Office of Undergraduate Research Summer Undergraduate Research Fellowship 2015 Final Summary: Matrix Metalloproteinase-19 plays a novel role in planarian stem cell dynamics

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Summary

My project is to study the role of matrix metalloproteinases (MMPs) in the regulation of stem cell activity and regeneration in the freshwater flatworm planarian *Schmidtea mediterranea*.

Abstract

MMPs are extracellular proteases that play diverse roles in human disease. Previous studies have focused on their contributions to disease pathogenesis, but little is known about the normal physiological role of MMPs. Planarians are excellent model systems for complete organismal regeneration and accelerated sexual development; thus, these flatworms offer a unique opportunity to study the physiological role of MMPs. Using a combinatorial approach, incorporating RNA interference, *in situ* hybridization, immunostaining, and quantitative RT-PCR, we have established a novel role for the conserved matrix metalloproteinase, MMP-19, in the regulation of planarian stem cell activity.

Introduction

Matrix metalloproteinases (MMPs) are a large family of regulatory enzymes that function in extracellular matrix degradation and thus facilitate a diverse range of cellular activities.

Inappropriate regulation of these proteases can result in a variety of human diseases; most prominently, MMPs play a salient role in cancer cell metastasis. Despite the significant focus on the role of these enzymes in the pathology of human disease, there is a lack of substantial knowledge regarding the physiological role of MMPs.

Planarians (*S. mediterranea*) represent an excellent system to study MMP function and their subsequent mechanism during a variety of dynamic events. These flatworms undergo rapid and complete regeneration and serve as a model for understanding stem cell activity. They have the innate ability to regenerate their whole body from a fragment of the original planarian, and a population of pluripotent stem cells called neoblasts governs this process. Much of the neoblast's ability to replenish tissues as well as maintain appropriate body size depends on the stem cell's proliferative capacity.

Planarian stem cells are surrounded by extracellular matrix (ECM), which comprises the medium through which signaling molecules and bioactive substrates diffuse, forming a fluid communication network between cells. The coordination of appropriate neoblast activity relies on complex signaling cascades, and these signals must sometimes travel great distances to reach their targets. Previous studies have shown that MMPs serve as the main regulatory enzymes within the ECM and facilitate many of these signaling events.

My project focused on a conserved matrix metalloproteinase, MMP-19. In humans, MMP-19 is one of the most widely expressed MMPs and plays a diverse role in the pathology of a number of diseases. Using available functional genomic tools I have worked to elucidate the role of MMP-19 in the planarian.

Results

I used dsRNA-mediated RNA interference (RNAi) to inhibit *mmp-19* expression and thus evaluate the function of MMP-19 under homeostatic conditions. I cloned out the *Smed-mmp-19* gene and generated double-stranded RNA (dsRNA). RNAi knockdown planarians exhibited a marked reduction in size, despite normal nutritional intake. This phenotype is indicative of neoblast dysfunction, suggesting that MMP-19 may be required for regulating signals involved in stem cell proliferation.

The planarian undergoes two critical proliferative bursts in response to injury with the first peak occurring at approximately six hours following insult. To assess whether RNAi knockdown affected stem cell proliferation, I injured *mmp19(RNAi)* animals, waited six hours for the animals to reach the first burst, then used an antibody against phospho-histone H3 to detect for actively dividing cells. My results showed that there was a 30% decrease in the number of mitosing cells in the knockdown animals as compared to the controls, which implied that *mmp-19(RNAi*) attenuated the critical proliferative response that the planarian normally undergoes in response to injury.

To observe where the transcript is expressed in the planarian, I made riboprobes for whole-mount colorimetric in situ hybridization (ISH). I found that *mmp-19* mRNA localizes to a specific subset of muscle cells, which I confirmed by following up with fluorescent ISH on single cell dissociations of planarians. These muscle cells extend dorsoventrally and sit in close proximity to large populations of planarian stem cells, providing further evidence for the hypothesis that MMP-19 influences stem cell activity.

I investigated the role of MMP-19 during regeneration by amputating the tails of planarians and assaying relative *mmp-19* expression levels using real-time quantitative

PCR (RT-qPCR). In addition, I used ISH to track changes in expression of *mmp-19* throughout the course of regeneration. My RT-qPCR results indicated that *mmp-19* mRNA levels were upregulated in the budding blastema of the animal, which was supported by ISH. This indicated that MMP-19 could play a novel role in regeneration.

Most recently, I have successfully developed an antibody that recognizes planarian MMP-19. To do this, I cloned out and expressed *mmp-19*. By affinity chromatography I purified the recombinant protein to be used for antibody development. After receiving highly specific polyclonal antibodies to MMP-19, I performed immunostaining on whole planarians and, again, I saw that MMP-19 is expressed in planarian muscle fibers.

To confirm specificity of the MMP-19 antibody, I used RNAi to knockdown *mmp-19* expression in the planarian and performed fluorescent in situ hybridization in combination with immunostaining on the *mmp19(RNAi)* animals. By confocal microscopy, I observed loss of both the *mmp-19* mRNA and MMP-19 protein as compared to the control animals. This confirms both the specificity of the MMP-19 antibody and the penetrance of the RNAi.

My work on this project has suggested that MMP-19 plays a novel role in the plasticity of these unique flatworms. This work is currently being written up as a first-author paper and a Senior Thesis to be submitted upon graduation from the University of Illinois.