Abstract:

For this project, I will be conducting biological research on Human Papillomavirus (HPV). HPV research is important due to the alarming increase in the rate of infection associated cancers and HPV’s contribution to those numbers. In fact, HPV infection is estimated to cause a third of the 1.5 million cases of these cancers. Specifically, this virus is the leading cause of cervical cancer in women, and is also the cause of many other cancers in men and women. Much about the virus lifecycle is known, however not much is known about the beginning stages and initial mechanisms that the virus uses for infection. The lab’s goal is to determine the process of penetration and intracellular trafficking that leads to HPV infection. My current project is determining the role of Tetraspanins in HPV infection. To achieve my objective, I am observing these proteins using biological research methods such as Western Blot analysis, Flow Cytometric analysis, Immunoflorence, and siRNA-Mediated knockdown. I will be conducting this research at Fordham University in Larkin Hall under the guidance of Dr. Patricio Meneses. I believe the information achieved from this research can potentially be used to develop ways to prevent infection and reduce the burden of cancer and HPV disease.
Project:

My main goal of this research project is to learn more about Human Papillomavirus (HPV) and better understand how it infects the human body. The Human Papillomavirus (HPV) is the most common sexually transmitted infection in the United States. Approximately 20 million Americans are currently infected with HPV and another 6 million acquire HPV every year. In fact, the CDC estimates that close to 50% of all sexually active individuals will contract HPV at one point. Also due to its asymptomatic tendencies, many fail to detect and treat it in time ultimately leading to cervical cancer, penile cancer, genital warts, oropharyngeal cancer or other cancers involving the genitalia of both sexes. However, when symptoms or signs exist, they are presented as genital warts or as warts in the throat.

There are over hundreds of different viral genotypes of HPV, of which more than forty are sexually transmitted. The most common HPV strains known today include 6, 8, 16, and 18. Strains 6 and 8 are associated with condylomata acuminata, also referred to as genital warts. Strains 16 and 18 are tied to high-risk cervical cancers. There are currently two FDA approved vaccines available to prevent HPV infection: Gardasil® and Cervarix. These two vaccines protect against HPV 16 and 18. This project’s contribution is significant because current vaccines targeting HPV 16 and 18 cover 70-80% of the HPV related cancers, and thus are ineffective at preventing a minimum of 150,000 cancer cases worldwide. This number can possibly be even greater due to the lack of cost-effectiveness of the vaccines. These vaccines are too expensive for those in a low socioeconomic status or in underdeveloped nations where incidences of cancers such as cervical cancer are the highest worldwide. HPV related cancers are consistently on a rise
and the inability to prevent infection by the strains unaffected by the current vaccines will certainly have a significant impact. These facts highlight the need for a low cost and broad-acting antiviral.

While it is evident that HPV is the cause for many health issues, not much is known about the beginning stages and initial mechanisms that the virus uses for infection. Those of the lab postulate that HPVs utilize similar and maybe even identical routes of entry into human cells. Thus, determining these common steps will provide the knowledge needed to develop a broad-acting antiviral that will protect people from much more than the couple strains covered by the current vaccines. These HPV interactions with the human body, when identified, can serve as targets for prevention of infection regardless of HPV strain. There are a multitude of factors, which need to be determined to have a complete understanding of the early stages of HPV infection.

Dr. Patricio Meneses of Fordham University is the principal investigator of a research program that is observing different types of HPV. His research deals with the multitude of mechanisms that may be involved in the complex infection process of HPV, and what proteins and molecules are essential for infection. These projects are all divided among the researchers in the lab. Dr. Meneses is an expert in his field with extensive biological research experience and his guidance has been very helpful. He has been an ideal mentor during my project with him.

My project’s objectives were to first determine the expression and localization of tetraspanins CD9 and CD63 in human keratinocytes, the predominant cell type in the human skin and mucous membranes, through western blot analysis and immunofluorescence. These tetraspanins are being studied because they have been
implicated in epithelial cell wound healing, a process that is likely taken advantage of by HPV during infection. CD37 is used as a negative control in IF to determine if signal location is specifically due to the localization of the tetraspanins. CD37 is found only in Leukocytes so in a working confocal microscope there will be no signal of CD37 in keratinocytes. I will then determine the importance of each tetraspanin using siRNA-mediated knockdown. If tetraspanins are critical for HPV infection, knocking down specific tetraspanins by siRNA targeted for those specific tetraspanin genes should decrease or prevent the infection of keratinocytes by HPV. In theory if tetraspanins are critical for HPV infection, knocking down specific tetraspanins by silencing RNA targeted for those specific tetraspanin genes should decrease or prevent the infection in of human keratinocytes, the predominant cell type in the human skin and mucous membranes, by HPV. Silencing RNA knockdown is a biological research technique I will perform that interferes with the expression of the gene that the siRNA targets. Therefore if the gene for a tetraspanin were interfered with, there would be none of that tetraspanin protein in the cell.

So far I have had these results since I started working on this project last year. The expressions of tetraspanins CD9 and CD63 in HaCaT cell lysates were confirmed by Western Blotting analysis. Lysates from uninfected HaCaT cells were ran on an SDS-PAGE and incubated with CD9 and CD63 antibodies. Actin staining was used as a loading control. My data confirms the presence of tetraspanins CD9 and CD63 in HaCaT cells. My next set of results determined the localization of tetraspanins CD9 and CD63 in HaCaT cells. Tetraspanins could be clustered on the plasma membrane and on cytoplasmic vesicles such as those in lysosomes and endosomes. Immunofluorescence
studies were carried out to detect the specific localization of the tetraspanins in the HaCaTs. CD9 was found to be strongly concentrated near the plasma membrane (Figure 1) and according to prior knowledge, the intercellular junctions. CD63, a lysosome glycoprotein, was shown to be distributed evenly in the cytoplasm in small clusters that are possibly vesicles. CD37 acted as the negative control tetraspanin. Further IF studies with phalloidin will allow me to further confirm the localization of these tetraspanins.

I am still performing diligent work on my extensive research project but there are still some steps that need to be completed for the role of tetraspanins CD9 and CD63 in HPV16 infection to be determined. The siRNA Knockdown of the tetraspanins has yet to be completed. Once knockdown of the tetraspanins have been confirmed by a shift in expression level determined by flow cytometric analysis, their respective role in HPV16 infection can be determined. Future directions for this project will also include repeating the experiments already completed in order to obtain more usable data that I can further confident in.

For many viruses, endocytic entry into their host cells occurs in a series of tightly controlled, consecutive steps involving binding to the cell surface, lateral diffusion, signaling, and internalization. In the case of HPV, it is unlikely tetraspanins present a separate entry pathway but they are viable candidates for a possible secondary receptor after the primary attachment to heparan sulfate proteoglycans. Due to the versatile roles of the tetraspanin-enriched microdomains in adhesion, migration, and signaling pathways, it is very possible that they are part of the endocytosis complex necessary for initial HPV infection.
I believe my project on tetraspanins is considerably important in real life application and scholarly significant. Determining the role of tetraspanins in HPV infection can contribute to the knowledge needed for a more cost effective and broad-acting antivirals. A vaccine that is more financially feasible for those too obtain that protects against virtually all HPVs will significantly reduce the continuously increasing rate of HPV related cancers and diseases. I believe the results of my project and those of the rest of Dr. Meneses’ lab have a momentous real life application by improving the health of the world. My contribution to the field virology research has incredible scholarly significance. The results of my project in further understanding how HPV infects cells will likely provide new targets for preventative interventions in HPV and even other infectious agents, sparking further research studies.
Preliminary Bibliography:


**Budget and Budget Justification:**

1. **Financial Stipend**

Time spent in the lab conducting research – Tuesday, Wednesday and Friday- 6 hours each day

Because of the time commitment (18 hours a week), I will not be able to take another job to facilitate monetary income. I will not be able to work at my job at Staples, a job I had to abandon in order to conduct my research, where the hourly wage is 12$/hr. I have already been working on the research since January 3\(^{rd}\) with consistent hours.

   January 3\(^{rd}\) – mid May = 20 weeks x 18 hrs/week = 360 hours

**Total:** 360hrs x $12/hr= $4320

**Total Budget Justification** – Financial Stipend ($4320)

Maximum amount offered for Student Undergraduate Research - $1000

**Amount Considerately Requested-** $1000
**Anticipated Outcomes:**

I have already accomplished so much in the lab since I started last year. Researching with Dr. Meneses, I hope to continue gaining valuable research experience and techniques that will be critical in my goal of obtaining an M.D. and Ph.D. degree. I specifically want the training in both the M.D. and Ph.D. degrees in my plans in becoming a doctor for many reasons. As a physician, I will appreciate and value the relationships I will forge with my patients. The doctor I will become will focus on this doctor-patient relationship, because I believe the most optimal health care will be delivered if this is kept in mind. As a researcher, I want to forge new connections with the laboratory and the clinic by bringing knowledge and new discoveries from the former and applying it in the latter. I think fundamental knowledge from scientific research are important in how medicine moves forward in helping patients across all domains of disease.

I am also excited to present my research at the FCRH Undergraduate Research Symposium and other conferences in order to bring awareness to the problems of HPV and the possibilities research can bring in preventing infection and reducing the burden of cancer and HPV disease. After this semester I will publish my research into a scientific journal. The experience from the lab, conferences that I would attend, and the publication, will be incredibly valuable when I apply to M.D./Ph.D professional schools this year.