This summer, I interned at the University of the South under the supervision of Dr. Julie Lively. I worked in collaboration with M. Haley Shepherd. It was an amazing experience that allowed me to learn about laboratory skills on basic and specific levels. I studied cancer biology by examining mouse mammary tumor cells in addition to previously isolated RNA and proteins from the cells. I compared the differences between two types of cells and their tendency to produce vasculature.

The cells we cultured over the summer were originally created at MIT laboratories by Dr. Lively. The cells were genetically altered to produce tumors at an accelerated rate, so that they could be more easily studied. These cells were referred to as MMTL cells. In my research, I studied two different cell lines. In one cell line, the cells were normal MMTL cells. The other cell line had been genetically altered to lack the gene that produces alpha-v protein. Alpha-v protein is an integrin that is thought to accelerate tumor cell growth when removed.

One of the first techniques I learned was how to culture cells. It is necessary to have an actively growing culture of cells to use in order to collect data. The first step to growing a cell culture is to thaw previously frozen cells. It is important to thaw cells as quickly as possible to allow their survival. After thawing cells, they were placed on a flask coated with a collagen matrix. Only living cells will adhere to the surface of the flask. They were fed with MMTL media every few days. Once the cells cover approximately 80 percent of the flask, they need to be divided. Trypsin is used to detach the cells from the collagen and then they are divided into new flasks where they will have more time to grow. As the culture enlarges, it may be necessary to freeze down some cells for future use. Contrary to the thawing of cells, cells must be frozen very slowly. Once mastering these cell culture techniques, culturing cells became a daily task in the laboratory.

The main problem that affected our research was the cell culture. There was a vicious outbreak of yeast and bacteria in the laboratory. With ampicillin treatment, the bacteria were easily killed. Yeast, however, were much harder to remove from a cell culture. In most cases it was necessary to throw away the growing cells contaminated with yeast. In all cases, we had to dispose of our cells. This posed a very large problem in the research. Luckily, there were already tissues, RNA and DNA isolated from the cells, so that some experimentation was possible.

Another technique that I acquired through my summer internship was western blotting. A western blot is a technique used to separate protein by molecular weight. It is possible by placing protein at one end of a gel matrix and then running an electrical current through the gel to separate protein fragments by size. The protein bands are then transferred from the gel to a membrane. We were able to find FLK1 and VEGF by using antibodies that highlight their presence in the membrane. The finalized membrane can be scanned and analyzed on the computer. We looked at the presence of FLK1 and VEGF (proteins found in blood cell lines) in the proteins found in the cell and tissue lysates of alpha v null and wild type cells. Mastering this technique was rather difficult and we were unable to have consistent results when comparing alpha v null to normal cells. We found that the information from western blotting was useful, but not as crucial to our research as some of the other techniques.

The red blood vessels were also found by looking at tissue cross sections from the mice. These tissues were taken from MIT by Dr. Lively. By using a staining process known as immunohistochemistry, I was able to find areas of cancerous tissues heavily surrounded by red blood vessels. The results of the immunohistochemistry supported the hypothesis that alpha v null tumors would contain more red blood vessels (Figure 1). Blue staining represents cytoplasm and brown staining represents the outside of blood vessels. It can also be seen that the vessels are wider in alpha v null tumors.

A

B

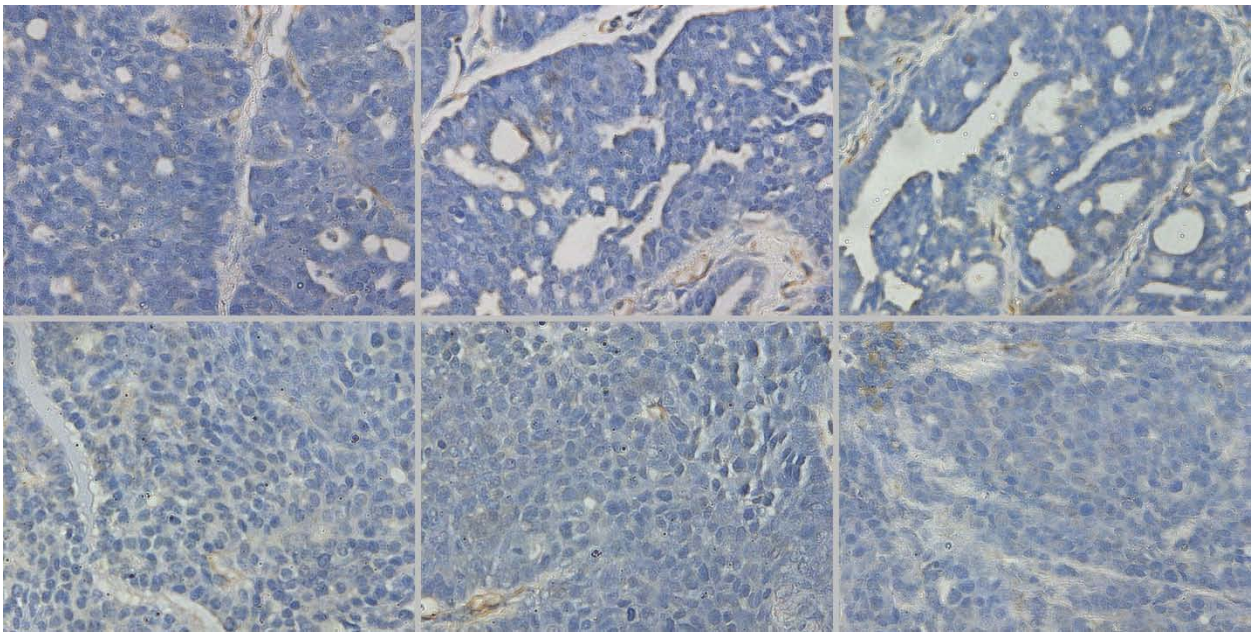

Figure 1. The images in row A are those of an av null tumor. They have 23, 28, and 27 vessels, respectively. The images in row B are those of a wild type tumor. They have 6, 7, and 3 vessels, respectively.

In the end of the summer, we began searching the cells' RNA for coding regions that produce FLK1 and VEGF, which would suggest they are genetically programmed to produce more blood vessels. It is possible to find this information through real time PCR. In real time PCR, we treat the RNA samples with a DNase treatment to remove any excess DNA. Then, a specific primer is used to amplify only regions containing FLK1 or VEGF during a heat cycle. If the amount of DNA/RNA increases, then there are coding regions for FLK1 and VEGF. We expect there to be more FLK1 and VEGF sequences in the DNA of alpha v null cells than

normal MMTL cells. Trials of this have successfully proven our hypothesis valid, but we plan on continuing this area of research this fall.

The research that I conducted this summer was one of the most valuable experiences I have ever had. I was able to work hands on in a laboratory and truly see what it is like to conduct scientific research. I was able to learn so much from Dr. Lively that will continue to help me throughout my career. One day, I hope to be a doctor. I believe this training will help my skills in laboratory classes. I also believe the knowledge I have gained will be useful in interviews. It has inspired me to continue researching this topic to further understand the relationship between alpha v integrin and the expression of vasculature in the cells.