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B.S. Health Sciences with a Pre-medical Studies Designation
Examining Protein Expression as a Biomarker in Cancer-associated Fibroblasts Activated by HPV Negative Cervical Cancer Cell Exosomes
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Cervical cancer is diagnosed when the cells of the cervix proliferate abnormally, uncontrollably and infiltrate neighboring tissues to metastasize to other organs. Only about a decade ago, cervical cancer was one of the leading causes of deaths-by-cancer in American women. According to the American Cancer Society, it is estimated that there will be about 13,170 new cases of invasive cervical cancer in 2019 and about 4,250 women will die from it, with around 10% of those accounts being HPV negative.[1] Although most cases of cervical cancer are HPV positive, a rarer form of cervical cancer can be triggered by HPV negative cervical cells, as those cells exist in every type of cervical cancer and have the potential to become malignant. However, not only is HPV Negative Cervical Cancer (HNCC) undetectable to the standard screening methods of Pap Smear and HPV tests, but also, HNCC is under-recognized and under-reported.[2] Thus, many aspects of HNCC are unclear such as the molecular mechanisms that are involved in the development of HNCC.

This research endeavor will focus on the interaction between HNCC cell exosomes (from C-33A and DoTc2 4510 cell lines) and normal human fibroblasts cells (MRC-5 cell line) that could help explain how those exosomes activate cancer-associated fibroblasts (CAFs), which are said to promote tumor cell proliferation, by examining the expression of proteins. Because in the central dogma of biology, the genes in the DNA become translated into RNA and transcribed into protein, so the proteins are the functional factors that determine the final genetic characteristics and behavior of the individual, as the expression of a gene does not necessarily mean that a protein is expressed.

It was hypothesized that: If exosomes of the HPV negative cervical cancer cells C-33A and DoTc2 4510 are used to treat the normal human fibroblast cells MRC-5, then the MRC-5 cells will become CAFs, because there will be an increased expression in the CAFs of at least one of the three proteins used as a CAF marker, whether it be alpha-smooth muscle actin (αSMA), fibroblast activation protein (FAP) or fibroblast specific protein (SV100A4).

After multiple Western Blots and a Flow Cytometry reading of cell lysates with purified proteins of MRC-5 cells that have been treated with the C-33A and DoTc2 4510 exosomes, the outcomes of my research was that CAFs were initiated. The results suggest that the hypothesis was supported because, not only does this provide insight on the microenvironment of HNCC, but also, the Western Blots produced both qualitative data with the distinct protein bands and quantitative data to show that the expression of aSMA and FAP in MRC-5 cells increased over a

[1] https://www.cancer.org/cancer/cervical-cancer/about/key-statistics.html

[2] http://medicalrepublic.com.au/know-hpv-negative-cervical-cancer/5616

span of 72 hours, as well as, the Flow Cytometry displayed that the cell cycle of the MRC-5 cells remained normal even after the exosome treatments.

The projected future aspects to study are conducting a proliferation assay of the MRC-5 cells, investigating a third protein called Fibroblast Specific Protein, obtaining more data to calculate the statistical significance, and publication. I was able to conduct this research with the help of the University of New Haven's SURF Program Coordinators, Mr. McHale's generous donation, and Dr. Zito.

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