Research exercise: Characterization of WNT Signaling During Dorsal versus Ventral Iris-Derived Newt Lens Regeneration

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Introduction

Newts have an exceptional capability of regenerating an entire organ via transdifferentiation. Of particular interest is their ability to regenerate the lens from iris pigment epithelial cells (PEC). Interesting enough, this ability to regenerate lens from the iris is a highly topological process, meaning that the lens will always form from the dorsal iris and never from the ventral. Thus, differences in gene expression of dorsal versus ventral iris have been suggested aid in finding the mechanism of lens regeneration. This implies that the regeneration process is controlled by the action of spatial organized signaling systems and different signaling pathways must be studied for better understanding. The Wnt/β-catenin signaling pathway represents one of the major pathways that was demonstrated to play a role in dorsal iris transdifferentiation (Figure 1 below).

Figure 2: β-catenin staining during lens regeneration in dorsal and ventral iris.

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<th>Day 0</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 22</th>
<th>FH535 Day 22</th>
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Methods

Following lens removal, newts were treated with the corresponding Wnt-related drugs (Wnt activator agonist 681665, GSK3 alpha/beta inhibitor CHIR 99021, β-catenin/TCF inhibitor FH535) at 1, 10 and 25 micromolar concentration in 30 microliter of 0.1%DMSO/0.6% NaCl by intra-peritoneal injection for the complete time period of 20 days. Controls animals were treated with 0.1%DMSO/0.6% NaCl solution. Following 20 days of lens regeneration, eyes were dissected and fixed in 4% paraformaldehyde/phosphate buffer saline followed by histology analysis. Histology analysis included paraffin embedding of eye specimen followed by sectioning. Sections were deparaffinized and stained using Hematoxylin/Eosin staining.

In addition eyes from untreated newts were obtain in 0, 8, 9, 10, 11, 12, 13, 14 and 22 days post lentectomy for immunohistochemistry. Those sections were stained with a β-catenin antibody in order to observe if the antibody is present in the nucleus of the iris (Figure 2) or the retina (Figure 4).

Conclusion

Wnt activator 681665 was first used to increase beta catenin concentration in the nucleus in order to turn the pathway “ON” in the ventral iris and develop an additional lens. The Wnt activator 681665 was hypothesized to bind in the membrane receptor Frizzled (Fzd) and inhibit the formation of the beta-catenin destruction complex in the cytoplasm. Lens expression was observed only from the dorsal iris (Figure 3A). Then CHIR 99021 was used in order to inhibit the GSK-3 kinase which phosphorylates beta-catenin leading to its degradation. The results showed only dorsal iris-derived lens development (Figure 3B). Lastly FH535 was used in order to inhibit the beta-catenin in the nucleus and thus inhibit any lens formation from both dorsal and ventral iris. The results were again lens formation from the dorsal iris (Figure 3C).

After staining with beta-catenin antibody it was found that the protein was found in both dorsal and ventral iris nucleus from day 0 till day 13 (Figure 2). Thus, the original hypothesis that Wnt pathway is in the “ON” state in the dorsal and “OFF” state in the ventral iris was disproved, since beta-catenin was present in both side of the iris indicating the “ON” state.

Acknowledgments

I thank Dr.Lancaster and Dr. McDougall for their support from the Lancaster-McDougal award. I also thank every member of Dr. Tsonis laboratory and Dr. Andrea Hoffmann.