* siRNA and *ptc* siRNA. Conversely, a decrease in dendritic arborization was observed in larvae with increased Hh signaling, UAS-ptc. While these results appear promising, a larger sample size must be analyzed to determine whether the data can be replicated and the differences are statistically significant, as the number of neurons and larvae currently examined are in the single digits. Furthermore, experiments are underway to study the effect of reducing other Hh signaling pathway proteins, including Smoothened and Fused.

Conflicts of Interest: Authors have no conflicts of interest.

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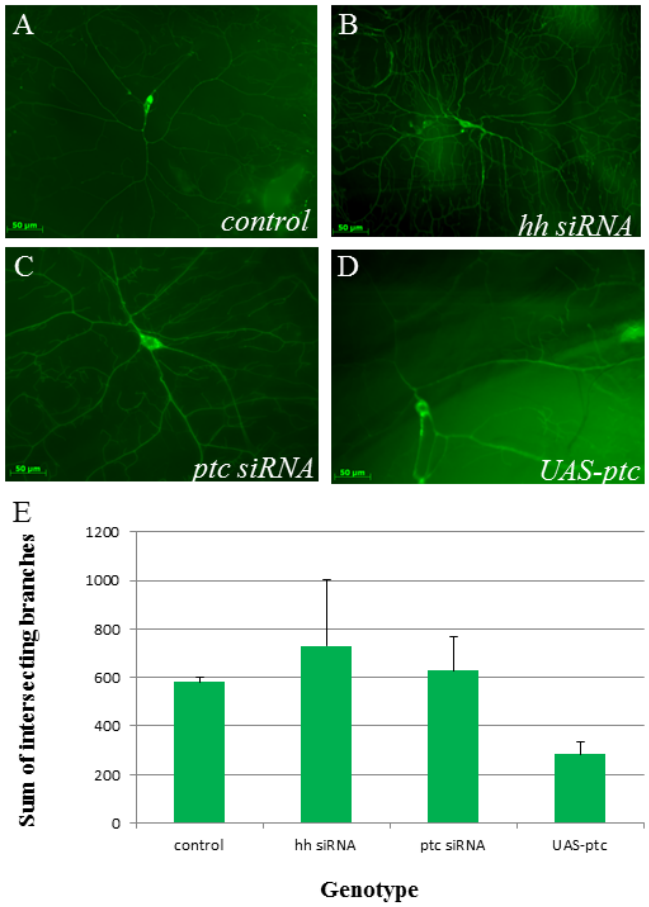

Fig. 1: Dendritic arborization of multidendritic neurons. (A) Control neuron, expressing a control siRNA construct which does not target any mRNA sequence. (B-C) Neurons expressing siRNAs designed to reduce Hh signaling (B) *hh* siRNA and (C) *ptc* siRNA. (D) A neuron with increased Hh signaling: UAS-*ptc*. (E) Sholl analysis of dendritic arborization (average sum of intersecting branches) for different genotypes. Error bars represent standard deviation.

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