Glucocorticoid Signaling and Immune Cell Function

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MDIBL molecular genetics short course led by Ian Gans; completed by 11 SMCC students and 2 SMCC instructors.

Abstract

The hypothalamus-pituitary-adrenal (HPA) axis directs homeostasis by regulating the secretion of glucocorticoids. In zebrafish, the hypothalamus-pituitary-inter renal axis performs an equivalent role. Cortisol is the active glucocorticoid in humans and zebrafish. Studies suggest that exposure to elevated cortisol at an early developmental stage alters immune system activity. The glucocorticoid receptor (GR) is the intermediary between an organism's metabolic, immune, nervous and cardiovascular system response to cortisol. The GR regulates a network of genes, including the kruppel-like factor 9 (klf9) gene which is linked to the regulation of immune genes. Neutrophils are immune cells and first responders to injuries/infection and signal other immune cells. To be able to track neutrophil activity after trauma, fish with neutrophils expressing green fluorescent protein (GFP) were crossed with klf9 knockout mutant fish. Tailfin amputations were performed and neutrophil response was compared to response in GFP expressing wild type fish.

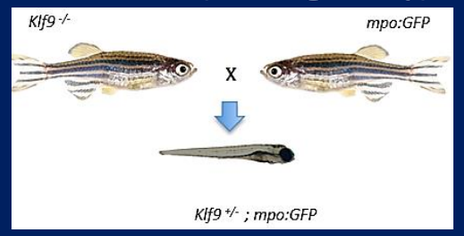

Fig.1 - Breeding figure on heterozygous klf9 +/- ; GFP

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Results

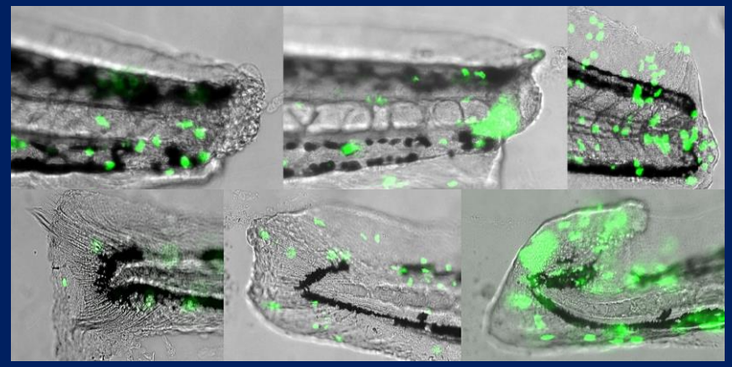

Fig.1 – (Top) Brightfield overlaid fluorescence microscopy of GFP tagged neutrophil activity in wild type larvae with vehicle treatment at 0, 4, and 48 hours past injury. (Bottom) Brightfield overlaid fluorescence microscopy of GFP tagged neutrophil activity in wild type larvae with cortisol treatment at 0, 4 and 48 hours past injury.

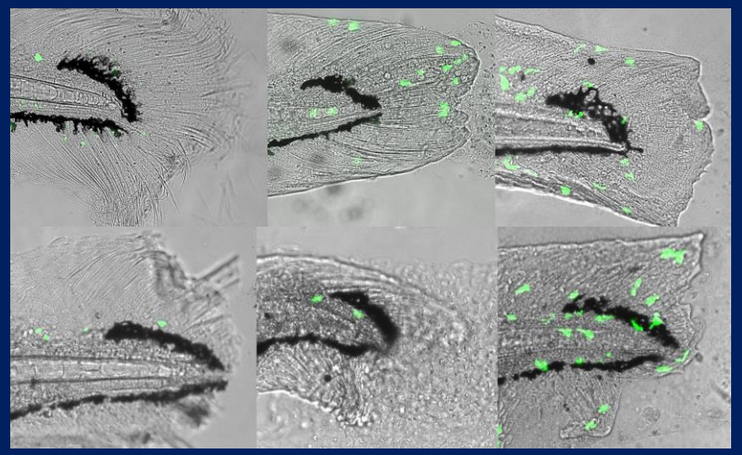

Fig.2 – (Top) Brightfield overlaid fluorescence microscopy of GFP tagged neutrophil activity in Klf9 +/- larvae with vehicle treatment at 0, 4, and 48 hours past injury. (Bottom) Brightfield overlaid fluorescence microscopy of GFP tagged neutrophil activity in Klf9 +/- larvae with cortisol treatment at 0, 4 and 48 hours past injury.

Methods

The three-day old klf9+/-; mpo:GFP larvae were anesthetized with 0.4% tricaine and positioned on their side to allow surgical cutting of the caudal fin. Animals were immobilized with 1% low melt agarose to allow visualization at multiple timepoints. The tail fins were imaged at 0, 4 and 48 hours post injury using brightfield and fluorescent microscopy.

Conclusions

Neutrophil activity did appear to be affected by the lower amount of klf9 in the heterozygotes, but a greater number of larvae would need to be examined to quantify this well enough to demonstrate haploinsufficiency. Further research is needed to understand the role of klf9 in regeneration and the immune system response.

Fig.3 - The MDIBL and SMCC genetics short course participants and instructors.

References

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