Final Internship Report

This summer I had an amazing opportunity to work hands-on in the biology lab on Sewanee's campus. I worked for Dr. Lively in collaboration with Stephanie Lussier, continuing some of Lively's research in cancer biology.

Dr. Lively uses mouse mammary cells that have been extracted from genetically modified mice that were bred at MIT. These mice have been altered so that they are more prone to developing breast cancer tumors, which makes it easier to see the tumor progression and its effects in a shorter time period. Half of the mice have also had the gene that encodes for their alpha – v integrin removed. These mice cells are referred to as the null cells. The null cells are thought to have accelerated tumor development and growth because without the alpha-v integrin, we hypothesized that they would have an increase in vasculature, a key factor in tumor growth and development. The effect of the lack of alpha v integrin on the protein VEGF and its receptor protein Flk-1, along with the signaling pathway that these two proteins control, is what we think causes this increase in vasculature.

My typical day included feeding and maintaining our cell cultures and observing them under the microscope to ensure their vitality and check their progress. To feed them, we would aspirate out the old cell media from the flask and add new cell media – the cells were attached to the bottom of the flask and therefore did not get sucked out with the old media. We had to feed these cells every 4 days or so, as well as splitting them occasionally when they had populated so much that they were running out of room in their cell culture flasks. Splitting just means that you take an existing flask's population, remove the cells from the surface by trypsinizing them, and then divide the cell solution into two new cell culture flasks.

One of the issues we encountered was that our cells became contaminated. This occurred consistently throughout the summer. We autoclaved, or sterilized, all of the utensils in the hood and cleaned the incubator, and yet the cells still became contaminated. At this point

we think that we either had a bad batch of plastic storage containers or that our hood apparatus, the area where we culture our cells, was contaminated.

Because of our lack of uncontaminated cells, we had to utilize some previously stored protein, RNA, and tumor slides in order to run various tests for our hypothesis. Our results are somewhat affected by the fact that we used older samples to experiment with, and ideally we will eventually obtain healthy cells and fresh samples with which we can confirm our results. We analyzed protein levels to see how the lack of the alpha-v integrin affected the production of the VEGF protein and the Flk-1 receptor protein and therefore the downstream pathway following this receptor. We studied RNA in order to discover if the protein increase was due to more protein expression of RNA or more copies of RNA being made. We dyed the tumor cross section slides in order to examine how the null cells appear compared to their normal counterpoints as far as the amount of vasculature present in the tumors themselves.

In order to analyze the amount of protein produced, we performed multiple Western Blot analyses. These tests involve a protein assay, where we measured the amount of protein in each sample obtained from a different mouse, and then used that data to properly distribute each sample based on how much total protein we wanted in each well. We then pipetted this exact amount of protein into separate wells on a graduated agarose gel. We put this gel into an apparatus by which we ran an electric current through the gel, separating out the proteins based on their size. We then took this gel, transferred it onto a special kind of membrane using more electric current, and scanned the membrane onto a special scanner that detects proteins and sends the picture to a computer program. We then analyzed these scans based on the intensity of the detected protein to see if our hypothesis was supported by the data. We had several of these experiments because we had to figure out how much protein to initially add to the gel by a process of trial and error, but eventually concluded that the null cells had more protein than the normal cells.

When a protein is being expressed more, the root cause can be one of two things. RNA codes for proteins, and this RNA is a template of sorts of the cell's DNA. In the case of proteins, the RNA can either be transcripted more from the DNA, resulting in more actual copies of RNA, which then results in more protein, or the proteins could have been translated more from the RNA, which means the RNA was "read" more times by the cell in order to increase the protein production. Either scenario results in more protein, but the distinction between the two scenarios can potentially help in the determination of what exactly alpha v integrin effects in the cell. QRT –PCR is a technique in which a machine basically counts how much RNA is in a particular sample. In a test tube, we combined an RNA sample, two primers that coded for our gene of interest, a chemical that degraded any contaminating DNA, and then a chemical that turned the RNA into cDNA, a more stable form that the machine can recognize by a dye and then quantify. We ran several QRT –PCR tests in order to quantify the RNA in our samples and compared the results of the null cells versus the normal cells. We eventually found that, as we had predicted, the null cells actually produced more RNA than the normal cells.

Another illustration of the effects of alpha v integrin is in the actual visual characteristics of the tumors themselves. We used immunohistochemistry to dye the cross sections of the tumors and view them under a microscope, looking for any differences in the frequency, size, and/or shape of the blood vessels and other vasculature. This process included several steps where we dipped the slides into various chemicals in order to dye the nuclei of the cells blue and the vessels brown. The slides were then dried and observed under a microscope. This particular microscope is connected to a computer and can take pictures of the slides. An example of our dyed slides of tumor cross sections as seen by the microscope can be seen below, as well as a count of the vasculature for both the null animal's tumors and the normal animal's tumors (Figure 1). As you can see, there are many more brown vessels in the null tumors than in the normal tumors.

Although we did not get as far as we originally hoped due to our cells becoming contaminated, we still learned invaluable lessons about research methods, cancer biology, and the scientific process of experimenting, observing, and reevaluating. Many of these experiments were performed several times, mainly because we did not get the results we expected and had to try different conditions, or we inadvertently made an error, or the test simply did not work. Each time we failed, we had to reevaluate everything that went into that experiment and then try to change it for improved results. This was an exhausting process, but when we were successful, it was that much important. Through this process of trial and error, we also learned more about ourselves and what we might want to pursue as a future career. I learned that although I enjoyed research and the process of scientific inquiry, I am still not completely convinced that this is what I want to do in the future. However, I loved this experience, and I cannot express enough how valuable this opportunity was for me.

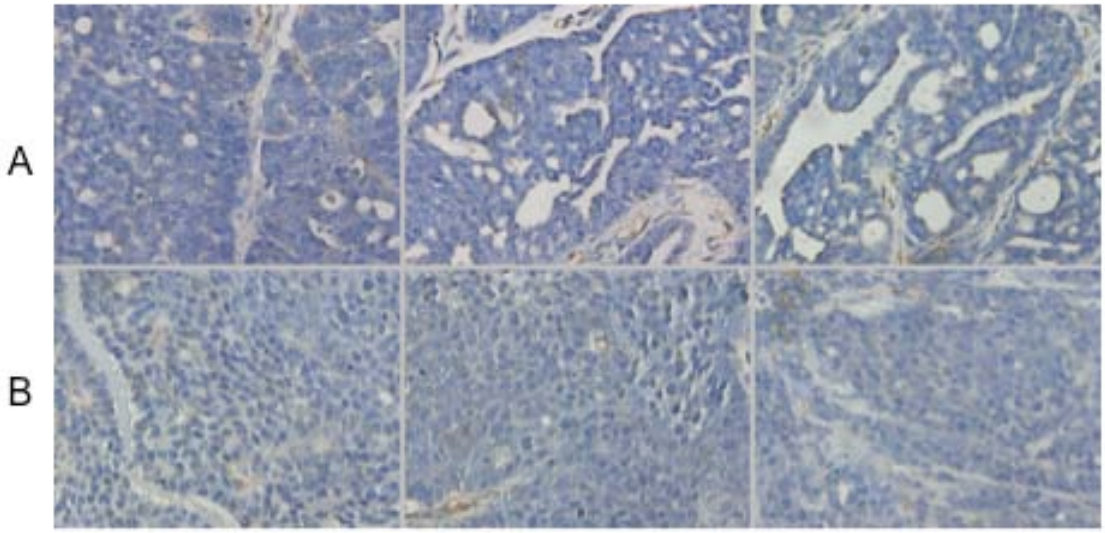

Figure 1. The images in row A are those of an av null tumor. They have 23, 28, and 27 vessels, respectively. The images in row B are those of a wild type tumor. They have 6, 7, and 3 vessels, respectively.