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Study of alpha mangostin as a chemoprotective agent for breast cancer via activation of the p53 pathway

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Literature Review

Breast Carcinoma & Treatments

The development of cancer is a multistep process that involves complex interactions between host and tumor tissue. The process involves oncogene activation as well as immunosuppression, leading to uncontrolled cell growth despite damaged DNA (Wang 2010). Genome instability contributes to cancer development due to mutations in DNA damage response pathways in which are mediated through the tumor suppressor p53 (Reinhardt & Schumacher, 2012).

Breast carcinoma is the most frequently diagnosed cancer among women and causes over 400,000 deaths yearly worldwide (Walerych, Napoli, Collavin, & Del Sal, 2012). Metastasis accounts for a large majority of the deaths that result from breast cancer, mostly to lymph nodes, lungs, and bones. Like many other cancers, the complex nature of breast cancer causes it to be difficult to treat. Treatment plans are dependent on the stage and characteristics of the cancer, as well as the age, menopausal status, and risk benefit analysis associated with each option. Based on a study in 2013, stage I and II patients most often receive breast conserving surgery. Stage III patients most often receive a mastectomy, radiation therapy, as well as chemotherapy. The five-year survival rate for women with stage III is about 72%. Stage IV patients most often receive radiation therapy and or chemotherapy with a five-year survival rate of about 22% (American Cancer Society, 2017).

In order to delay the progression of breast cancer and increase the longevity of patients, less toxic yet effective chemotherapeutic agents are needed to limit the harsh side effects of the treatments to the patients (Shibata et al., 2011). These side effects include pain, lymphedema, musculoskeletal symptoms, bone loss and osteoporosis, heart problems, new cancers, blood clots,

infertility, and concerns about memory loss and cognitive function. (American Cancer Society, 2017).

Currently, conventional treatments discussed as radiation, chemotherapy, and surgery have not been entirely effective against the high incidence and low survival rates of this cancer due to its complex nature (Moongkarndi et al., 2004). Research has established that combinations of drugs are more effective than one drug alone for the treatment of early-stage breast cancer. An example of such treatment is that of Trastuzumab, a monoclonal antibody that directly targets the HER2 protein. When combined with chemotherapy, this treatment was found to reduce the risk of recurrence by 52% and death by 33% for patients who over produce the growth promoting protein HER2/neu (American Cancer Society, 2017). This leads the scientific community to search for other potential therapeutic approaches including drug combinations to treating this malignancy and many other cancers that continue to evade conventional treatments.

P53 Gene & Cascade

The main function of p53 is to promote genetic stability and prevent the formation of tumors. When a cell is under severe stress, it induces cell death through apoptosis. P53 also plays a role in milder stress through cell cycle arrest and DNA repair. This is shown in Figure 1 (Vogelstein, Hughes, Kimmel, & Cancer, 2013). When stress occurs, p53 is phosphorylated, which disrupts its binding to the regulatory protein MDM2. P53 then accumulates and its stimulates transcription of numerous genes, including the gene that encodes the

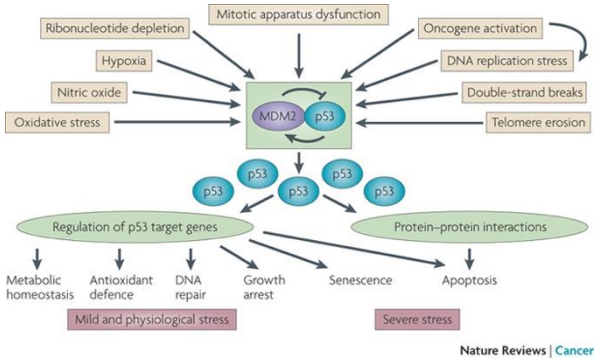

Figure 1. Various outcomes of the p53 pathway.

CKI protein p21. P21 binds and inactivates G_1/S-Cdk complexes, arresting the cell in G_1 for DNA repair. An alternate pathway for this gene results in apoptosis. This process is shown in Figure 2 (Alberts 2015).

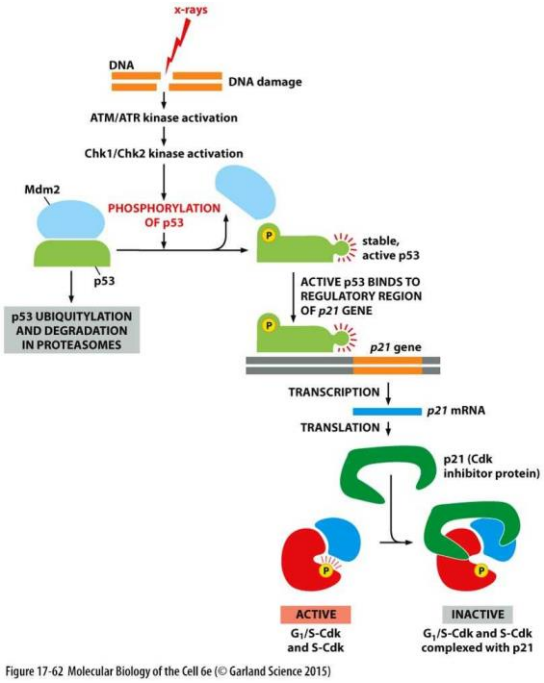

Figure 2. The p53 Cascade.

P53 Mutation & Treatments

Inactivation of p53 is an almost universal feature of human cancer cells (Lane, Cheok, & Lain, 2010). On average, p53 is mutated in 50% of all tumors and approximately 20% in breast cancer. Though the frequency of mutation is lower in breast cancer cells, p53 inactivation has been seen in some breast cancers without a mutation. The pathway has been shown to be affected by alterations in upstream regulatory proteins and downstream p53-induced proteins (Gasco, Shami, & Crook, 2002). In breast cancer, this mutation is associated with a more aggressive disease and worse overall survival according to several studies (Gasco et al., 2002).

The ability to activate the p53 pathway which protects cells from tumor formation is lost in cells with p53 mutations. Most of these mutations occur as a result of a substitution of single amino acids in the central region of the p53 protein, which causes many variants (Walerych et al., 2012). Indeed, rapid malignant cell growth which leads to many different cancer types often involves a defective p53 gene, which is the transcriptional activator that works to suppress tumors in normal tissues (Muller & Vousden, 2014). In breast cancer, mutant p53 is involved in many processes associated with the cancer development such as early tumorigenesis, tumor growth and

development, and metastasis (Walerych et al., 2012). In clinical practice, molecular pathological analysis of the tumors of the structure and expression and constituents of the p53 pathway is likely to have value in diagnosis, in prognostic assessment and, ultimately, in treatment of breast cancer (Gasco et al., 2002).

P53 Activator Current Research

Due to the prevalence of this mutation, p53 a uniquely valuable target for basic as well as applied research (Vogelstein et al., 2013). Therefore, much research has gone into both therapeutic strategies to restore wild-type activity to mutant p53 and pretreatment of cancer cells with p53 activators, which will arrest p53-normal proliferating cells without impacting the cell cycle of cells with a p53 mutation and allow for selective killing of cancerous cells. Furthermore,

Current research on this type of treatment has led to the discovery of small molecules that directly or indirectly activate the p53. P53 activation has also been reached in the clinic. The most advanced of these are the p53 mdm2 interaction inhibitors. As research continues and understanding of p53 response increases, development will continue allowing for powerful drug combinations that may increase the selectivity and safety of chemotherapy, by selective protection of normal cells and tissue (Lane et al., 2010).

Chemotherapy and Chemoprotection Treatment

Chemotherapy induces many adverse effects in patients because of normal cell toxicity, resulting at least in part from p53 activation and apoptosis induction in normal proliferating cells/tissues, such as bone marrow, lymphoid organs, hair follicles, and epithelium lining of the small intestine (Wang & Sun, 2010). An important aspect of chemotherapy is it kills actively

dividing cells. In particular, Paclitaxel (PTX) inhibits microtubule function, which kills cells as they enter mitosis (Blagosklonny, 2002). Microtubules are essential to the process of mitosis as they separate chromosomes to opposite sides of the cell during anaphase. When PTX inhibits the ability for the chromosomes to be separated during the division process, the cell is inactivated and eventually is killed. Therefore, wild type cells treated with the p53 activator are arrested in G1 and do not enter into mitosis and therefore the chemotherapy selectively kills p53-deficient cancer cells. This mechanism can be experimentally controlled with the use of p53 activators to arrest p53 wildtype cells and protect against the harmful effects of chemotherapeutic agents on noncancerous cells.

Alpha Mangostin as a Chemoprotectant

My study will focus on the p53 activator, alpha mangostin. This compound is isolated from the carp of the *Garcinia mangostana* (Mangosteen fruit), which is native to Thailand and traditionally used for medicinal purposes such as an antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral (Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, & Pérez-Rojas, 2008). This extract is known to inhibit the binding of p53 to MDM2, a negative regulator of th

In a particular study in 2011, alpha mangostin was used to reduce tumor growth and lymph node metastasis in an immunocompetent xenograft model of metastatic mammary cancer with a p53 mutation. The study showed that treatment with 20 mg/kg/day alpha mangostin resulted in prolonged survival rates and increased inhibition of tumor growth and lymph node metastasis (Shibata et al., 2011). This reveals that this extract at high concentrations can potentially be a successful treatment for p53 mutated cancer types. Meanwhile, at lower concentrations, alpha

mangostin has the potential to act as a chemoprotectant to wild-type cells. One study tested the chemoprotection of alpha mangostin on wild type BHK cells. The results supported the hypothesis that the alpha mangostin can be used to protect cells from the cytotoxic effects of chemotherapy (Wojciechowski, 2017). However, the effect on breast cancer cell lines at low concentrations is not known. My research seeks to determine whether or not the alpha mangostin pretreatment would be an effective strategy for chemoprotection of wildtype cells by testing whether or not the cancer cells are also protected. If the data indicate that the cancerous cells are not protected, this p53 activator could potentially be a successful pretreatment before chemotherapy for selective cancer killing.

Hypothesis

I hypothesize that alpha mangostin, a p53- dependent chemoprotectant, protects wild-type cells, but not those with a p53 mutation from the chemotherapeutic agent Paclitaxel.

Materials and Methods

In this research, two variations of a cell line will be used to test the chemoprotective qualities of alpha mangostin. The two lines will consist of p53 knockout (-/-) and wild type human breast cancer cells, MCF10A. Cell culture protocol was based up the ATCC© Thawing, Propogating, and Cryopreserving Protocol ("Thawing, Propagating, and Cryopreserving Protocol: MCF10A-JSB Breast epithelium," 2012).

These cells were treated with various levels of alpha mangostin and paclitaxel concentration, based on the results for the toxicity curve and dose response curve. Data was collected on cell viability using differential fluorescent staining. The differences through fluorescent microscopy and Hoechst, Propidium Iodide, and YO-PRO-1 stains. The first stain I will use, the Hoechst stain, only stains normal healthy cells. The next two, Propidium Iodide and YO-PRO-1, only stains necrotic and apoptotic cells, respectively. Through these three stains, I will be able to delineate the three populations of cells. Representative pictures at 10x were taken blindly by a professor in order to eliminate bias. Cells were counted using a fluorescent cell counting program.

The goal of this research is to gather data to support or reject the hypothesis that breast cancer cells will not be protected by the p53 activator alpha mangostin when treated with chemotherapeutic agents and thus have the potential to be used as a selective cancer treatment and will allow for higher doses and therefore more effective treatments of chemotherapy.

Results

Results for Pence Boyce Research, not honors program. I am almost to the data collection point for honors where I will be able to collect a lot of meaningful data. For now, these are the results I have.

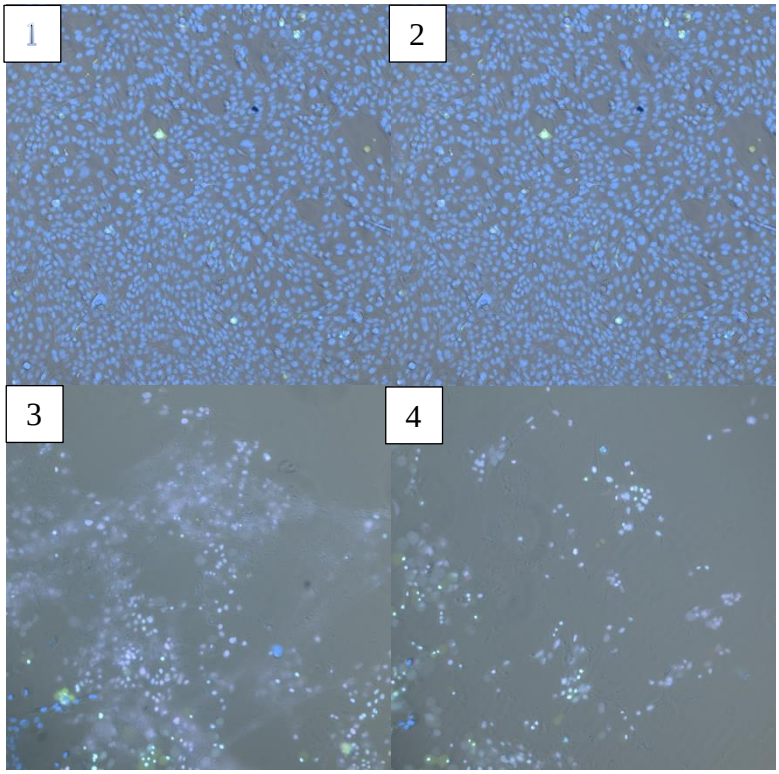

Figure 3. Fluorescent Microscopy of MCF10A TP53 (-/-) Cells after treatment with Alpha Mangostin (1 is 0 uM, 2 is 0.1 uM, 3 is 0.5 uM, 4 is 1 M uM). Blue cells indicate live cells stained with Hoechst, and green cells indicate apoptotic cells, and red cells indicate necrotic cells. Increasing concentrations led to a decrease in live cell count.

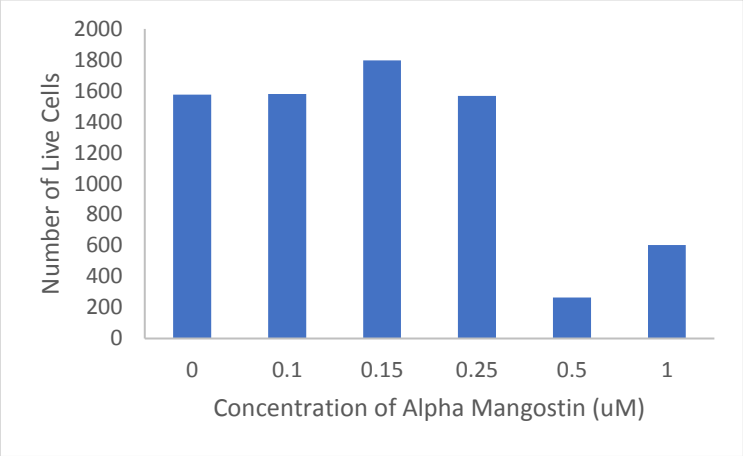

Figure 4. Live Cell Counts using Fluorescent Cell Counting Program.

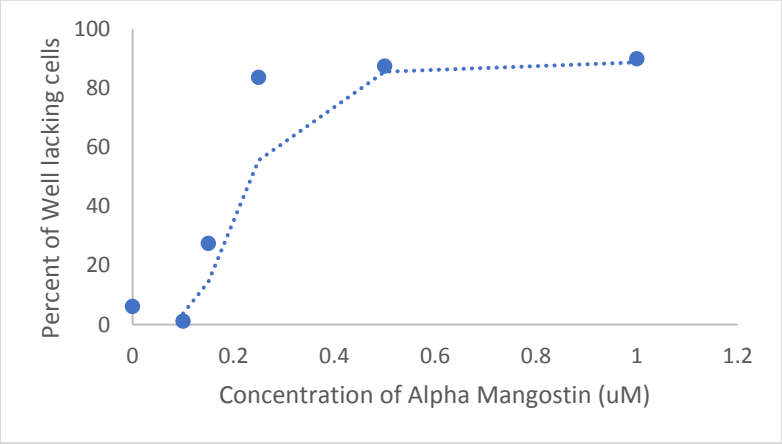

Figure 5. Qualitative observation of percent of wells lacking cells for increasing treatments of Alpha Mangostin. Observations of this percentage were estimated using a blind study method.

Discussion

Through observations of the wells after treatment, the wells with the higher concentration of Alpha Mangostin had many more floating dead cells, which is not represented well in the photos. These dead cells indicate that the low number of cells at high concentrations is not due to cell arrest, but rather from cell toxicity. The decrease in cell number is shown through both figure 4 and 5. Figure 4 shows quantitative data that show a relative decrease in cell number as the concentration increased. Figure 5 shows a qualitative analysis of the percentage of the well that does not have cells. This was done as a blinded study to avoid bias. If the same concentration can be used to arrest normal cells, pretreatment with Alpha Mangostin would be an effective method of selectively killing cancer cells using Paclitaxel, a chemotherapeutic agent that only kills actively dividing cells.

Limitations on this research included time. Time became a important factor when mostly the entire summer was troubleshooting protocol and learning how to keep this particular cell line alive. This research is an ongoing process with many unanswered questions to be addressed. The upcoming trials with this research will include treatment with paclitaxel, a microtubule inhibiting chemotherapy, as well as dual treatment with alpha mangostin and paclitaxel. These will be performed on both MCF10A TP53 (-/-) and MCF10A wild type breast cancer cell lines. These results will be quantified using the 10x representative picture and counted using a cell counting program. This will give a better indication of the chemoprotective qualities of Alpha Mangostin.

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