

This summer, I was provided funds by the Yeatman Science Research Internship Fund to work under the guidance of Professor Alyssa Summers in the Biology labs at Sewanee: The University of the South. For seven weeks, during the months of May through July, I performed research on a mouse model project entitled: **Effects of HDAC 3 on the Development of Mammary Glands** in Sewanee, TN. Additionally, during the seven weeks of my internship I was given the opportunity to accompany Professor Summers on a weekly basis to Nashville, TN, to observe and participate in her research project at the Department of Cancer Biology at Vanderbilt University. My time spent at Vanderbilt allowed me to experience not only the environment of a graduate lab, but also to experience a lab that holds a place at the forefront of cancer research innovation in this nation.

The purpose of the project that I worked on this summer was to elucidate the role that the protein histone deacetylase 3, HDAC3, plays during normal mammary gland development. The next phase of research that Professor Summers' lab will conduct is to investigate the effects that HDAC3 has on tumorigenesis within the mammary gland, in order to develop a better understanding of the process of tumor development and to investigate a possible direction for new cancer treatment.

The premise for my research this summer was based on the following:

Research over the last decade has shown that histone deacetylase inhibitors (HDACi) regulate and strongly inhibit breast cancer cell proliferation. HDACi target HDAC (histone deacetylases) and inhibit their function within an organism. Histone deacetylases (HDACs) are proteins that regulate post-translational modifications on histones. Histones are a family of proteins around which DNA is coiled to form the nucleosome, the basic unit of chromatin. Deacetylation of histone proteins condenses chromatin and represses transcription of certain genes (1). Importantly, HDACi are not specific inhibitors and are considered global inhibitors, meaning they target multiple HDAC proteins. Moreover, the role of individual HDACs in cancer is not completely understood. The need to determine the exact effects of individual HDACs, such as

HDAC3, in tumorigenesis is extremely important given the current implications of HDACi usage in patients. HDAC inhibitors are of interest to the medical community because they can trigger cell growth arrest, differentiation, and/or apoptosis and can restrict tumor growth in animal models (2). Additionally, several HDAC inhibitors are currently in clinical trials, treating leukemia and solid tumors (2). However, there are some side effects that more specific targeting of individual HDACs might be able to alleviate.

Furthermore, normal growth within the mammary gland is indicated by the branching and the elongation of the mammary gland ducts, and the potential for growth within the gland is marked by the presence of terminal buds on the ducts. Mammary (breast) cancer can arise from many sources within the gland. There are several different types of mammary (breast) cancer. One type of mammary cancer, Ductal carcinoma, begins in the cells lining within the ducts that bring milk to the nipple (3). Another type, Lobular carcinoma, begins in the milk-secreting glands of the breast, however ultimately it is similar to ductal carcinoma (3). Other varieties of breast cancer can arise from abnormalities of the skin, fat, connective tissues, and other cells present in the breast (3). Ultimately, there is the potential for developing cancer in any of the breast tissues. According to the American Cancer Society it is suspected that one in every eight women will develop breast cancer, in the United States (4). From these numbers it can be seen that it is of the utmost importance for us to discover better treatment methods for breast cancer.

It was my daily responsibility to maintain our mice colony. This consisted of feeding and watering the mice, changing the bedding for their cages, and monitoring the overall health of the mice. This was the first summer that Professor Summer began the mouse model; therefore, I took part in setting up the mouse lab and the mouse model project. Additionally, as mice became available it was my responsibility to genotype the mice using DNA isolation and PCR techniques. This is a crucial procedure used to determine whether a mouse expresses the gene for HDAC3, along with other genes targeted in our study.

The first week of my internship was spent reading prevalent literature, in order to learn more about normal mammary gland development, tumorigenesis within the mammary gland, and common methods and experiments conducted when studying tumorigenesis. Throughout my internship, the literature that I read served as a reference point whenever I had any questions. Familiarizing myself with research literature helped me to understand not only the customary

methods of research, but also helped me to understand the problems faced by the cancer research community today. The second week the mice arrived at Sewanee and I began maintaining the colony and determining the genotypes of our mice. In the weeks following, and as mice of the appropriate age and genotype arose, I performed experiments to examine mammary gland development.

In my time working on this study, we examined the normal growth of the mammary gland; we studied the development of the gland at time points 5 weeks, 8 weeks, 9 weeks, and 20 weeks. Additionally, we examined the effects that histone deacetylase 3 (HDAC3) has upon the development of the mammary gland within a mouse of 9 weeks of age (as this was the only viable null that arose during my time at Sewanee). The glands from this null mouse were compared to the glands of mice that express HDAC3 in order to analyze the effects that HDAC3 has on the mammary development. Our experiments consisted of whole mount assays and protein expression assays. In the whole mount assays, ductal architecture (or branching) and lymph node development were examined to determine the level of development that had occurred within each gland. Additionally, the number of terminal buds was examined to determine the potential for further development within the gland at each time point. The protein assays were used to investigate the differences in cell cycle processes within the glands of different ages and genotypes.

Ultimately, our study of normal gland development revealed that the glands of 5-week old mice had very little ductal architecture originating from the lymph node, yet the gland had very prominent terminal buds, indicating the potential for ductal growth. 8-week and 9-week old mice had developed a more extensive ductal architecture. The terminal buds of 8-week old mice were still prominent; however, the 9-week old glands had fewer terminal buds than the 8-week

old mice. The 20-week old gland had extensive branching, forming a ductal architecture that was fully developed; however, the 20-week old gland lacked terminal buds on the ducts, indicating that the gland had completed its development. The results gathered this summer, for normal development within the gland, will be used in future research for studying gland growth without the presence of HDAC3.

Furthermore, our results showed that the mouse with the null phenotype (lacking HDAC3) has lymph nodes that are significantly smaller than the average lymph node size. Additionally, the null mouse has no ductal architecture development within the mammary gland. Based on these results, it can be deduced that the lack of HDAC3 in the mammary gland severely stunts the development of the ductal architecture within the gland. Additionally, the lack of HDAC3 has an effect on the development of the lymph nodes within the gland. Ultimately, the elimination of HDAC3 from the gland could reduce the likelihood of developing cancer within the ducts and lymph nodes by influencing the growth of the ducts and the lymph nodes. However, for a young woman, who may one day wish to have children, the implications of lacking ductal architecture affects her ability to feed and nourish any future children. It is necessary to further investigate the effect of the lack of HDAC3 on the gland at older time-points in development, and to investigate the effects that the lack of HDAC3 has on an individual and on tumor development within the gland.

I am extremely grateful for the opportunity provide by this internship to explore the world of cancer research. This summer as significantly helped me determine the course I wish to pursue in the future for my career. Working in the lab at Sewanee and at Vanderbilt has allowed me to pursue my interest in studying cancer, and has helped assure me that I would enjoy a career spent investigating questions that have yet to be answered by science and research. I

learned that contributing in research, especially research that is innovative and relevant to the medical world today, is exciting and thrilling. Research is a frontier unlike any other, where a scientist is constantly probing the unknown, searching for answers. Furthermore, this summer has sparked my interest in pursuing a career in medicine, treating patients. I hope to continue to work with Professor Summers on this mouse model project, and I hope to further the progress made in elucidating the function that HDAC3 performs in tumorigenesis.

Literature Cited:

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