The Evaluation of Interferon-beta Levels in HPV-positive Cervical Cancer Cell Lines

Olivia M. Ford and Jennifer T. Thomas, Ph.D.

Human Papillomavirus (HPV) is the most common viral sexually transmitted disease in the United States, with over forty different types known to cause infection in the genital areas of both males and females. Previous studies have shown that the presence of IRF-3, a transcription factor that regulates anti-viral proteins, is reduced in HPV-positive cervical cancer cell lines. Our study is examining a downstream target of IRF-3, Interferon-beta. In an effort to see the effect of HPV on the presence of Interferon-beta, we grew HPV-positive and HPV-negative cervical cancer cell lines. We then performed an ELISA and looked at the levels of Interferon-beta in the supernatant. We found that the HPV-positive cell lines showed decreased levels of Interferon-beta, indicating that the presence of HPV can lead to suppressed anti-viral responses, and, potentially, carcinogenesis.

INTRODUCTION

Cancer

Cancer is a term used for a disease of the cells in which there is uncontrolled cell growth resulting in malignant tumors (NIH - MedlinePlus, 2014). Tumors can be benign or malignant. If they are malignant, they become harmful to the tissues surrounding the tumor, which can result in metastasis, or the spread of cancer from one part of the body to another (NIH - MedlinePlus, 2014). There are over one hundred different types of cancer, which, according to the CDC, is the second leading cause of death in the United States (CDC - Gynecologic Cancers, 2014).

Among these types of cancers is cervical cancer. Cervical cancer, like most other cancers, is named after the place of origin of the cancer; therefore, cervical cancer originates in the cervix. The cervix is the narrow, lower end of the uterus. It connects the uterus to the vagina. Cervical cancer is most commonly found in women over thirty and, in ninety-nine percent of cases, is associated with Human Papillomavirus (HPV) (Yao, 2013). HPV is the most common sexually transmitted disease for both males and females, although it does not always result in cancer.

Human Papillomavirus

Human Papillomavirus is the most common sexually transmitted viral infection. According to the CDC, nearly all people who are sexually active get HPV in their lifetime, but it does not always exhibit symptoms or lead to cancer; HPV can lay dormant in a person’s system for years (CDC - Gynecologic Cancers, 2014). There are over one hundred known types of human papillomavirus. The most common symptoms are warts, usually benign, in the genital area. These warts are also known as papillomas, giving the disease its name. The types of HPV with papilloma are known as non-oncogenic, or non-cancerous (NIH - National Institute of Allergy
and Infectious Diseases, 2014). Other types of HPV do not cause genital warts and are associated with specific forms of cancer. These types of HPV are known as oncogenic, or cancerous.

Both men and women can be infected by HPV, as it is passed along through intimate contact. While the most common transmission is oral, vaginal, or anal sex, HPV can also be transmitted through skin-to-skin contact during sexual activities. Human papillomavirus works by infecting epithelial cells. It is not known to spread throughout the body, leading scientists to believe that it never actually enters the bloodstream (Tluckova, 2013). Once the infection enters the cell, it produces viral proteins. When the infection is oncogenic, these viral proteins start to affect the functions of the cell causing it to reproduce at an uncontrollable rate. Most of the time, the immune system will be able to eliminate the infected cell and clear the body of the virus, but in some cases the immune system is not successful and the infection continues (Hawkins, 2013).

**Interferon-beta**

A major component of the body’s immune response is interferons. The National Center for Biotechnology Information (NCBI) describes the interferons as anti-viral proteins that are crucial in the body’s ability to clear a virus (Dunn, Koebel, and Schreiber, 2006). Type I Interferon is secreted by virus infected cells. Interferons interact with specific cellular receptors, which promote production of second messengers ultimately leading to expression of antiviral genes (NCBI, 2014). Two subcategories of Type I Interferon are Interferon-alpha and Interferon-beta. The main difference between these two subcategories is the second receptors that they are associated with.

There have been previous studies that have focused on the levels of IRF-3 in HPV infected cells. They found that in these cells, there were decreased levels of IRF-3 indicating that HPV is able to affect the anti-viral immune response by reducing the presence of interferons, causing a less effective anti-viral state (Everhart, 2010). Another study was then conducted to examine the impact of HPV on the expression of Type I Interferons. The antibody used in the western blot was not able to clearly show the difference between HeLa cell lines and C33A cell lines causing the results to be insignificant (LeBlanc, 2014). Given the outcome, we wanted to test the Interferon-alpha and Interferon-beta separately, focusing on Interferon-beta. We thought this to be important in order to see how exactly HPV works to evade the immune response. The more scientists know about the virus and how it works, the better scientists will be able to treat and prevent the spread of it all together. Because Interferon-beta is a downstream target of IRF-3, we hypothesized that there would be lower levels of Interferon-beta in HPV infected cells lines, indicating the role of HPV in suppressing anti-viral immune response.

**MATERIALS AND METHODS**

**Cell Culture**

Cell lines were derived from cervical cancer epithelial tissue, HeLa cell lines and C33A cell lines. They were grown in the tissue culture laboratory at Belmont University and obtained by
thawing out previously frozen cells purchased from American Type Culture Collection (ATCC, Manassas, VA). The HeLa cell lines have HPV-positive cervical cancer cells, while the C33A cell lines have HPV-negative cancer cells. These lines were chosen because they are immortal and only differ in the presence of HPV, which made them ideal for this experiment. Once thawed, the cells were aseptically transferred to tissue culture treated petri plates containing 10 mL of EMEM (Eagle’s Minimum Essential Media) (Life Technologies Corporation, Grand Island, NY) enhanced with 10% fetal bovine serum and 0.5% gentamycin antibiotic (Life Technologies Corporation, Grand Island, NY). The goal of the media was to provide all the essential organic molecules needed for proper growth in order to simulate a real life scenario. We used gentamycin antibiotic to ensure the absence of any bacterial contamination. The two cell lines, HeLa and C33A, were then incubated at 37°C with 5% CO₂ in order to promote cell proliferation.

When the cells reached 70-80% confluency, we split them by first aspirating the media. We washed the media with 10 mL of Phosphate Buffered Saline (PBS) (Life Technologies Corporation, Grand Island, NY) and then broke the cell adhesion to the plate by adding 2 mL of 0.05% trypsin EDTA (Life Technologies Corporation, Grand Island, NY). Once the cells were no longer adherent, we pipetted the 2 mL of trypsin (Life Technologies Corporation, Grand Island, NY) and cells into a new tissue culture petri plate containing 10 mL of EMEM (Eagle’s Minimum Essential Media) (Life Technologies Corporation, Grand Island, NY). They were then incubated at 37°C with 5% CO₂ in order to promote cell proliferation.

http://www.isogen-lifescience.com/cancer-antigen-elisa-kits

**Fig.1** Step by step guide to using an ELISA

**ELISA - enzyme-linked immunosorbent assay**

When we were ready to run the ELISA we harvested the media. We pipetted the media from the plate with HeLa cell lines and the plate with C33A cell lines into their own test tubes (ATCC, Manassas, VA). They were then centrifuged at 1050 rpm at 4°C for four minutes in order to expel any extra cells from the media which might interfere with the ELISA.
Once the cells had been harvested and centrifuged, it was time to use the ELISA kit (Pestka Biomedical Laboratories, Piscataway, NJ). The Enzyme-Linked Immunosorbent Assay (ELISA) is a technique used to detect antibodies or infectious agents in a sample (BioBest Laboratories, 2014). In order to test for Interferon-beta, we put the antigens on the plastic surface and added samples to the ninety-six welled plate as shown in Figure 1. We then added a second antibody that contained a marker. With a positive reaction, the solution was expected to change color, indicating the presence of Interferon-beta. We then ran the samples through a spectrophotometer in order to get their absorbance readings.

RESULTS

After eight runs of the ELISA, our preliminary results suggest that IFN-beta levels are reduced in HPV positive HeLa cell lines compared to HPV negative C33A cell lines, as shown in Figure 3, agreeing with our original hypothesis. Before running our samples through the spectrophotometer we were able to see a slight color change, but the absorbance readings were what we used for our calculations. We did a cell count and used those results along with the absorbance readings to find the pictogram per cell, which are the numbers represented on the bar graph in Figure 3.

![IFN-beta ELISA](image)

**Fig. 2** This bar graph compares the pictograms of Interferon-beta per cell for C33A and HeLa.

DISCUSSION

HPV is the most common sexually transmitted viral infection and works by evading the immune response long enough to cause infection. In order to see exactly how it is able to evade the immune response, we performed an ELISA to evaluate the levels of Interferon-beta in HPV infected cervical cancer cell lines, in this case HeLa cells. We expected that Interferon-beta levels would be decreased in the HPV positive cervical cancer cell lines. The experiment was a learning experience and there was some troubleshooting that took place throughout the duration of the experiment in order to produce a reliable standard curve. We tried incubating the media for eighteen hours and then 24 hours, but neither yielded significant results. We also tried letting
the supernatant stand at room temperature for 15 minutes rather than centrifuging it at 4°C. That too yielded no significant results. The only solution that proved to work was when we decreased the amount of media from 10 mL to 5 mL, which gave us amore concentrated supernatant with which to run the ELISA. Eventually we were able to obtain some results in the form of trends.

When looking at the preliminary results, we can conclude that HPV does have a negative impact on the production of proteins essential for viral clearance. Unfortunately, due to the fact that we only ran the assays in duplicate instead of triplicate or more, we were unable to run a statistical analysis. Had we run a statistical analysis, we would have used a simple t-test and been able to see standard deviation and standard error, which would have showed us if our results were statistically significant. While we did not run the analysis, we did see trends that were consistent enough to report our findings. The trends were consistent, though it would still be beneficial to repeat this experiment and run it in triplicate in order to run that statistical analysis.

After running the test again in triplicate and seeing the results, we think it is important to conduct future studies that examine the impact of bacterial and fungal infections on cervical cancer cell lines to see if IFN-beta levels are reduced even further. According to the trends evident in this study, the presence of HPV does inhibit the production of IFN-beta in cervical cancer cell lines, in turn inhibiting the body’s immune response. Studying how and if bacterial and fungal infections further this inhibition is the next clear step in learning the effects of HPV on the body’s immune response. It would also be interesting to see if different strains of HPV have different effects on the IFN-beta levels. Future studies might look into other cervical cancer cell lines that are associated with different strains of HPV.
LITERATURE CITED


