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This summer I had the chance to work in the Sewanee biology lab for ten weeks under the supervision of Dr. John Palisano, continuing research that he had previously started. My internship was a memorable experience that taught me not only basic laboratory skills but also many advanced applications of those skills. Along with Dr. Palisano, I used the immortal cervical cancer HeLa cell line to study confronting cisternae, organelles of debated origin which are observed in close association with the nuclear envelope in many rapidly proliferating cells, including cancer cells.

There are two existing hypotheses regarding the origin of confronting cisternae in rapidly proliferating cells: one suggests that confronting cisternae arise from the stacking of endoplasmic reticulum cisternae; the other suggests that the confronting cisternae arise from the nuclear envelope. Dr. Palisano suggests that confronting cisternae are an intermediate stage between the nuclear envelope and the vesicles that arise from the breakdown of the nuclear envelope during mitosis. In rapidly proliferating cells, nuclear envelope degradation does not occur as quickly as mitosis occurs[1]; consequently, the NE folds back upon itself as it begins to break up and takes the appearance of the stacked cisternae referred to as confronting cisternae. As the nuclear envelope does during routine mitosis in normal cells, the confronting cisternae eventually break down into vesicles; these vesicles are then used as a source for rebuilding the nuclear envelope of daughter cells via association with complexes of the nuclear envelope-specific proteins chromatin and lamin, which are thought to initiate nuclear envelope formation[2]. Further microscopic studies show that rapidly dividing fetal cells lose the ability to express confronting cisternae once their rapid proliferative stage ceases, supporting their nuclear envelope origins[3].

During my internship, in order to investigate the hypothesis that confronting cisternae arise from the breakdown of nuclear envelope in rapidly proliferating tumor and select fetal cells, I labeled the nuclear envelope-specific protein lamin B in cancerous HeLa cells with monoclonal antibody raised to lamin B, followed by a secondary antibody conjugated to the flourochrome rhodamine, which fluoresces red when the cells are exposed to UV light. However, the nuclear envelope cannot be seen using light microscopy and rhodamine alone; to visualize membranes, light microscopy must be correlated with electron microscopy of HeLa cells labeled with quantum dots---electron-dense, fluorescent semiconductor nanocrystals that fluoresce a specific color depending on the particle size of the nanocrystal ---a process which allows the nuclear envelope and the presence or absence of the NE-specific protein lamin B in confronting cisternae to be observed.

Thus, my experimental goal this summer was to label lamin B in HeLa cells with secondary antibody conjugated to a specific wavelength quantum dot, a procedure that no one has successfully done because lamin B is found on the inner aspect of the nuclear envelope, and an effective method has not been devised to get quantum dots into the inner aspect of the nuclear envelope. This quantum dot labeling would mark the presence or absence of lamin B in the confronting cisternae; since lamin B is a nuclear envelope-specific protein, the presence of lamin B in the confronting cisternae, shown by the presence of the chosen quantum dot color and its correlation to light microscopy results, would support Dr. Palisano's hypothesis that they are the breakdown product of the nuclear envelope, while the absence of fluorescence would indicate that confronting cisternae do not originate from the nuclear envelope.

Over the ten weeks that I spent in the biology lab, I learned four major skills. First, I learned how to culture healthy HeLa cells for use in my immunostaining experiments. This consisted of feeding and splitting the cells on a regular basis. The cells were fed if needed by

aspirating old media from the cell culture flasks and immediately adding new media. When the cells in a particular culture flask became too crowded or confluent, I would split the cells, removing a specific amount of cells from the overcrowded culture flask and adding them to a new culture flask. Trypsin was used to remove the cells from the flask surface to which they were adhering so some cells could be used to continue the cell line in a new, non-confluent flask.

Second, I learned immunostaining, labeling desired cellular components with fluorescent particles using monoclonal and secondary antibodies. This procedure allowed me to stain and visualize the nuclear envelope of the HeLa cells under UV light. This nuclear envelope staining was performed over the course of five hours and consisted of labeling the nuclear envelope-specific protein lamin B with a secondary antibody associated with the flourochrome rhodamine. Later, Dr. Palisano and I developed a procedure through which we were able to label lamin B with the desired quantum dots. This procedure involved the use of a buffer containing potassium chloride and magnesium chloride, which permeabilized the nuclear envelope enough to allow quantum dots to pass through it.

Closely associated with immunostaining is the third skill I had the privilege to learn: using the two light microscopes available in the Sewanee biology lab. Although at first I had many problems on the Zeiss microscope capturing photographs of the immunostained cells, I eventually learned to use the microscope well after many hours of trial and error with the photographic software. This skill will be useful for both the rest of my research and my future classes here at Sewanee.

Finally, the fourth and most valuable skill that I learned is how to properly search for and extract information from scientific articles. At the beginning of the summer, scientific articles seemed all

the same to me: long, confusing jumbles of large and intimidating words. Now, after an entire summer, I can easily breeze through the mazes of available articles, picking and choosing which articles are most beneficial to me.

Being a part of the summer research program at Sewanee was an amazing experience. Although I do not want to go into professional research, I have learned skills that I can hopefully one day take with me into the medical profession.