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Summer 2010 Internship Report

Researching the Tumorigenesis Effects Caused By the Genetic Ablation of α-v Integrins

During my summer long internship, I focused on the role the α-v integrin has on cell proliferation and, if mutated or deleted, tumor progression. Integrins are part of the family of heterodimerix transmembrane proteins that serve as receptors, which mediate cell-to-cell and cell-to-matrix interactions. It has been previously discovered that the α-v integrin plays some sort of role in regulating epithelial cell proliferation and homeostasis and helps activate other proteins such as transcription growth factor beta (TGF- β). The TGF- β is a latent transcription factor that is known to be both an oncogene and a tumor suppressor gene, meaning it helps suppress tumors when expressed one way but also assists in tumors growth when expressed by other genes.

In normal cells, one way the TGF- β prevents uncontrolled cell proliferation is by activating SMAD 2/3 proteins by phosphorylation. SMAD 2/3 proteins, once phosphorylated, are able to dissociate with their receptor and bind to SMAD, making a polymer, SMAD 4. Once this SMAD 4 polymer is formed, it is able to enter into the nucleus and recruit other gene regulatory proteins and activate transcription of a specific target gene that prevents uncontrolled cell proliferation. SMAD proteins are therefore transcription factors that behave as mediators of TGF-β signaling and act as tumor suppressor genes that prevent tumorigensis or formation of tumors. When TGF- β becomes mutated or loses the phenotype of tumor suppression, it becomes an oncogene. Rather than restricting cell growth, differentiation and cell death, the mutated TGF-B assists in cell proliferation, which in turn create tumors. The question remains of what

specific role the α-v integrin plays in regulating TGF- β and therefore in suppressing uncontrolled epithelial cell growth and tumorigenesis which in turn suppresses cancer. Throughout this summer I focused on trying to better understand the complicated relationship that α-v integrins have with TGF- β and its effect on SMAD to prevent uncontrolled cell growth. In that, I focused on how the cell and the signaling events that are downstream of the α-v integrin are affected when the a-v integrin is removed from the cell.

To study this relationship between the α-v integrin and the cells, several procedures were preformed throughout the summer. These procedures include Western Blot, Immunohistochemistry (IHC), Quantitative Real Time Polymerase Chain Reaction (q-rtPCR), cell lysing, and cultivation, care, and uptake of the cultured mouse mammary tumor epithelial cells. The cells grown throughout the summer were from the mouse model created by Dr. Lively during her sabbatical at MIT. This mouse model consisted of mammary specific MMTV-Polyoma middle T antigen transgenic mice crossed with α-v floxed mice and MMTV-cre. From these mouse models, cells were extracted and grown in vitro which allowed us to study the biochemistry of the cell more easily. The cells were then treated with Cre-Recombinase Adenovirus, which deleted the α-v integrin making the cells α-v integrin null ($\alpha\text{-v}^{-/-}$), specifically in the mammary epithelium. Throughout the summer I cared for the cells. This involved 'feeding' the cells regularly with fresh media and washing the cells with phosphate buffer saline (PBS) to remove any debris or dead cells. As the cells grew, they needed to be monitored daily so that cell number could be observed and when the desired density was achieved, the cells could be trypsinized and either split into another flask or frozen down. Before splitting could

occur, flasks had be coated with collagen combined with an acetic acid and water mixture so that the cells can attach to the coating once they are transferred.

One analytical technique that was preformed this summer to determine the function that α-v integrins play in tumorigenesis is a Western Blot. During a Western Blot, null and wild type tissue samples were tested to detect specific proteins. In this case, we tested for phosphorylated-SMAD (phospho-SMAD) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). By testing for phospho-SMAD, we were able to observe whether TGF-B was present and functioning inside the null or wild type cells because the amount of phospho-SMAD can be correlated to the amount of TGF-B. By comparing tissue samples that either had the α-v integrin functioning (*wt*) or had the α-v integrin deleted (null), I was able to deduce whether the α-v integrin had a role in managing TGF-B. GAPDH is an enzyme located in every cell type that catalyzes one of the steps in glycolysis to break down glucose for cellular energy. Therefore GAPDH served as a control in that it would be located in every tissue sample used, whether null or wild type, and could ensure that all samples were loaded in the same concentration.

Another procedure I conducted during the summer is IHC. IHC is a method to detect the localization of specific expressed proteins on a sample of tissue by staining through an antigen-antibody interaction. By staining both wild type and null tissue samples with phospho-SMAD, I was able to detect the presence of TGF-B, if any, and also where the TGF-B was located on the tissue sample.

One of my favorite procedures, partially because I was able to see the steps that it takes to lead up to this experiment is quantitative real-time PCR (q-rtPCR). Before I could even begin to run rtPCR, I had to isolate the RNA from tissue samples. This was

done in the beginning of the summer when I worked at MIT with Dr. Lively. Once the RNA was isolated, it was transported back to Sewanee. Yet before the rtPCR could be run, I had to calculate the annealing temperatures and sizes of the primers that would be used during the rtPCR. Once this was done, the rtPCR could than be preformed by converting the RNA into, first, cDNA and than into DNA. By using specific primers, I was able to amplify and quantify particular sections of the DNA molecules. In this case my primers were specific to the DNA sequences of beta molecules that are involved with the signaling cascade of the α-v integrin and TGF-B. Therefore I could detect the amount of gene expression or how much mRNA transcript was made for α-v integrin and TGF-B in the RNA tested. This was one of the many experiments where I was able to understand the amount of research and the numerous tasks that first must be completed before the experiment can even begin

This realization of the amount of detail and planning that takes place before an experiment even starts was one of the most important lessons I learned this summer. In several of the experiments described in this report, calculations had to be done to determine the amount of a reagent needed, or cells had to be grown in advance to a specific cell count. Whichever experiment was conducted it involved not only extensive planning but also collaboration with my lab partner, Luca, and with Dr. Lively. Yet perhaps the most important lesson I learned is that working in the lab is not necessary an isolated job with no interaction with other researchers. Although I preformed experiments alone at times I received direction and advice not only from Dr. Lively but also from other professors or from other researchers from MIT. In addition, from my

time at MIT, I realized that the lab is a friendly place and although everyone may have his or her own specific research, everyone is willing to assist everyone else.

In all, the Yeatman Fellowship allowed me to further develop my lab skills and knowledge that will be beneficial for me in future endeavors. I was able to form a better understanding of how a lab operates and appreciate the work commitment involved in an experiment of this caliber. Throughout the summer I either worked on my own or with my lab partner. Through this I learned independence and personal motivation when on my own, but also teamwork and how to collaborate on a project when working with Luca. In either instance, I was able to learn specific techniques and procedures that are used in examining the biochemistry of the cell. Most importantly, this opportunity allowed me to realize that I truly do enjoy working in the lab. Although we made advancements in Dr. Lively research, these projects are still ongoing and I plan to continue to work on these projects throughout the school year until the results can become published.