Undergraduate Thesis Prospectus

Using the CRY2:CIBN Optogenetics Construct to Model Ovarian Cancer Migration

(technical research project in Biomedical Engineering)

Telemedicine: Improving Cancer Treatment in Underserved Areas

(STS research project)

by

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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General Research Problem

How can cancer treatment be improved in the United States?

2011 marked the point where Baby Boomers, people born between 1946 and 1964, have started reaching retirement age, and this will continue until 2030 ("The Baby Boomer Generation|Baby Boomers are Reaching Retiring Age," 2011). With growing age, comes chronic disease, and as the largest subset of the population, Baby Boomer healthcare will pose an immense toll on the rest of the United States population. One of those chronic diseases is cancer, and Baby Boomers are predicted to drive up cancer incidence. In 2010, people over age 65 had a cancer incidence of a little less than 1 million, but in 2030 the incidence is predicted to be over 1.5 million (*CPR12_Slides_PDF.pdf*, n.d.). Statistically, the older generation has the most of any type of cancer over all age groups, and this will only increase as time goes on (*Cancer-treatment-and-survivorship-facts-and-figures-2019-2021.pdf*, n.d.). With the impending strain on the United States healthcare system, it is important to develop novel ways of treating and detecting cancer in people. Cancer treatment varies not only in how it is treated (drugs, chemotherapy, etc.) but also in how it is implemented across communities, cultures, and societies. Both are extremely important in improving to make cancer care more accessible to everyone and to make the treatments more effective in destroying cancer.

Using the CRY2:CIBN Optogenetics Construct to Model Ovarian Cancer Migration

How can the CRY2:CIBN optogenetics plasmid construct be used to model ovarian cancer migration as a result of PTP4A3 intercellular localization?

This project is being investigated in the biomedical engineering department in conjunction with the Pharmacology department, specifically the Fiske Drug Discovery Research Laboratory.

Ovarian cancer is the fourth most common cause of death for females in the world. The cancer starts from abnormal cells in the ovaries that multiply and form a tumor. It can become metastatic and spread to other parts of the body, typically starting with the stomach. Its high death rate is because of the late stage presentation of the cancer. Currently therapies for ovarian cancer treatment are surgery, chemotherapy, and drug treatments (Jayson, Kohn, Kitchener, & Ledermann, 2014). Drug treatments for cancer usually target specific proteins involved in the pathway of cancer progression, like protein kinases. There are currently, 49 FDA-approved small molecule protein kinase inhibitors, but only six drugs that target phosphatases ("FDA-approved protein kinase inhibitors/US Food and Drug Administration approved small molecule protein kinase inhibitors," n.d.). The lack of research on phosphatases is because they were regarded as "undruggable" due to their conserved structures and reactive catalytic sites, however they can no longer be ignored, as kinases and phosphatases aid in the regulation of tissue homeostasis, so phosphatases should have just as high an importance as kinases ("Phosphatases start shedding their stigma of undruggability | Nature Reviews Drug Discovery," n.d.).

Protein Tyrosine Phosphatases, specifically the overexpression of Protein Tyrosine Phosphatase 4A3 (PTP4A3), are suspected to play a role in the cancer progression pathway. PTP4A3 had elevated levels in 79% of human ovarian cancer samples. It appears to regulate several cancer processes like invasion, migration, and angiogenesis (McQueeney et al., 2017).

Even though PTP4A3 is known to be overexpressed in many cancers, it is difficult to detect its expression using western blot techniques. Concentrating the protein through

immunoprecipitation has also failed in yielding viable results. The problem was hypothesized to be attributed to antibodies that were unable to properly detect PTP4A3. One of the ways to solve this problem is through the lipofectamine transfection of Myc-tagged and GFP-tagged PTP4A3 into knockout murine colorectal cancer cells. The Myc-tagged PTP4A3 yielded better results in western blots because the Myc antibody was more specific than the PTP4A3 antibody. The GFP-tagged PTP4A3 allowed the visualization of PTP4A3 location in the cell. This model works if the researcher is only interested in determining the effects of small molecules on the expression of PTP4A3 within the cell. The optogenetics model will allow researchers to quantify cell migration as a result of PTP4A3 localization to the cell membrane or to the cytoplasm. It is also a reversible method, so one stable cell line can account for localization to the membrane and localization the cytoplasm based on whether blue light is shined on the cells. In a lipofectamine transfection, two stable cell lines would have to be created for each localization area. The optogenetics method uses the proteins CRY2 and CIBN to localize PTP4A3 to the membrane or to the cytoplasm. CIBN and a green fluorescent protein marker will be attached to the membrane, while CRY2, a mCherry marker, and PTP4A3 will be attached together and floating in the membrane. In this situation, the PTP4A3 is localized to the cytoplasm. When blue light is shown over the cells CRY2 will attach to CIBN on the membrane, effectively localizing PTP4A3 at the membrane. Once the blue light is removed the CRY2 and PTP4A3 goes back to the cytoplasm.

The project has two aims. The first is the design and test the optogenetics vector to express the genet of interest, PTP4A3, within ovarian cancer cells. This aim will be accomplished by first creating the optogenetics construct, transfecting it into an ovarian cancer cell line, and using fluorescent microscopy to confirm the success of the transfection. The second

aim is to quantify the migration of these transfected ovarian cancer cells by designing a Transwell insert 3D migration assay. This aim will quantify how PTP4A3 localization influences cell migration.

Until recently, kinases have been the primary focus of pharmacological drug targeting in cancer (Bhullar et. al. 2018). Phosphatases were believed to be "undruggable" because active sites are conserved among many protein tyrosine phosphatases (PTPs) and most active sites are positively charged meaning inhibitors found through screening are negatively charged which have difficulty entering cells (Zhang 2016). However, the precise balance of PTPs and protein tyrosine kinases (PTKs) are better understood now than ever before and the relevance of up- or down-regulated downstream signaling pathways caused by PTPs sheds light on the druggability of PTPs (He et. al 2014). As of 2018, six PTP inhibitors were in clinical trials (Mullard 2018). PTP4A3 is one such protein that has been upregulated in many forms of cancer, including ovarian (McQueeney 2017).

PTP4A3 localizes to the cell membrane after posttranslational prenylation occurs (Zheng et. al 2000). The effects of the importance of this localization have not been studied as the downstream effects are not well understood. PTP4A3 is believed to be involved with extracellular matrix protein secretion and ultimately cell migration and metastasis (McQueeney 2018). Optogenetics has been used to study protein localization downstream of a known signaling pathway (Karunarathne et al. 2015). We will instead use this technique to study cell migration, a phenotypic characteristic, in response to the localization of PTP4A3.

The blue light-activated CRY2:CIBN construct has been used to study the localization of proteins downstream of receptor tyrosine kinases (RTKs) including mitogen-activated protein kinases (MAPKs) (Karunarathne et al. 2015). Optogenetics have been applied to PTPs for the

first time in HEK293T cells to study the effects of Extracellular Signal-regulated Kinase (ERK) translocation in response to increased MAPK phosphatase 3 (MKP3) activity (Courtney & Deiters 2019). We plan to add fluorescent tags onto both CRY2 and CIBN to view protein localization using a spectrophotometer. Implementing a fluorescent CRY2:CIBN complex in an ovarian cell line is a novel method of quantifying protein localization.

Three-dimensional cell migration assays vary across literature and report very different metrics depending on the study. In ovarian cancer, multicell spheroids break from ovarian tumors invade different regions of the body (Habyan et al. 2018). We would like to create a novel assay that occurs in three dimensions in conjunction with fluorescently-tagged CRY2 and CIBN that measures the number of cells that migrate a known distance and the rate at which they travel. One of the standard methods of studying cell migration is utilizing Transwell inserts in tissue-treated plates. Cells migrate through an extracellular matrix and through micropores to the chamber below where they can be counted. There is no instance of using light to induce an optogenetic effect in cells that will affect migration in the literature. We would use the Operetta Imaging System to shine blue light on the cells while simultaneously fluorescing the tagged proteins. There is also a potential to fix and immunostain the cells during and after PTP4A3 membrane localization (Smyrek & Stelzer 2017).

The plasmid that will be used in this proposed study will be designed to express four different proteins. Each segment of expressed DNA will be separated by an Internal Ribosome Entry Site (IRES). This will allow each protein to be expressed on its own without being attached to the others. A viral CMV promoter will also be used for all four proteins. The main proteins are an ampicillin resistance gene, a geneticin resistance gene, the CIBN protein attached to pmGFP, and CRY2 protein attached to PTP4A3 and mCherry. Before transfection, the

plasmid must be amplified in bacteria. The plasmid will be grown on LB agar plates with ampicillin, and slowly scaled up. Bacteria that do not have the plasmid will not be ampicillin resistant and will die, selecting for bacteria containing the plasmid. The plasmid DNA will be collected and separated from the bacteria using the Qiagen Plasmid Mega Kit. A lipofectamine transfection will then be done to put the plasmid into the ovarian cancer cells. Once there are enough cells to for a stable cell line they will be frozen down for future studies.

OVCAR4 cells transfected with the CRY2:CIBN complex as well as PTP4A3 knock-out cells and wild type cells will be added onto a collagen-coated Transwell insert. Sample groups include 1) CRY2:CIBN with blue light, 2) CRY2:CIBN without light, 3) PTP4A3 knock-out with light, 4) PTP4A3 knock-out without light, 5) wild type with light, and 6) wild type without light. After incubation, the Transwell insert will be removed, the media washed out with phosphate buffer saline, and the cells stained with crystal violet for 10 minutes. Cells will be counted from microscopy images using ImageJ and the rate of travel can be measured knowing the thickness of the filter and height from the bottom.

If the cells can be successfully transfected, then the ovarian cancer cell line can be used to determine how multiple variables effect cancer cells. Varying PTP4A3 location can be combined with small molecule inhibitors to determine how both affect various aspects of cancer cells such as migration, viability, colony formation, etc.

Telemedicine: Improving Cancer Treatment in Underserved Areas

What are the effects of telemedicine on cancer diagnosis and treatment in remote and underserved areas?

In 2019, there will be an estimated 1.8 million people who will be diagnosed with cancer and an estimated 60,880 people will die from it (“Common Cancer Sites—Cancer Stat Facts,”

n.d.). Although we have been developed lots of new hospital treatments for cancer a huge issue is access to care. People who live in large cities or near hospital systems are able to reap the benefits of having nearby healthcare facilities, but those who live in remote or underserved areas do not have those same resources. One-fifth of the United States population lives in rural areas, but only one-tenth of all physicians choose to practice in rural areas. A bigger problem is that a majority of physicians who choose rural areas are family medicine doctors and thought they might be helpful for yearly checkups and small problems; they do not have the resources to treat chronic disease like cancer. Oncologists, and other specialty-specific doctors, typically choose to practice in urban areas because there are more educational opportunities and a larger population to treat. However, this leaves a glaring hole in rural healthcare and forces rural residents to travel great distances and pay a lot of money in order to receive treatment ("Challenges of Rural Cancer Care in the United States | Cancer Network," n.d.). Typically, people in underserved and remote areas are socioeconomically disadvantaged and are not able to just leave work to go to a clinic. They have to take huge spans of time off of work in order to go to a hospital, leading to lost wages, travel costs, and childcare expenses ("What is Telemedicine?," n.d.). It is important to implement a method that can break the barriers people in rural community's face so that they can have equal access to healthcare as people in urban areas. One answer is telemedicine.

Telemedicine is a healthcare method that uses electronic communications and software to provide patient services, without needing an in-person appointment. It is a form of remote clinical services that are extremely helpful for those who do not have easy access to healthcare services. Patients get to spend less time away from work, they do not have travel or time expenses, and they are able to better handle their child or elder care responsibilities. There is also the added benefit, even for urban patients, of confidentiality and safety. If the patient-physician

interaction ins one-on-one through teleconferencing, patients will feel more comfortable sharing sensitive information because there is no one around to eavesdrop and pass judgement. Patients are also safer because there is no exposure to potentially contagious patients ("What is Telemedicine?," n.d.). Overall, telemedicine is able to dissolve a majority of the barriers keeping rural patients from getting adequate healthcare.

STS Framework

The STS Framework that will be used in this thesis is the Actor-Network Theory (ANT). ANT is a social theory that considers all the aspects surrounding a technological achievement and how they helped that technology be successful. There was a network of human and non-human actors that helped create this achievement. A great example is Isaac Newton founding the theory of gravitation. This assumes that Newton did this completely by himself, but ANT puts into the perspective that his network of colleagues, past work from previous scientists, his tools, his labs, and culture factors, is what led to this accomplishment, not just him by himself. ANT does not tend to explain why a network exists, but instead focuses on how they formed, how they work, and how they could fall apart ("Actor-Network Theory (ANT)," 2007). ANT was developed by two French scholars, Michel Callon and Bruno Latour. Sociologists deemed the theory as creating material-semiotic networks. For example, a bank is itself a network with many actors within it, but it can also be an actor in a larger scale network. Networks are also delicate structures that require a certain process to be followed constantly or they will dissolve ("Actor-network theory (ANT)—Stswiki," 2016). The success of telemedicine is a network that is extremely dependent on the actors involved in order to make it a viable healthcare method. Telemedicine has three main human actors involved: the patient, a nearby rural doctor, and a clinical specialist at a hospital far away, and sometimes there can be more specialists involved

("How Does Telemedicine Work?," n.d.). The communication between these three actors is what makes up the basis of telemedicine but there are other actors as well. The non-material actors can include the different technology needed to diagnose patients and have internet conference with physicians, health insurance companies to help pay for these services, local clinics, and large hospitals. While the thesis will mainly focus on the telemedicine network, it will also cover how telemedicine will have to adapt to successfully implement itself in different societies, cultures, and even subsets of the population. This brings in the STS framework, the Social Construction of Technology (SCOT). This theory states that just because a technology is deemed the best, if it does not consider societal factors it will not be successful. Telemedicine relies heavily on health and technological literacy. Population health literacy levels, not only change from area to area, but also between age groups. The older generation typically has lower health literacy levels than the younger generation. Although in the United States, basic technological literacy like using computers and phones is extremely high, in other countries that have extremely poverty-stricken areas like India or some parts of Africa, do not have this same benefit. These two factors require telemedicine to introduce a new actor into the network that focuses on educating people in how to properly use and understand the resources related to telemedicine.

Plan for the Thesis

The main body of the thesis will explore the different actors that are involved in making the telemedicine network work. It will talk about each actor and their importance in the network individually as well as how they interact with other actors in the network. It is also important for the thesis to consider actors that could be their own network. For example, three of the actors in the telemedicine network could also be considered their own networks: insurance companies, local clinics, and urban hospitals. In the sections of the thesis about these actors, I will

incorporate information about what makes them successful networks so that they can be actors in the telemedicine network.

The beginning of the thesis will introduce two human actors, the patient and the physician. This will be the platform to introduce the healthcare problem that telemedicine can be used to solve. It will go over the problems stated above which is the reason why this telemedicine network was formed. The problem section will talk about the disparities and barriers in healthcare access that those in remote and underserved communities face and how there is a shortage of physician specialists who choose to practice in those rural communities. It will also focus a little more on the side of the patient than the physician. It will describe the problems that patients face and then how telemedicine affects their well-being specifically.

The next topic will be introducing the rural physician and the urban physician as actors in this network. Physicians who choose to practice in rural areas are typically generalists, meaning they treat the whole person on a surface level instead of delving deep into a specific part of the body. These physicians might be generalists who specialize in rural or community medicine. They have a good grasp on how to handle cultural and societal differences and use patient experiences to bridge the gap between their background and the patient they are treating's background. This is an aspect that may make patients more comfortable trusting and speaking to their local physician, unfortunately, they do not have the same knowledge in treating cancer as an oncologist does. Oncologists, and other specialists, tend to prefer practicing in cities and other urban locations. They pick areas with more opportunities to expand their practices and people to treat. The number of people in rural areas who require specialists is much lower than in urban cities, but that discrepancy reduces rural access to these specialists. While an easy solution to the patient's problem would be to get more specialists to become rural physicians, that is not

practical as there are various reasons why they choose to stay in their preferred environment. Telemedicine was almost a compromise between the patient and physician so that they can both reap benefits while reducing the effort involved in seeking and giving care.

The next actor will be the technology itself. It will talk about the different types of technology involved in cancer telemedicine and how it evolved over time. This is an extremely important actor in the network because without communication or diagnostic technology the network would not even exist. The thesis will then cover local clinics and large hospitals and how they play parts in the telemedicine network. It will cover questions like: how have they had to adapt to using technology as a way to treat remote patients, how are they able to streamline this process to make it as efficient as possible, what are the financial risks and benefits that are taken to be a part of this network, and how does telemedicine overall benefit the clinics/hospitals. The last section about actors will cover the role of health insurance companies. This will not only cover the companies themselves and how they have had to change to include telemedicine into their plans, but also how telemedicine might affect taxpayers and those paying for these plans. Since there is low access to specialist resources, a lot of people in rural communities do not go to see a doctor until their symptoms become severe and then they are forced to go to an emergency room. This actually costs taxpayers far more money than if they were able to have regular visits to their local clinic.

The last part of the thesis will consider SCOT and talk about aspects of telemedicine like health and technological literacy. This will cover how telemedicine will have to adapt to societal and cultural differences.

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