

Investigating the Effect of Changing the Growth Conditions (pH and Salt Concentration) on the HER2 Pathway in Gastric Cancer Cells

Abstract:

Gastric cancer is one of the leading causes of cancer deaths worldwide and is prevalent in East Asian countries. The HER2 protein, which is often overexpressed in gastric and breast cancer, plays an important role in signaling for cell growth and proliferation in breast cancer, but its function in gastric cancer is less characterized. By culturing gastric cancer cells, the HER2 protein pathway can be examined in detail. This project proposes to determine the effects of different pH and salt environments on gastric cell growth, effect on HER2 expression, and signaling pathways in gastric cancer cells. Using flow cytometry, western blot, and microarray analysis, this research aims to identify the role of the HER2 expression and signaling in gastric cancer cells. This information will be useful to better understand the role HER2 protein has in gastric cancer cells.

Personal Statement:

When I was ten years old, I remember reading a letter written in Japanese from my Uncle Tsuguharu, who died from cancer at 27 years old, three months before I was born. Because I arrived so soon after his death and have heard about him throughout my life, I have always felt connected to him. My bilingual uncle, adept in intercultural skills, was the key person in the family who advocated for my Japanese mother to marry my American father. Without my uncle, I would not be here, and so for him I have carried this ambition to find solutions to help people survive cancer.

To learn and advance cancer research in some way, I made choices that will help me become a cancer researcher. My first step was being accepted into the online program of North Carolina School of Science and Mathematics (NCSSM), a highly selective, publicly-funded school in Durham. I studied in the Summer Accelerator program titled "Genetic Analysis of the Signaling Networks in Cancer" prior to my junior year of high school. That was my first exposure to higher-level experiments involving genetics, a curriculum that was acquired from MIT. I earned top grades in both Molecular and Classical Genetics courses through NCSSM during my senior year. In that same year, I also studied in introductory level courses as a dual-enrolled student at Elon. Taking those courses provided me the opportunity to speak to professors about research, since I knew that I wanted to conduct research in college.

My research interest was further inspired by last year's Voices of Discovery speaker, Dr.

Mukherjee, who wrote a book that examined the history to the present field of cancer research. He explained that cancer research is becoming more personalized as scientists are able to observe the specific gene and protein levels in a patient's cells. My goal for my postgraduate education is to be accepted into an MD/PhD program and become a researcher who can directly impact patients. In line with my career goals, I will be participating in a summer research program in the Department of Pharmacology and Cancer Biology at the Duke University School of Medicine. I hope to use the knowledge I gain from the summer experience and apply it to my research at Elon.

The Lumen Prize will aid in carrying out my proposed research as I work to develop my skills to pursue a career as a physician and cancer researcher. I have developed a project that I am excited to start. The Lumen Prize will enable me to be able to purchase cells lines and reagents specific to my project and pay for methods to collect more sophisticated data. The funds will also support attendance and travel to conferences in the United States and Japan. From these conferences, I hope to talk to scientists in this field and become familiar with the most current cancer research. By having these experiences, I want to advance myself as a scholar and develop my skills towards finding solutions in cancer research. With the grounding that the Lumen Prize offers, I would move closer to my ambitions to directly help patients with medical research, and to honor those who were affected by cancer, such as my Uncle Tsuguharu.

Project Description

Focus:

Gastric Cancer

Cancer is characterized by uncontrolled cell growth and division. Gastric cancer is one of the leading forms of cancer-related death in the world and is especially prevalent in Asian countries (Yoshioka et al., 2018). In Japan, gastric cancer is the second-leading cause of cancer death (Cancer Statistics in Japan, 2017). Gastric cancer, similar to other cancers, is caused by a number of factors. It has specifically been linked to higher salt and salted-food consumption (D'Elia et al. 2014). One study found that Japanese immigrants in Hawaii had lower incidence rates of gastric cancer than Japanese people who had remained in Japan; the country associated with an overall higher salt diet (Tsugane et al., 1990). Another potential cause of gastric cancer is *H. pylori*, a species of bacteria known as the highest risk factor for gastric cancer incidences (D'Elia et al., 2014; Wang et al., 2009). *H. pylori* infection rate is associated with higher salt consumption, since salt enables *H. pylori* to thrive (Wang et al., 2009). Since gastric cancer is often related to high salt, investigating the response of cells in high salt environments will reveal the relationship of high salt or differing pH conditions to the growth of cells.

HER2 Protein

Signal communication within cells occur through proteins. Specific proteins in the cell will cause the cells to grow and divide. Investigating the signaling pathways of proteins can reveal how they are involved in these processes. A protein, suggested to be involved, in gastric cancer that I am proposing to study is human epidermal growth factor receptor 2 (HER2). This protein has mainly been associated with tumor formation in gastric and breast cancers (Feizy et al., 2018; Kaufmann et al., 2011). It is part of a protein family that function as cell-signaling mechanisms for cell growth and proliferation (Zhang et al., 2009). Out of the HER family proteins (EGFR, HER2, HER3, and HER4), HER2 is the only protein that does not bind to any known ligand (Zhang et al., 2009). A ligand will bond to a protein and activate the function of the protein. The HER2 protein forms a heterodimer with another member of the HER family; HER2 is the only protein in this family that forms heterodimers, which is a connection between two different proteins to create a larger structure (Oliayioye, 2001; Yoshioka et al., 2018) Only when HER2 forms a heterodimer, can the cell signaling cascade -- the chain of signals from a receptor to the nucleus -- occur and the result is foreboding. When this heterodimer is created, the signaling pathway becomes magnified and increases the cell growth signals, allowing the cancer cells to grow faster (Zhang et al., 2009; Kanayama et al., 2018).

HER2 Protein in Cancer

Research has focused on the links between HER2 and gastric cancer cell growth. One

outcome has been the development of a chemotherapeutic drug, called Herceptin, to inhibit HER2 proteins in gastric and breast cancer (Hu et al., 2012; Hofmann et al., 2008). This is an advancement, but this type of chemotherapy often comes with side effects, such as lung damage and skin reactions (Hansel et al., 2010). Beyond chemotherapy, an area of on-going research is investigating specific relationships between proteins and cancer (Yoshioka et al., 2018). I intend to conduct experiments that would help characterize the role of HER2 protein in gastric cancer cells.

Research Gaps

My project aims to study the influence of HER2 expression in gastric cancer development by investigating the response of cell growth in relation to HER2 expression in varying growth conditions. The HER2 protein has been better evaluated in breast cancer, but it has been found to be a significant indicator for poor prognosis in patients with gastric cancer (Ye et al., 2014). One apparent gap in the literature is the response of HER2 amplified gastric cancer cells to different cellular environments. Although there is an association between regions with high salt diets and increased incidence risk of gastric cancer, little research has been conducted to determine the molecular-level interaction. Most research studies for HER2 in gastric cancer have been conducted on patients. However, I will use gastric cancer cell lines as a model system and alter the cellular environment to measure changes in HER2 expression and cell behavior. This will help determine a link between HER2 amplified gastric cancer and cellular environments.

Scholarly Process:

The proposed research is to test how gastric cells with HER2 protein expression responds to environmental changes and how that may affect cell proliferation. This research will be carried out over four steps and each part involves different experimental techniques and data collection.

Step 1: Cell culturing and HER2 expression on cells

I will first learn sterile cell culture techniques to grow and maintain the NCI-N87 gastric cancer cell line reported to have amplified HER2 expression (Jorgensen, 2014). Flow cytometry will then be used to confirm if HER2 is present on the cells. HER2 must be present in order to continue with experimentation. If the initial gastric cancer cell line does not have HER2 expressed, another type of gastric cell line will be purchased. If the NCI-N87 cells culture successfully and HER2 is present, the experiment will move to step 2. A similar cell line without HER2 expression will be used as a control.

Step 2: Altering cell culturing conditions-pH & salt conditions | Flow Cytometry

The gastric cancer cell line will be cultured in media with different pH and high-salt environments. The changes would deviate from normal growth conditions and cell

proliferation will be measured through using a hemocytometer. Cell growth will be compared across the different conditions and if changes are determined, further analysis on specific proteins will be completed. In addition to monitoring cell proliferation, the level of HER2 will be also analyzed. The next step will be carried out to determine if specific protein activity is altered. Flow cytometry will be used to detect protein levels of HER2 and reveal the correlation between cell cycle phases and HER2.

Step 3: Western Blot Analysis & siRNA

Western blot analysis will help identify specific proteins in the signaling pathways that are associated with HER2 signaling in cell proliferation under different pH or salt concentrations. The data from Western blot analysis will further support the link between HER2 expression and gastric cancer cell growth. I will initially evaluate the PI3K/Akt pathway, a commonly researched pathway for HER2 (Sukawa et al., 2014).

Small interfering RNA (siRNA) can be used to inhibit HER2 expression and will show how cells without HER2 would also respond in different conditions. Use of siRNA will also require learning a new technique of optimally introducing siRNA into cells. Analysis using flow cytometry and Western blot will evaluate the proteins previously identified to be affected by altered growth conditions.

If results demonstrate that there is a connection between changing the environmental conditions for gastric cancer cell growth and HER2 expression, the next experimental approach will be used.

Step 4: Microarray and Gene Expression

Microarray analysis provides measurements of gene-level expression and can reveal possible changes in groups of genes associated with specific functions in the cell. This type of analysis will indicate the genes that are affected when pH or salt concentration is changed and affects cell proliferation.

To carry out microarray analysis, I will send isolated RNA from cells grown in different conditions that were shown to change growth or affect HER2 expression. The microarray analysis will be outsourced to Phalanx Biotech Inc. because that technology is expensive and not available at Elon. If the higher pH- or salt-environments changes the expression of the HER2 protein and cell proliferation, identifying what groups of genes are affected can help to narrow down the genes related to HER2 function in gastric cancer cells. The microarray analysis will potentially identify new proteins linked to HER2 signaling in gastric cancer development.

Laboratory Techniques

For the previously mentioned methods, I will need to gain expertise in each to perform the experiments. I will learn, from Dr. Yuko Miyamoto, how to culture cells and western blot analysis. Dr. Tonya Train will guide me on how to use flow cytometry machine and data

analysis. Both are routinely used in research and will greatly advance my knowledge and expertise in analyzing cells. The research skills will also help me develop critical problem-solving skills as I continue on my track to apply to MD/PhD programs in the future.

Proposed products:

- 1) Present poster or oral presentations at undergraduate conferences, such as: Spring Undergraduate Research Forum (SURF), National Conferences on Undergraduate Research (NCUR), West Coast Biological Sciences Undergraduate Research Conference (WCBSUR).
- 2) Submit a proposal to participate in Summer Undergraduate Research Experiences (SURE).
- 3) Present a poster presentation at national conferences, such as: American Society for Cell Biology (ASCB) or American Association for Cancer Research (AACR).
- 4) Submit a paper to an undergraduate journal.

Feasibility

Feasibility statement:

My proposed project requires learning several key experimental procedures, but after much research and discussion, I am confident that it is feasible. Included in my design is the ability to adjust should I encounter unexpected problems. Elon has the lab equipment needed for the techniques and the reagents, cells, and antibodies required are commercially available. For the lab techniques, specifically flow cytometry, I am grateful for Dr. Train's willingness to help me.

For my timeline, I will have two semesters, including my summers, to conduct research. This summer, I will be focused on learning and carrying out research in my fellowship in the Department of Pharmacology and Cancer Biology at the Duke University School of Medicine.

In my fall semester in Osaka, Japan, which houses the Osaka International Cancer Center, I intend to pursue more knowledge about gastric cancer as it applies to Japanese cases. I hope to make contact with scientists at conferences there and potentially visit their labs. I speak conversant Japanese and know enough scientific language to communicate on these topics, but hope to expand my ability to converse about research. I will also read research papers for my Lumen research project during my time in Japan. During the semester, I will submit a SURE proposal for the summer of 2020. After my semester abroad, I will continue my research in winter term, and through my senior year at Elon.

As mentioned, an aspect of my experiments is the ability to adjust if necessary. In the "Scholarly Process" section, I outlined my proposed multilayer process and proceeded throughout with the assumption that each experiment would yield results. However, I have considered the potential obstacles that each step may encounter. In step 1 and 2, the gastric cancer cells may not grow as desired in the various conditions or do not have HER2. This can be overcome by either purchasing a different gastric cancer cell line or by adjusting the conditions that may be more cell growth friendly. In step 3, if I find no correlation in terms of cell growth to HER2 levels, I would test a different cell line that relies on HER2. If the proteins selected for Western blot analysis do not reveal any change, other proteins can be studied. Since HER2 binds with other HER family proteins, other signaling pathways can be analyzed. Once I arrive at findings in step 3, step 4 is a potential option. This research will not proceed directly from step 1 to step 4 because it is important to validate and identify key pathways before doing a genomic analysis. Conducting the other steps will establish the interaction between the HER2 protein and gastric cancer in altered environments, and the microarray will be able to further support this evidence.

Budget:

Total Budget – \$18,629

Materials: Total - \$6,695

- NCI-N87 gastric cancer cell line - \$400
- Additional cell lines-\$800
- RPMI-1640 media - \$100
- Additional media- \$100
- Pre-cast gels - \$150
- Flow Cytometry - \$1,525
 - HER2/ErbB2 (29D8) Rabbit mAb #2165 - \$260 (per 100 microliters)
 - HER2/ErbB2 (29D8) Rabbit mAb (PE Conjugate) #98710 - \$305 (per 100 microliters)
 - EGF Receptor (E746-A750del Specific) (D6B6) XP® Rabbit mAb #2085 - \$420
 - SiRNA for HER2 - \$290
 - Lipofectamine® RNAiMAX™ Transfection Reagent - \$250
- OneArray RNA Microarray - \$1,600 (\$400 per sample)
- RNA isolation kit - \$500
- Western Blot materials - \$1,520
 - Most of the materials are available (solutions, blotting paper, and nitrocellulose)
 - Specific reagents needed are the antibodies to HER2 and additional signaling proteins - \$1,280 (\$320 each)
 - Secondary antibodies used in Western blot analysis - \$300 (\$100 each)

Conferences: Total – \$4,619

- 2nd International Conference on Cancer Science - Osaka (10/17-18/2019)
<https://worldcancer.cancersummit.org/>
 - Registration Fee: \$499
 - Travel: \$25
 - Food: \$75
- 13th World Congress on Hematology and Oncology - Tokyo (10/23-24/2019)
<https://hematology.global-summit.com/>
 - Registration Fee: \$400
 - Travel: \$140
 - Food: \$75
 - Hotel: \$500
- AACR - San Diego

<https://www.aacr.org/Meetings/Pages/future-annual-meetings.aspx>

- Registration Fee: Free for students
- Travel: \$450 for flight
- Food: \$150
- Hotel: \$1,000
- ASCB - Washington D.C

<https://www.ascb.org/meetings-events/future-ascb-meetings/>

- Registration Fee: \$75
- Travel: \$80
- Food: \$150
- Hotel: \$1,000
- NCUR 2021
 - Registration Fee, travel, hotel: \$100 (Through Elon NCUR Travel Grant)
 - Food: \$80

Study Abroad Expenses:

- Roundtrip flights between Raleigh-Durham and Osaka: \$3,000

Applying to graduate programs total - \$4,315

- MCAT registration fee: \$315
- MCAT Study materials: \$3,000
- Medical School application fees: \$1,000

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	Proposed Experiences	Proposed Product(s)
First Summer Term	Research fellowship: Duke School of Medicine, Department of Pharmacology and Cancer Biology	- Independent research project - Final presentation at Duke
First Fall Term	Study abroad in Japan: attend international conferences on cancer biology	- Conference notes and visit labs - Improve Japanese language skills, especially in scientific/research contexts - Submit application for SURE Submit Honors Proposal
First Winter Term	Begin experiments 2 sh 498	- Culture cells, learn flow cytometry, test for HER2 expression - Alter conditions for cell culture - Preliminary Data
First Spring Term	Present at SURF 2 sh 498	- Learn Western Blot analysis - Test for activity of cell growth pathway proteins
Second Summer Term	SURE Submit abstract to ASCB or AACR	- Participate in SURE - Continue signaling pathway protein analysis
Second Fall Term	Present at ASCB 2 sh 498	- Continue Western blot analysis - Evaluate data - siRNA analysis
Second Winter Term	Continue experimentation 1 sh 498	- Continue siRNA analysis - RNA isolation for microarray analysis
Second Spring Term	Present at SURF, NCUR, AACR 1 sh 498	- Presentations - Honors thesis - Draft undergraduate journal submission