Indiana State University
Sycamore Scholars

All-Inclusive List of Electronic Theses and Dissertations

2022

Chemoresistance In Breast Cancer: Acquisition, Microtubule-Associated Proteins, And Combinatorial Therapy

Keeley Cleghorn
Indiana State University

Follow this and additional works at: https://scholars.indianastate.edu/etds

Recommended Citation

Cleghorn, Keeley, "Chemoresistance In Breast Cancer: Acquisition, Microtubule-Associated Proteins, And Combinatorial Therapy" (2022). *All-Inclusive List of Electronic Theses and Dissertations*. 1885.
https://scholars.indianastate.edu/etds/1885

This Dissertation is brought to you for free and open access by Sycamore Scholars. It has been accepted for inclusion in All-Inclusive List of Electronic Theses and Dissertations by an authorized administrator of Sycamore Scholars. For more information, please contact dana.swinford@indstate.edu.

CHEMORESISTANCE IN BREAST CANCER: ACQUISITION, MICROTUBULE-ASSOCIATED PROTEINS, AND COMBINATORIAL THERAPY

A Dissertation

Presented to

The College of Graduate and Professional Studies

Department of Biology

Indiana State University

Terre Haute, Indiana

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Philosophy

---

by

Keeley Cleghorn

May 2022

© Cleghorn 2022

Keywords: Acquired Chemoresistance, Paclitaxel, KIF14, Epigallocatechin-3 Gallate, Triple-Negative

Keeley Cleghorn

Indiana State University

Terre Haute, Indiana 47809

EDUCATION AND CERTIFICATIONS

May 2022 Ph.D. Biology, Indiana State University, Advisor Dr. William Mitchell

May 2017 B.S. Biology, Indiana State University

PROFESSIONAL POSITIONS

Teaching

Graduate Teaching Assistant, Department of Biology, Indiana State University, (August 2017 – May 2022)

Research

Porter Research Fellow, Rich and Robin Porter Cancer Research Center, Department of Biology, Indiana State University, (May 2021 – May 2022)

Student Affiliate Member of The Center for Genomic Advocacy, Department of Biology, Indiana State University, (May 2014 – May 2022)

RESEARCH GRANTS

2020 - Graduate Student Research Grant, CGPS, *Evaluation of KIF14 in a Chemotherapeutic Resistant Breast Cancer Cell Line*. $550

2019 - Graduate Student Research Grant, CGPS, *Diving into Chemoresistance: Investigation into the CCKR Signaling Map*. $600

2018 – Senior Research Grant, IAS. *Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance*. $2,300

- Graduate Student Research Grant, CGPS. *Evaluating Changes in Metastatic Potential in Acquired Paclitaxel Resistance*. $505

2017 - Graduate Student Research Grant, CGPS. *Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance.* $350

PUBLICATIONS

Cleghorn, Keeley and Steding, Catherine E. 2021. “Evaluation of KIF14 in a chemotherapeutic resistant breast cancer cell line.” *Cancer Research Proceedings*

CONFERENCE RESEARCH PRESENTATIONS

2021 - *Evaluation of KIF14 in a chemotherapeutic resistant breast cancer cell line.* (American Association for Cancer Research Annual Conference)

2021 - *A Mechanism of Acquired Chemoresistance: An Exploration into the Function of KIF14*. (Midwest Graduate Research Symposium)

2020 - *Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance.* (Indiana Academy of Science)

- *Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance.* (Student Research Exposium)

2019 - *Critically Evaluating in Vitro Conditions: Implications in Combating Cancer Derived from Long Term Treatment with Commonly Used Compounds.* (Annual Biomedical Research Conference for Minority Students)

2019 - *Critically Evaluating Culture Conditions and the Long-Term Implications on Gene Expression.* (Indiana Academy of Science)

- *Defining Critical Differences Between Epigallocatechin Gallate and Match Tea as Therapeutic or Dietary Supplements in Cancer*. (Indiana Academy of Science)

- *Investigation of Acquired Chemoresistance Against Paclitaxel*. (Summer Undergraduate Research Experiences Symposium)

- *Evaluating Changes in Metastatic Potential in Acquired Paclitaxel Chemoresistance.* (A Celebration of Student Research & Creativity, Exposium)

2018 - *Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance.* (In A Celebration of Student Research & Creativity)

- *Linking Tumor Heterogeneity to Tissue-Specific Metastasis: A Preliminary Exploration into Extracellular Vesicles.* (Exposium In a Celebration of Student Research & Creativity)

COMMITTEE MEMBERS

Committee Chair: William Mitchell, Ph.D.

Associate Professor, Department of Biology

Indiana State University

Committee Member: Shaad M. Ahmad, Ph.D.

Assistant Professor, Department of Biology

Indiana State University

Committee Member: Kristopher Schwab, Ph.D.

Assistant Professor, Department of Biology

Indiana State University

Committee Member: Scott G. Canfield, Ph.D.

Assistant Professor of Anatomy, Cell Biology & Physiology

Indiana University School of Medicine

Committee Member: Yunlong Liu, Ph.D.

Director, Center for Computational Biology & Bioinformatics

Indiana University School of Medicine

Committee Member: Catherine E. Steding, Ph.D.

Assistant Professor of Biochemistry and Molecular Biology

Marian University

ABSTRACT

Breast cancer remains the most diagnosed cancer in American women with a projected 44,000 women that will succumb to advanced metastatic disease in the next calendar year. Advanced and recurrent breast cancers frequently exhibit clinical resistance to therapeutic interventions including many that exhibit resistance against multiple drugs or therapies and results in chemoresistance. Chemoresistance, a common cause of therapeutic failure, can be classified as either innate or acquired, both of which result in dramatically different results following therapeutic intervention. Work in our laboratory has emphasized evaluating acquired chemoresistance against the microtubule-stabilizing drug, paclitaxel, and has demonstrated the ability to generate cells more resistant than cells treated with solvent controls. These paclitaxel resistant cells provide a critical model system for evaluating mechanisms of acquired chemoresistance. Findings from preliminary investigations of these chemoresistant cells indicate both novel growth patterns and dose-dependent reactions to paclitaxel. These changes in cellular behavior warrant further investigation as they imply a previously unreported mechanism of chemoresistance.

To study acquired chemoresistance, it is necessary to have a model system. In the first part of my research (Chapter 2), I show that MDA-MB-231 cells respond to paclitaxel in a dose-dependent manner, and I create a long-term acquired paclitaxel resistant cell line. I then characterized this cell line and demonstrated that although they are resistant to paclitaxel their basic phenotypic traits remain unaltered. However, there are significant changes to these cells on

a genotypic level which could be the beginning of investigation into the mechanisms of acquired chemoresistance.

In the second part of my research (Chapter 3), I demonstrate that reducing expression of KIF14 within a triple-negative breast cancer (TNBC) cell line causes a reduction in cellular growth including proliferation potential and colony formation abilities. I also determine that when treated with paclitaxel, the TNBC cells are more responsive to paclitaxel treatment. When KIF14 expression is also reduced within resistant cells, they express a reduction in growth capabilities and are more responsive to paclitaxel treatment. This information thus provides a deeper understanding of KIF14's role in the mechanism of chemoresistance.

Appearing in the third part of my research (Chapter 4), I find that epigallocatechin-3 gallate (ECGC) can suppress the growth of paclitaxel-resistant hormone responsive breast cancer cells in a dose-dependent manner. I also found that EGCG has the capacity to decrease the viability of paclitaxel resistant TNBC cells when treated with both EGCG and paclitaxel thus demonstrating the potential for this compound. This information thus supports further investigation into the molecular mechanisms of EGCG activity in paclitaxel-resistant cells and the potential as EGCG as a combinatorial treatment with paclitaxel.

ACKNOWLEDGEMENTS

I would like to express my deepest thanks to my committee for their support and assistance. I am grateful for Dr. William Mitchell for your willingness to lead my committee and all the assistance you have provided. Thank you, Dr. Shaad M. Ahmad, and Dr. Kristopher Schwab, for your comments and advice. Thank you, Dr. Scott G. Canfield, for your time, mentorship, consistent guidance, and support. Thank you, Dr. Yunlong Liu, for your assistance with guiding me through my bioinformatics. Thank you, Dr. Catherine E. Steding, for your mentorship and guidance, as well as teaching me about research and helping me discover my passion for cancer research.

I would like to thank Rich and Robin Porter and the Rich and Robin Porter Cancer Research Center for their financial support via the PCRC fellowship. I would also like to thank the Indiana Academy of Science, Indiana State University College of Graduate and Professional Studies, the Department of Biology, and The Center for Genomic Advocacy.

I would like to thank my fellow graduate students for their advice and new perspectives when needed. I would especially like to thank Dr. Jennifer Smith and Dr. Ashley Clodfelder for your continual guidance; Dr. Andrew Kump, Dr. Manoj Panta, and Nykara Brown for assisting me with experiments. I would like to thank Danielle Muse for the support you have provided from countless hours of labeling tubes to discussing potential research aspects. I would also like to thank Angela Chamberlain for aiding in keeping me sane all these years.

It is also my privilege to thank all of my family for their love, support, and care. I would also like to thank Charlie, for his patience and love. Finally, I could not have completed this degree without the unwavering love and support from my husband, Kenneth Cleghorn, thank you for all you do.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

AN INTRODUCTION TO BREAST CANCER AND THE FIGHT AGAINST CHEMORESISTANCE

Epidemiology of Breast Cancer

Cancer is a disease that will affect every person. When normal cells no longer respond to their regulatory controls, they become cancerous. The most commonly diagnosed cancer in women is breast cancer with about 30% of all new cases diagnosed as breast cancer (Siegel et al., 2021). It is estimated that this year alone approximately 44,000 women will perish from breast cancer, a trend that is steadily increasing in numbers with a global rise of 20% since 2008 (DeSantis et al., 2014; Jemal et al., 2008; Siegel et al., 2021; Siegel et al., 2017).

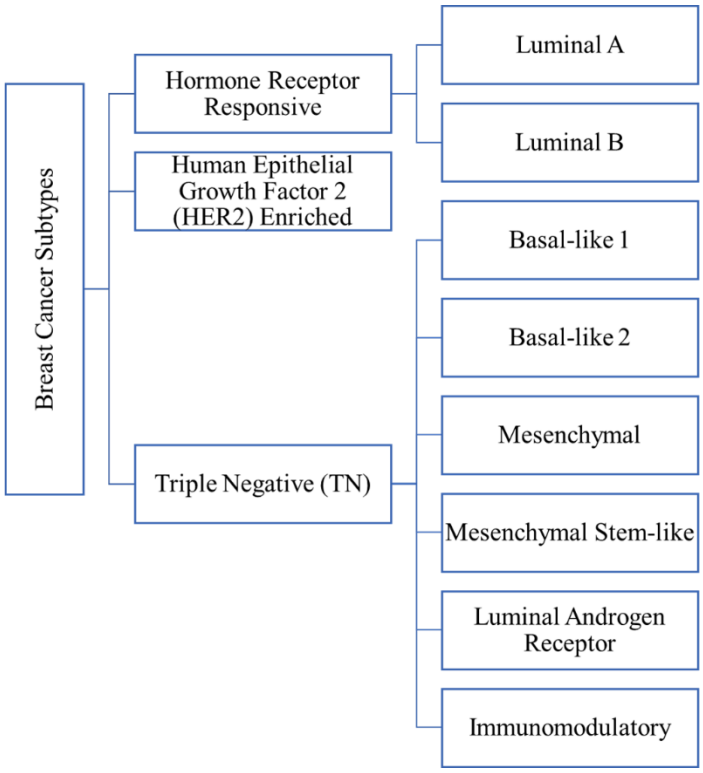
Breast cancer is a heterogenous disease in which breast tissue changes and divides uncontrollably (Society, 2019) and is divided into three main groups. The first group is hormone receptor positive in which the cancer is identified for being positive for either or both estrogen receptors (ER) or progesterone receptors (PR). The second group known as HER2-enriched as it is classified by being positive for the human epithelial receptor 2 (HER2). Finally, the third group is negative for PR and ER while simultaneously lacking an amplification of HER2 receptors and is known as triple-negative breast cancer (TNBC) (Perou et al., 2000; Sørlie et al., 2001).

Hormone positive breast cancer can be further divided into two groups luminal A or luminal B. Luminal A subtype is positive for ER and/or PR and is negative for HER2, while luminal B subtype is positive for all three hormone receptors.

With the use of gene expression analysis, TNBC can be classified with six different subtypes each with unique ontologies. These six subtypes include: a basal-like 1 and 2, a mesenchymal, a mesenchymal stem-like, a luminal androgen receptor, and an immunomodulatory subtype (Lehmann et al., 2011) (Figure 1.1).

Figure 1.1

Molecular Subtypes of Breast Cancer



Note. Molecular subtypes of breast cancer based on gene expression analysis.

On average 78% of all female breast cancer is hormone receptor responsive with only 4% being HER2-enriched and 10% of cases being triple-negative (Anderson et al., 2002; Cleator et al., 2007; Konecny et al., 2003; Kumar & Aggarwal, 2016; Surveillance, November 2020). TNBC is a highly proliferative subtype with a higher incidence of metastasis and results in poor overall survival (Mir et al., 2020). Identification of the hormone receptors located within the breast cancer is necessary as different treatment options are dependent upon the presence or absence of these key receptors.

Model Systems

With a plethora of different breast cancer subtypes, work in our laboratory has focused on studying the MDA-MB-231 cell line and the MCF7 cell line as these are well characterized breast cancer cell lines that will allow for comparison of molecular changes. MCF7 cells are a luminal A subtype, positive for both ER and PR, that was initially isolated in 1970 from a patient with a malignant adenocarcinoma and is commonly used to study early stage breast cancer (Kao et al., 2009; Welsh, 2013). This cell line has low metastatic potential as a non-invasive, non-aggressive breast cancer (Gest et al., 2013; Shirazi et al., 2011). Usage of this cell line allows for a reliable model to study changes in response to chemotherapy in hormone responsive cancers (Koutsilieris et al., 1999).

MDA-MB-231 cells are a TNBC line, ER-, PR-, and HER2-, that was initially harvested in 1973 from a patient with invasive ductal carcinoma and is a common cell line to study late stage TNBC (Cailleau et al., 1978; Welsh, 2013). This cell line is significantly more invasive and therefore a more aggressive cell line. Both cell lines are cultured the same in a low glucose Dulbecco's modified Eagle's medium with cosmic calf serum, glutamine, and insulin. The cells need a medium renewal twice a week with weekly passages. Both cell lines are aneuploid and

are typically responsive to chemotherapy. With the use of both a hormone receptor positive (MCF7) and a TNBC (MDA-MB-231) cell line critical investigation of breast cancer can be defined on a broad spectrum.

Treatment

Along with the different subtypes of breast cancer, physicians have multiple treatment options depending on the patient's need. Treatments for breast cancer can be divided into two groups: local treatments or systemic treatments. Local treatments are isolated treatment options that can terminate cancer cells; however, their effect on neighboring cells is minimal. Local treatments include radiotherapy or surgery.

Radiotherapy is radiation therapy that uses high energy radiation to minimize tumors and terminate cancer cells (DePolo, 2021). Surgery for breast cancer includes the removal of breast tissue in hopes of eradicating the cancer cells from the patient. There are different surgical options depending on the stage of the cancer and even the patient's peace of mind. The two main types of surgical options include: lumpectomy, in which only the tumor is removed, or a mastectomy, where all of the breast tissue is removed.

Systemic treatments can be used in conjunction with local treatments or used on their own. Systemic treatments make use of pharmaceuticals to eliminate the cancer cells. However, one of the drawbacks of systemic treatments are that they are not localized only to the cancer cells and can have a damaging effect on neighboring or distant cells that are not cancerous. Common systemic treatments include: hormone therapy, immunotherapy, and chemotherapy.

Immunotherapy encourages the patient's immune system to eliminate the cancer cells; however, since cancer cells begin as normal cells it can be difficult for the immune system to identify them as cancerous (Simonian et al., 2021). There are two main types of immunotherapy;

active immunotherapies, which train the patients' immune system to respond on its own, or passive immunotherapies, in which patients are provided with immune system components to fight the cancer cells (Emens, 2021). Immunotherapies have been found most beneficial to patients expressing with HER2-enriched breast cancer cells (Plevritis et al., 2018; Wolff et al., 2019).

Hormone therapy is a systemic treatment specifically for breast cancer patients that have hormone receptors on their cells (hormone receptor responsive cancers). Typically, there are two main mechanisms for hormone therapy targeting. The first is to lower the amount of hormones within the body (Jennifer Armstrong, 2020). The second option is to block the existing hormones mechanism of action (Jennifer Armstrong, 2020). Hormone therapy is typically selected to treat breast cancers that are ER+ and PR+, like the MCF7 cell line.

With TNBC lacking ER, PR, and HER2 amplification, there currently is not a standard treatment option when a patient presents with this type of cancer, and their systemic options are limited to chemotherapy (Kumar & Aggarwal, 2016; Luque-Bolivar et al., 2020). Chemotherapy is the use of pharmaceuticals that may be given intravenously or by mouth. Chemotherapy weakens and destroys cancer cells at both the location of the original tumor and cancer cells that could have metastasized. However, since chemotherapy can target metastasized cells there is the possibility that it will have off target effects. Chemotherapy is the best option when cells are ER-, PR-, and HER-, like the MDA-MB-231 cell line.

One of the most common classes of chemotherapeutics utilized in metastatic breast cancer therapy is the taxanes. The taxanes were initially discovered as part of a National Cancer Institute program to find new natural therapeutics for cancer. In 1963, the bark of the Pacific Yew tree, *Taxus breviflia*, was harvested and tested for its antitumor properties. It was not until

1971, that paclitaxel was identified as the active ingredient with cytotoxic effects against tumors (Rowinsky & Donehower, 1995; Wani et al., 1971). In 1992, the Food and Drug Administration (FDA) approved paclitaxel to treat ovarian cancer and then in 1994 approved paclitaxel for breast cancer treatment (Gueritte, 2001; McGrogan et al., 2008; McGuire et al., 1989; Sarosy et al., 1992). The taxanes are approved therapeutically in breast cancer when the cancer presents with lymph node involvement and further metastasis (Crown et al., 2004; Hassan et al., 2010; Jordan & Wilson, 2004; Sparano et al., 2008). Paclitaxel treatment has also been used to treat lung, pancreatic, gastric, and cervical cancer (Massey et al., 2019; Sakamoto et al., 2009; Tuma, 2003; Wang et al., 2016). Treatment with taxanes has been shown to increase survival rates in patients although not necessarily cause complete tumor regression (Gradishar et al., 2009; Tabuchi et al., 2009). Paclitaxel, in particular among the taxanes, is one of the most common chemotherapeutic pharmaceuticals utilized against advanced metastatic TNBC; however, it has significant draw backs since it is easily tempered by resistance (Alves et al., 2018; Goldblatt et al., 2009; Stage et al., 2018).

Mechanisms of Chemoresistance

Cancer that forms, grows, and spreads quickly is known as aggressive. Aggressive cancer is typically difficult to treat with these characteristics. Despite the aggressive behavior of TNBC, there is a good initial response of patients to chemotherapy, as this treatment is not localized and could reach cells that may have invaded other tissues in the body (metastasized). Unfortunately, patients that have residual disease result with a worse prognosis (Carey et al., 2007). Residual disease occurs when some of the cancer cells evaded treatment. This evasion of treatment could occur for many reasons: the cells could have metastasized or they could exhibit resistance to therapeutic intervention.

Resistance to chemotherapy signifies that the cancer cells, or a subpopulation therein (pre-existing), can still grow in the presence of the pharmaceutical thus rendering the treatment ineffective. When a patient first undergoes chemotherapy, there is the possibility that their cancer cells contain mutations allowing them to be resistant to the therapeutic. When this occurs, it is classified as innate resistance, as the resistant cells existed prior to the full regimen of chemotherapy (Groenendijk & Bernards, 2014). After a full round of chemotherapy, if the patient experiences a recurrence of the cancer, there is the possibility that the cells will be resistant to chemotherapy as they have now gained mutations to be unresponsive to the chemotherapeutic agent. This classification is known as acquired chemoresistance as the resistant phenotype appears after being exposed to the therapeutic (Luqmani, 2005). Both innate and acquired chemoresistance comes with dramatically different results following therapeutic intervention (Catherine E Steding, 2016).

Several mechanisms have been proposed on chemoresistance including ATP-dependent efflux transporters, signaling pathways like PI3K/AKT/mTor and RAS/MAPK/ERK, microRNAs, and cancer stem cells (Fruci et al., 2016; Giltnane & Balko, 2014; Page et al., 2000; Pavlopoulou et al., 2016; Wang et al., 2015). In addition to these proposed mechanisms of chemoresistance, there are also mechanisms specific to acquired resistance based on the chemotherapy selected. For example, mechanisms unique to the taxanes are alterations in microtubules and key microtubule-associated proteins (Kavallaris, 2010).

Microtubules and Associated Proteins

To be able to combat chemoresistance, it is necessary to know how the pharmaceutical works and their targets for potential gain of resistance. The taxanes, including paclitaxel, affect cancer cells by blocking them in G2/M phase of the cell cycle and they do this by interacting

with the microtubules within the cells (Horwitz et al., 1986). Microtubules are long, filamentous, tubular protein polymers that are an important part of the cytoskeleton (Goodson & Jonasson, 2018). They are involved in cellular proliferation, shape, and movement, making them ideal targets for chemotherapy (Etienne-Manneville, 2013). Microtubules are long protofilaments consisting of alternating α- and β- subunits that are in a constant state of dynamic instability (Desai & Mitchison, 1997). This constant state of change is regulated by microtubule-associated proteins (MAPs).

MAPs regulate microtubules by binding to multiple different locations on the microtubules (Shi & Sun, 2017). If any changes in the MAPs occurred, it can cause a deregulation of microtubules that can lead to resistance to chemotherapeutic agents like paclitaxel (Shi & Sun, 2017). For example, there are MAPs that function in the polymerization and stabilization of the microtubule, including MAP2, EB1, and EB3 (Bauer et al., 2010; Gouveia et al., 2010; Luo et al., 2014). There are also MAPs that function in destabilization of the microtubule, including SIK2 and MCAK (Ahmed et al., 2010; Ganguly et al., 2011). Motor proteins within the cell can also function as MAPs, including KIF14, which functions in destabilizing the microtubules (Arora et al., 2014; Corson & Gallie, 2006). With a wide variety of MAPs, changes in any of them could result in the deregulation of microtubules that can lead to chemoresistance (Rodrigues-Ferreira et al., 2020; Shi & Sun, 2017).

Paclitaxel works by stabilizing cellular microtubules and blocking chromosome segregation. Paclitaxel binds specifically to the β-subunit within the microtubule where interactions with adjacent β-tubulin occur (Horwitz et al., 1986; Manfredi & Horwitz, 1984; Manfredi et al., 1982; Orr et al., 2003; Snyder et al., 2001). The stabilization ability of paclitaxel comes from this location of binding. When paclitaxel binds to this location, it strengthens the

lateral contacts between protofilaments by creating a conformational change within the structure (Jordan & Wilson, 2004; Nogales, 2001). Under normal circumstances, when the microtubule would need to be stabilized the microtubule-associated protein, tau, would bind to this location to stabilize the microtubule. However, when paclitaxel is present within the cell it competes with tau for this location resulting in tau being displaced. EB3 is another protein that is affected by the addition of paclitaxel. Studies have shown that when paclitaxel is added to a cell *in vitro,* EB3 is found to no longer be binding to the microtubule (Rovini et al., 2010; Shemesh & Spira, 2010) while *in vivo* studies have shown no change in levels of EB3 when paclitaxel is added (Benbow et al., 2016; Benbow et al., 2017). This could also affect the localization of another MAP, KIF2C, because the binding of EB3 to the microtubule promotes KIF2C binding, therefore, if EB3 is no longer binding then it is possible that KIF2C is also no longer binding.

Paclitaxel Resistance

Paclitaxel is effective in eliminating a majority of tumor cells; however, it is easily tempered by drug resistance and the mechanisms leading to resistance remains unclear (Bauer et al., 2010; Panayotopoulou et al., 2017). Many mechanisms have been researched to elucidate the mechanisms by which tumor cells can become resistant to paclitaxel. One proposed mechanism is through mutations in the binding with β-tubulin resulting in less stability of the microtubules (Ayers et al., 2004; Hari et al., 2006; Sledge et al., 2003). Some studies have reported different isoforms of β-tubulin being upregulated within a resistant population (Paradiso et al., 2005; Stengel et al., 2010; Tommasi et al., 2007). Another proposed mechanism is through the expression of the tubulin binding protein tau with expression indicating susceptibility to paclitaxel treatment (Rouzier et al., 2005; Smoter et al., 2011; Tanaka et al., 2009). Investigation into the expression of BRAC1, ER, and HER2 have also been investigated as possible

mechanisms for paclitaxel resistance (Estévez & Gradishar, 2004; Formenti et al., 1999; Formenti et al., 2003; Hess et al., 2006; Thuerigen et al., 2006). Resistance to paclitaxel has also been related to the increased expression of multidrug transporters, like p-glycoprotein 1 (Jeong et al., 2016). This research will focus on elucidating the mechanism of acquired paclitaxel resistance in relation to a microtubule-associated protein.

Combinatorial Therapeutics

There are many approaches to combat the development of resistance to chemotherapy and one of those avenues is with the use of a combination of treatment options. There are many different combinational therapies that can be administered as treatment options including: neoadjuvant therapy, in which a systemic therapy is delivered prior to local treatments, and adjuvant therapy in which systemic therapy is delivered post local treatments. TNBC is significantly heterogenous, with single-cell genomic analysis demonstrating that multiple different TNBC subtypes can be localized within one population, thus making effective treatment difficult (Lehmann, 2011). Therefore, using a novel and combinational approach could be more effective.

Combination therapy is starting to be used to increase efficacy of treatments for cancer. One of the common combinational therapy treatments with paclitaxel is combining it with immunotherapy. As early as 2006, paclitaxel was approved by the FDA to be combined with a recombinant monoclonal antibody for treatment of lung cancer (Cohen et al., 2007). Another combinational therapy is the combination of paclitaxel with inhibitors of signaling pathways or growth factors. Recently, paclitaxel was approved as a combinatorial with an inhibitor of an epidermal growth factor receptor (Kazandjian et al., 2016). One emerging treatment option is the use of epigallocatechin-3-gallate (EGCG). EGCG is the active ingredient in green tea and

previous work has demonstrated promising effects of a combination treatment of EGCG with paclitaxel (Ahmad et al., 2000; Mineva et al., 2013; Roy et al., 2005; Schröder et al., 2019).

Dissertation Focus

My research for this dissertation focuses on chemoresistance and a possible mechanism to chemoresistance to paclitaxel. To study chemoresistance in breast cancer, it is necessary to have an acquired chemoresistant breast cancer cell line. The status quo, as it pertains to chemoresistance, is to treat cells a few times before experimentation and deem them resistant; however, acquired resistance takes longer to achieve (Kenicer et al., 2014; Liu et al., 2014; Němcová-Fürstová et al., 2016; Sprouse & Herbert, 2014). By generating chemotherapeutic resistant breast cancer cell lines via incrementally increasing doses of a chemotherapeutic reagent, I have created a model system that can be utilized to define previously unidentified mechanisms of resistance. The extended treatments utilized in our laboratory are unique as they have been maintained in paclitaxel for an extended period. This better recapitulates the initial therapeutic suppressions that would be observed in a clinical setting. This technique also ensures that the cells retain a strong level of chemoresistance throughout the culture process.

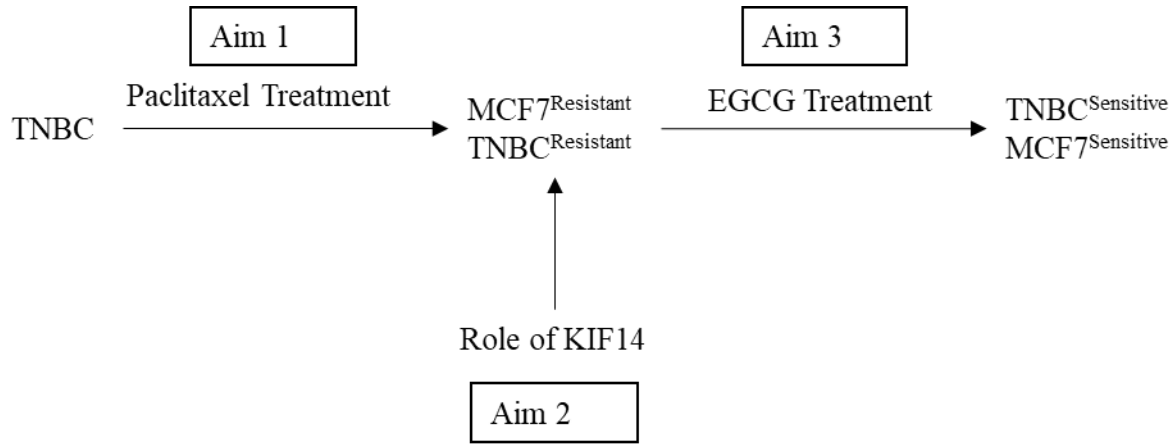
The first part of my research focuses on the creation of a TNBC cell line that has acquired resistance to paclitaxel (Aim 1) and any phenotypic changes these cells could possess compared to their sensitive counterparts (Figure 1.2). Genomic changes within this TNBC resistant cell line were evaluated using next-generation sequencing and preliminary analysis highlight a novel MAP to investigate the mechanism of acquired paclitaxel chemoresistance.

The second portion of my research delves into a MAP that could be a novel mechanism to acquired chemoresistance within a resistant cell line (Aim 2). Identification of the increased expression of a MAP, KIF14, led me to the hypothesis that the TNBC resistant cells upregulated

a destabilization MAP to counteract the stability of paclitaxel. Chapter 3 delves into the possible functions of KIF14 within TNBC as well as the resistant TNBC cell line.

Figure 1.2

Outline of Research



Note. Outline of research conducted within this dissertation.

Finally, I will investigate a potential adjuvant therapy to paclitaxel within both a TNBC and hormone responsive breast cancer cell line with the use of EGCG, the active ingredient in green tea (Aim 3) (Figure 1.2). There is a possibility that the co-treatment of paclitaxel and EGCG could result in the resistant cells being re-sensitized to paclitaxel.

The expected outcomes of this research will lead to a better understanding of the mechanism in which the acquisition of chemoresistance occurs. By having this understanding of acquired chemoresistance there is a potential to open new areas of translational research and clinical investigation.

CHAPTER 2

CHARACTERIZATION OF AN ACQUIRED CHEMORESISTANT BREAST CANCER CELL LINE

Abstract

Breast cancer remains the most diagnosed cancer in American women with a projected 44,000 women that will succumb to advanced, metastatic disease in the next calendar year. Advanced and recurrent breast cancers frequently exhibit clinical resistance to therapeutic interventions, including many that exhibit resistance against multiple drugs or therapies, resulting in chemoresistance being a cause for therapeutic failure. Chemoresistance can be classified as either innate or acquired, both of which result in dramatically different results following therapeutic intervention. Work in our laboratory has emphasized evaluating acquired chemoresistance against the microtubule-stabilizing drug, paclitaxel, and has demonstrated the ability to generate cells more resistant than cells treated with solvent controls. These paclitaxel resistant cells provide a critical model system for evaluating mechanisms of acquired chemoresistance. Findings from preliminary investigations of these chemoresistant cells indicate both novel growth patterns and dose-dependent reactions to paclitaxel. These changes in cellular behavior warrant further investigation as they imply a previously unreported mechanism of chemoresistance. Here, I show that MDA-MB-231 cells respond to paclitaxel in a dose-dependent manner and I create a long-term acquired paclitaxel resistant cell line. I then

characterized this cell line and demonstrated that although they are resistant to paclitaxel their basic phenotypic traits remain unaltered. However, there are significant changes to these cells on a genotypic level which could be the beginning of investigation into the mechanisms of acquired chemoresistance.

Introduction

Breast cancer is one of the most essential cancers to study, as advanced, metastatic disease is expected to claim approximately 44,000 American women this year alone (Siegel et al., 2021). The nature of breast cancer as a heterogeneous disease with a complicated and diverse tumor microenvironment makes it challenging to achieve significant, long-term efficacy in treatment. Although effective therapies exist, the combination of disease recurrence and the generation of resistance against multiple chemotherapeutic drugs make the development of new therapeutics essential. A key preliminary step in the generation of effective therapies is the development of a clear understanding of basic cancer cell behavior and mechanisms of cellular change in relation to chemoresistance.

Clinically, advanced and recurrent breast cancers frequently exhibit resistance to therapeutic interventions which often results in the failure of chemotherapy (Shi & Sun, 2017). Resistance to a chemotherapeutic drug signifies that the cancer cells, or a subpopulation therein (pre-existing), can still grow in the presence of the drug rendering the treatment ineffective. Chemoresistance can be classified one of two ways; the resistant cells existed prior to treatment, also known as innate resistance (Groenendijk & Bernards, 2014), or the cells gained the resistant phenotype after being exposed to the treatment, also known as acquired (Luqmani, 2005). Each of these resistant classifications come with dramatically different results following therapeutic intervention (Catherine E Steding, 2016).

While an understanding of innate resistance is important, it does not address the long-term consequences of therapeutic intervention that is characteristic of patient treatment. Acquired resistance by its nature can be considered an adaptive response over time. In patients, these adaptations can occur after few or many treatments and frequently results in recurrent, advanced disease with limited therapeutic options. As such, work in our laboratory has emphasized evaluating long-term treatments that better recapitulate acquired chemoresistance.

One of the most common classes of chemotherapeutics utilized in breast cancer therapy is the taxanes, a class of drugs that impact the dynamics of microtubules by stabilizing them within the cell (Goldblatt et al., 2009). Microtubules are long, filamentous, tubular protein polymers that are an important part of the cytoskeleton (Goodson & Jonasson, 2018). They are involved in cellular proliferation, shape, and movement (Etienne-Manneville, 2013). The taxanes are approved therapeutically in breast cancer when the cancer presents with lymph node involvement and further metastasis (Crown et al., 2004; Hassan et al., 2010; Jordan & Wilson, 2004; Sparano et al., 2008).

Treatment with taxanes has been shown to increase survival rates in patients although not necessarily cause complete tumor regression (Gradishar et al., 2009; Tabuchi et al., 2009). Paclitaxel, in particular among the taxanes, is one of the most common chemotherapeutic drugs utilized against breast cancer, however it has significant draw backs since it is easily tempered by drug resistance (Alves et al., 2018; Goldblatt et al., 2009; Stage et al., 2018).

Our laboratory has emphasized exploration of chemoresistance in relation to paclitaxel. Working with an acquired chemoresistant cell line is the long-established approach for studying the mechanisms of chemoresistance. Generation of a chemotherapeutic resistant breast cancer cell line via incrementally increasing doses of a chemotherapeutic reagent can be utilized to

define previously unidentified mechanisms of resistance (McDermott et al., 2014). Using a well characterized triple-negative breast cancer (TNBC) cell line, MDA-MB-231, we can evaluate molecular changes to a far greater degree. A TNBC cell line is vital for the study of chemoresistance as TNBC cells are characterized for being negative for key hormone responsive receptors: estrogen (ER), progesterone (PR), and human epithelial receptor 2 (HER2). By lacking these receptors, TNBC does not respond to hormonal treatment therapy and the best treatment option for TNBC is the use of chemotherapeutic agents, like paclitaxel (Luque-Bolivar et al., 2020).

Although chemoresistance to paclitaxel has been evaluated in the past, most of the model systems have utilized cells that have been maintained in paclitaxel for only brief periods of time and cells are considered to have acquired resistance if the cells survive after short-term assays (Dumontet et al., 1996; Guo et al., 2004; Han et al., 2018; Jeong et al., 2016; Lian et al., 2019; Liu et al., 2017; Sha et al., 2016; Sprouse & Herbert, 2014). However, the short-term treatments of these cells could be more indicative of innate resistance. It is estimated that creating an acquired chemoresistant cell line could take from three to eighteen months (McDermott et al., 2014). The extended treatments utilized in our laboratory are unique as they have been maintained in paclitaxel for multiple years; this better recapitulates the initial therapeutic suppressions that would be observed in clinic after several rounds of chemotherapy. This technique also ensures that the cells retain a strong level of chemoresistance throughout the culturing process.

Here, I show that MDA-MB-231 cells respond to paclitaxel in a dose-dependent manner and use this to create a long-term acquired paclitaxel resistant TNBC cell line. These cells generated using extended paclitaxel treatments have been designated as, MDA-MB-231^{Resistant}. I

then characterized this cell line and demonstrate that although they are resistant to paclitaxel their basic phenotypic traits remain unaltered. However, there are significant changes to these cells on a genotypic level which could be the beginning of investigation into the mechanisms of acquired chemoresistance.

Materials and Methods

Culture Conditions

The triple-negative breast cancer cell line (TNBC), MDA-MB-231, was maintained using traditional means (Steding et al., 2011). Cells were cultured in Nunc tissue culture flasks and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Cosmic Calf Serum (CCS) (Gibco and Fisher Scientific). Cells were maintained at 37℃ and 5% CO_2 at sub-confluent levels and passaged once cells reached a confluency between 75% - 95% via treatment with 0.25% Trypsin EDTA (Gibco). Images of the cells were captured at 50X magnification.

Table 2.1

Treatment Groups.

Name	Initiation of Resistance	T10	T20	T30	T40	T50	T60
MDA-MB-231^{Sensitive}	0.1% DMSO						
MDA-MB-231^{Resistant}	5 pg/mL Paclitaxel	10 pg/mL Paclitaxel	20 pg/mL Paclitaxel	40 pg/mL Paclitaxel	80 pg/mL Paclitaxel	90 pg/mL Paclitaxel	1.6 ng/mL Paclitaxel

Note. Table expressing treatment conditions of the cell line. MDA-MB-231 cells were passaged into two groups and treated with either Dimethyl Sulfoxide (DMSO), or paclitaxel. Once

treatments were initiated, treated cells were maintained as isolated cell lines designated as: MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant}. Cells were maintained with a concentration of 0.01% DMSO or 1.6 ng/mL of paclitaxel, respectively, weekly over the period of several years to maintain a resistant population.

Generation of Acquired Chemoresistant Cell Lines

MDA-MB-231 cell's dose response for paclitaxel was evaluated via a Methylene Blue Proliferation Assay. Briefly, cells were plated at a density of $1x10^5$ (short-term) or $1x10^4$ cells/well (long-term) then treated with 0.0005 ng/mL, 0.005 ng/mL, 0.05 ng/mL, 0.5 ng/mL, 5 ng/mL, 50 ng/mL, 500 ng/mL, or 5,000 ng/mL of paclitaxel diluted in Dimethyl Sulfoxide (DMSO), or 0.1% DMSO for control (Ctrl) and incubated at 37℃ and 5% CO_2 for 48 hours (short-term) or 7 days (long-term). Plates were then fixed with 100% methanol. Adherent cells were stained with 0.5% Methylene Blue and allowed to air dry. Treatment with 0.5 M hydrochloric acid (HCL) was used to redistribute stain into solution and absorbance was evaluated at 630nm using a spectrophotometer (BioTek). MDA-MB-231 cells were passaged into two groups and treated with either Dimethyl Sulfoxide (DMSO), MDA-MB-231^{Sensitive}, or 5 pg/mL paclitaxel, MDA-MB-231^{Resistant}, weekly over a period of several years. Every 10 treatments, the concentration of paclitaxel was increased until treatment 60 in which the MDA-MB-231^{Resistant} cells were maintained at a concentration of 1.6 ng/mL paclitaxel weekly indefinitely (Table 2.1).

Cell Invasion Assay

Cells were seeded in a 6-well plate with DMEM to be serum starved overnight at 37℃ and 5% CO_2. They were then placed within a Transwell^{TM} Multiple Well Plate with Permeable Polycarbonate Membrane Inserts (Corning) with the use of a .25% Trypsin. Cells were incubated

at 37℃ and 5% CO_2 for 48-hours at which point the DMEM was removed from the transwell. The transwell was then placed in 0.5% solution of Methylene Blue for 30 minutes with gentle agitation. The transwells were rinsed with Milli-Q water and allowed to dry overnight at room temperature. The transwells were placed in a 0.5 M HCL solution on a shaker for 30 minutes then the solution was placed in triplicate 96- wells and absorbance was read at 630nm using a spectrophotometer (BioTeK) (Kramer et al., 2013).

Cytotoxicity Assay

Following transfection, cells were assessed for cytotoxicity using CyQUANT™ LDH (lactate dehydrogenase) Cytotoxicity Assay following manufacturer's protocol (Invitrogen). Briefly, cells were seeded in a 96-well plate and incubated overnight at 37℃ and 5% CO_2. They were then treated with 10 ng/mL of paclitaxel or 0.1% DMSO for compound LDH release, or Milli-Q water, for spontaneous LDH release and incubated overnight at 37℃ and 5% CO_2. Wells serving as the maximum LDH release received 10x Lysis Buffer and the plate was incubated at 37℃ and 5% CO_2 for 45 minutes. Each sample was transferred to a 96-well plate in duplicate wells, Reaction Mixture was added, and the plate was incubated at room temperature for 30 minutes in the dark. Stop Solution was added to each well and the absorbance was measured at 492 nm and 680 nm using a spectrophotometer. LDH activity was determined by subtracting the 680 nm absorbance value from the 492 nm absorbance value before calculation of percent cytotoxicity. Percent cytotoxicity was calculated with the following equation:

$$Percent\ Cytotoxicity = \left[\frac{Compound\ treated\ LDH\ activity - Spontaneous\ LDH\ activity}{Maximum\ LDH\ activity - spontaneous\ LDH\ activity}\right] \times 100$$

Compound treated LDH activity was either those treated with DMSO as the control or paclitaxel. Paclitaxel treated cells were then normalized to the control.

Colony Formation Assay

Cells were seeded at 200 cells/mL in triplicate within 6-well plates and incubated at 37℃ and 5% CO_2 for 14 days. Plates were then fixed with 70% Ethanol and stained with 5% Geimsa for 1 hour at room temperature. Plates were rinsed with RO water and allowed to air dry, at which point stained colonies were counted.

Apoptosis Assay

The eBioscience™ Annexin V Apoptosis Detection Kit FITC (Invitrogen) was used per manufacturer's instructions to assess apoptosis following treatment with paclitaxel. Cells were treated with 10 ng/mL of paclitaxel or 0.1% DMSO. 48-hours following treatment cells were harvested, rinsed, and stained with Annexin V and Propidium Iodide. Flow cytometry was conducted on BD Accuri™ C6 Plus (BD) and data analysis was conducted using FlowJo software (Tree Star, Inc).

Proliferation Assay

Proliferation potential following treatment with paclitaxel was evaluated via a Methylene Blue Proliferation Assay. Cells were plated at a density of $1x10^5$ (short-term) or $1x10^4$ cells/well (long-term) then treated with 0.0005 ng/mL, 0.005 ng/mL, 0.05 ng/mL, 0.5 ng/mL, 5 ng/mL, 50 ng/mL, 500 ng/mL, or 5,000 ng/mL of paclitaxel diluted in DMSO, or 0.1% DMSO for control (Ctrl) and incubated at 37℃ and 5% CO_2 for 48 hours (short-term) or 5 days (long-term). Plates were then fixed with 100% methanol. Adherent cells were stained with 0.5% Methylene Blue and allowed to air dry. Treatment with 0.5 M HCL was used to redistribute stain into solution and absorbance was evaluated at 630 nm using a spectrophotometer (BioTek) (Oliver et al., 1989).

Trypan Blue Exclusion Assay

Relative population doubling and cellular viability was determined using a Trypan Blue Exclusion Assay. Cells were plated at a density of $5x10^4$ cells/well (short-term) or $2.5x10^4$

cells/well (long-term) and treated with 10ng/mL paclitaxel diluted in DMSO, or 0.1% DMSO. Cells were then incubated at 37℃ and 5% CO_2 for 48 hours (short-term) or 5 days (long-term). Cells were harvested using 0.25% Trypsin EDTA and the resulting cell population was evaluated using an automated cell counter (BIO-rad). Relative population doubling was calculated by the following equation and then normalized to control cells.

$$\frac{\text{Number of Live Cells}}{\text{Number of Plated Cells}}$$

Viability of cells was calculated by the following equation and was normalized to the control cells.

$$\frac{\text{Number of Live Cells}}{\text{Total Number of Cells}} * 100\%$$

RNA-Sequencing

MDA-MB-231$^{\text{Sensitive}}$, and MDA-MB-231$^{\text{Resistant}}$ cells were pelleted, and flash frozen in liquid nitrogen. RNA was extracted using the RNAeasy Mini Kit Plus (Qiagen). RNA levels were quantified using a NanoDrop spectrophotometer (ThermoFisher) and RNA quality was assessed using the TapeStation (Agilent Technologies). Next generation sequencing using the Illumina HiSeq4000 sequencing system was performed at the Indiana University Center for Medical Genomics Core Facility (Breese & Liu, 2013; Dobin et al., 2013; Liao et al., 2014; Robinson et al., 2010).

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Cells were harvested and RNA was isolated using the PureLink RNA Mini Kit (Invitrogen) and the quantity of RNA was assessed using the NanoDrop™ One Spectrophotometer system (Thermo Scientific). TaqMan gene expression assays were used to quantify the mRNA expression of MAP2 (Hs00258900_m1). Quantification of gene expression was performed in triplicate in a 50uL volume in 96- well plates on Applied Biosystems®

QuantStudio® 3 Real-Time PCR System using a TaqMan RNA-to-C_t 1-step kit (Applied Biosystems). MDA-MB-231^{Resistant} groups were normalized to the control to determine percent change in expression levels (Guan & Yang, 2008; Pfaffl, 2001; Tsai & Wiltbank, 1996).

Statistics. Data was expressed as the means ± the standard deviation. IBM SPSS Statistics Version 27 was used for statistical analysis. For two-group comparisons, normally distributed data with homogeneity of variance, an independent *t*-test was conducted. Data comparison between multiple groups was performed using one-way analysis of variance (ANOVA) (Brown, 2005; Kim, 2017). A Levene's test of homogeneity was used, if Levene's statistic was not significant, then homogeneity of variance was assumed, and a Tukey's post hoc test was conducted to determine individual group statistics (Abdi & Williams, 2010; Glass, 1966). If Levene's statistic was significant, then homogeneity of variance was not assumed, and a Welch's test of homogeneity was conducted with a Games-Howell post hoc test. *p*-values of less than 0.05 were considered significant (Sauder & DeMars, 2019).

Results

MDA-MB-231 Cells Respond to Paclitaxel in a Dose-dependent Manner with a Significant Decrease in Proliferation in a Long-term Setting

A methylene blue proliferation assay was performed with paclitaxel at concentrations of 0.0005 ng/mL, 0.005 ng/mL, 0.05 ng/mL, 0.5 ng/mL, 5 ng/mL, 50 ng/mL, 500 ng/mL, and 5,000 ng/mL to determine the optimal concentration to begin the process of acquired chemoresistance (Figure 2.1). The triple-negative breast cancer (TNBC) cell line, MDA-MB-231, was treated with paclitaxel for 48-hours to determine the short-term effect of paclitaxel on the cell's proliferation potential (Figure 2.1A). There was a minute decrease in proliferation between the concentrations of 0.0005 ng/mL (M = 0.44, SD = 0.14) and 0.05 ng/mL (M = 0.41, SD = 0.12) of paclitaxel, however at a concentration of 0.5 ng/mL (M = 0.37, SD = 0.12), there was a greater

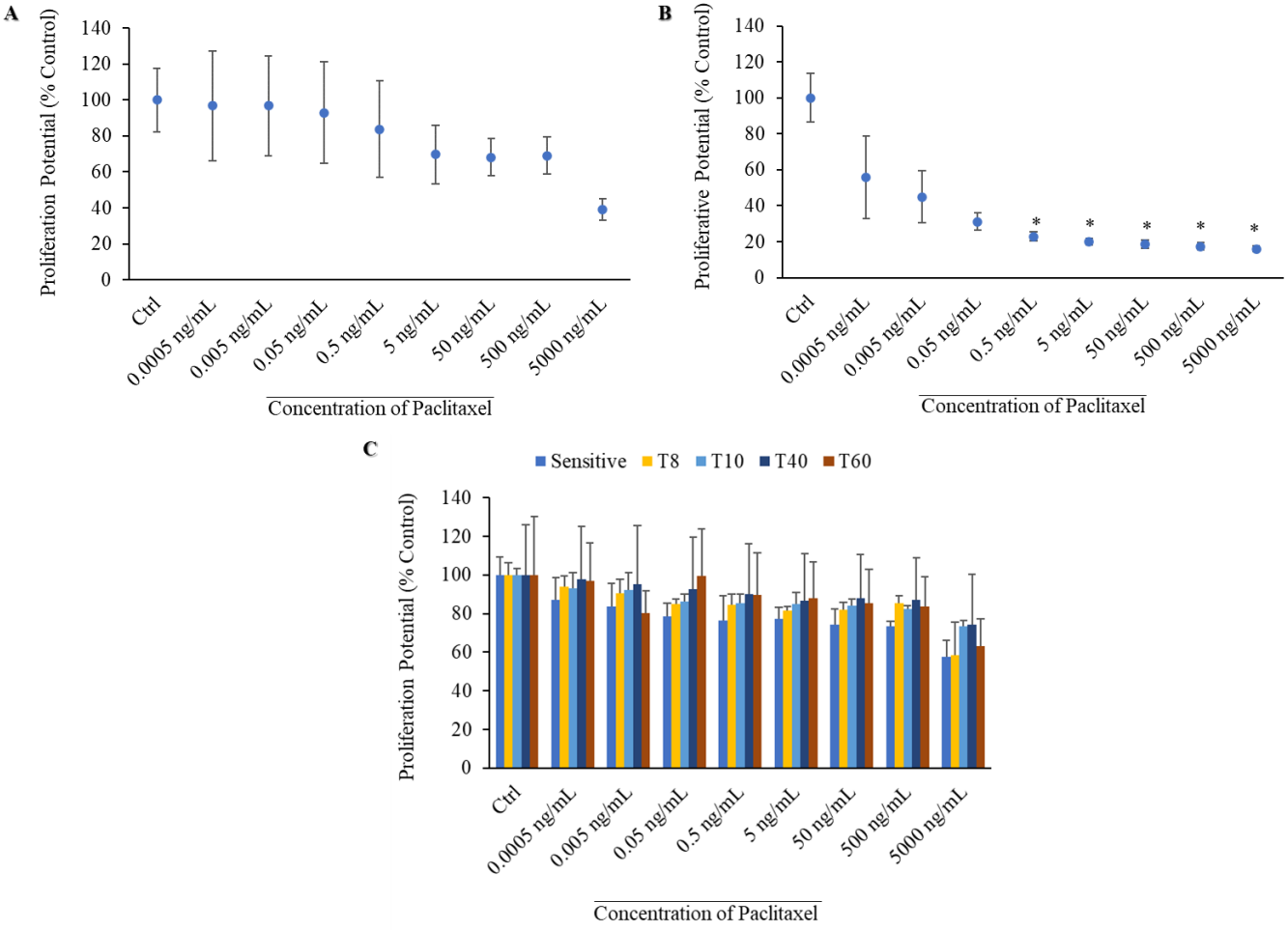
decrease in proliferation (16.32 ± 26.77%, p = .41) compared to the control (M = 0.44, SD = 0.08) though this was not a significant decrease as demonstrated by an independent *t*-test, $t(4) = 0.89$, $p = .41$. This trend continued with a decrease in proliferation (30.36 ± 16.11%, p = .82) at a concentration of 5 ng/mL (M = 0.31, SD = 0.07) compared to the control, however, this was not significant, $t(4) = 2.2$, $p = .82$. The decrease in proliferation compared to the control steadied at concentrations of paclitaxel between 5 ng/mL and 500 ng/mL (M = 0.14, SD = 0.02), however at a concentration of 5,000 ng/mL (M = 0.17, SD = 0.03) there was a steep decrease in proliferation (60.84 ± 6.08%, p = .13) compared to the control. Even with these decreases in proliferation with the short-term treatment of paclitaxel an independent *t*-test demonstrated that there was no significant decrease in proliferation between treatments, $t(4) = 5.64$, $p = 0.13$ (Tables 2.S1 – 8).

To evaluate the long-term effects of paclitaxel, a 7-day assay was conducted with the same concentrations of paclitaxel (Figure 2.1B). Unlike in the short-term there was a greater decrease in proliferation (44.24 ± 22.89%, p = .52) at a concentration of 0.0005 ng/mL (M = 0.44, SD = 0.18) compared to control (M = 0.79, SD = 0.11), however, similar to the short-term it was not a significant decrease, $t(4) = 2.88$, $p = .52$. This trend continued until a concentration of 0.5ng/mL of paclitaxel (M = 0.18, SD = 0.02), in which there was a significant decrease in proliferation (77.14 ± 2.51%, p = .04) compared to the control, $t(4) = 9.66$, $p = .04$. There was also a significant decrease in proliferation (79.87 ± 1.83%, p = .03) at concentrations of 5 ng/mL (M = 0.16, SD = 0.01) compared to the control, $t(4) = 10.08$, $p = .03$. This trend continued with a significant decrease in proliferation (81.29 ± 2.30%, p = .33) at a concentration of 50 ng/mL (M = 0.15, SD = 0.02) compared to the control and continued at 500 ng/mL of paclitaxel (M = 0.14, SD = 0.02), $t(4) = 10.21$, $p = .04$. The greatest decrease in proliferation (83.95 ± 1.58%, p = .03) occurred at a concentration of 5,000 ng/mL (M = 0.13, SD = 0.01) compared to the control, *t*(4)

= 10.62, *p* = .03 (Tables 2.S9 – 16). With this steady decrease in proliferation in the long-term, the concentration of 0.005 ng/mL of paclitaxel (M = 0.35, SD = 0.11) was selected to begin continuous treatment with as there was about a 50% decrease in proliferation (55.05 ± 14.44%, p = .49), cells were then designated MDA-MB-231^{Resistant}.

Figure 2.1

MDA-MB-231 Cells Respond to Paclitaxel in a Dose-dependent Manner with a Significant Decrease in Proliferation in a Long-term Setting



Note. Proliferation potential following treatment with paclitaxel at concentrations 0.0005ng/mL, 0.005 ng/mL, 0.05 ng/mL, 0.5 ng/mL, 5 ng/mL, 50 ng/mL, 500 ng/mL, or 5,000 ng/mL of

paclitaxel diluted in DMSO, or 0.1% DMSO for control (Ctrl) were evaluated via a Methylene Blue Proliferation Assay. (A) 48-hour short-term, (B) 7-day long-term. N = 3. (C) 48-hour short-term assay conducted at various treatments. N = 2.

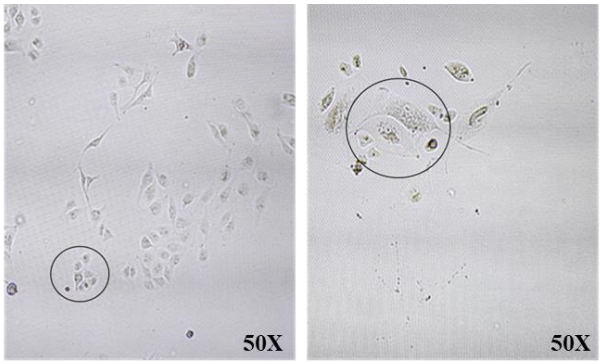
Cells were treated with this concentration of paclitaxel every six days until the 10^{th} treatment in which the concentration was increased and continuously increased every 10 treatments. At the 60^{th} treatment, the cells were increased to and maintained at a concentration of 1.6 ng/mL of paclitaxel. Periodically, at different treatments, preliminary investigation of acquired resistance was explored using a short-term Methylene Blue Proliferation Assay to monitor resistance levels (Figure 2.1C). At each concentration, there was a slight increase in proliferation potential of the resistant cells compared to the control until treatment 60; at this time, the increase trend steadied, so treatment was maintained at the concentration of 1.6 ng/mL of paclitaxel weekly for further experimentation.

MDA-MB-231^{Resistant} Cells Express Distinct Morphological Features Including an Increase in Cellular Size

Under normal culturing conditions of the MDA-MB-231^{Senesitve} and MDA-MB-231^{Resistant} cells it was observed that the MDA-MB-231^{Resistant} cells demonstrated an increase in cellular size. Normally, MDA-MB-231 cells are smaller and circular in shape as seen in the MDA-MB-231^{Sensitive} cells, however the MDA-MB-231^{Resistant} cells have developed a larger morphological size (Figure 2.2).

Figure 2.2

MDA-MB-231^{Resistant} Cells Express Distinct Morphological Features Including an Increase in Cellular Size



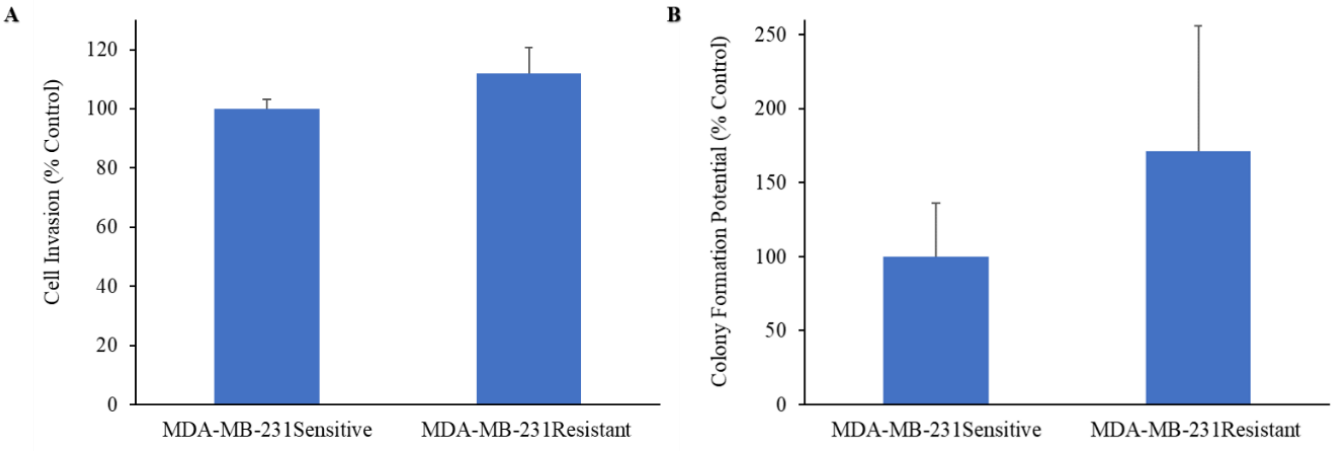
Note. Evaluation of Phenotypic Changes in Resistant Cells. MDA-MB-231 breast cancer cells were cultured under normal conditions and photographed at 50X magnification. Distinct morphological features, including increases in size, were observed for the resistant cells.

MDA-MB-231^{Resistant} Cells Express an Increase in Metastatic Ability as Demonstrated by an Increase in Cell Invasion and Colony Formation Potential

To investigate the metastatic ability of the MDA-MB-231^{Resistant} cells, a cell invasion and colony formation assay were conducted (Figure 2.3). MDA-MB-231^{Resistant} cells (M = 0.44, SD = 0.136) demonstrated an increase in cell invasion potential (11.94 ± 3.10%, p = .14) compared to the MDA-MB-231^{Sensitive} cells (M = 0.14, SD = 0.01) (Figure 2.3A), however, this was not a significant increase, $t(4) = 2.20$, $p = .14$ (Table S17). The MDA-MB-231^{Resistant} cells (M = 66.22, SD = 23.91) also expressed an increase in colony formation ability (71.56 ± 36.11%, p = .12) compared to the control (M = 113.61, SD = 56) (Figure 2.3B), however, as with the cell invasion it was not a significant increase, $t(4) = 1.35$, $p = .12$ (Table 2.S18).

Figure 2.3

MDA-MB-231^{Resistant} Cells Express an Increase in Metastatic Ability as Demonstrated by an Increase in Cell Invasion and Colony Formation Potential



Note. (A) Cells were serum starved for 24 hours. Cell invasion assay was plated using a Transwell^{TM} Multiple Well Plate. Methylene blue and HCL was used to stain and de-stain transwells then absorbance was read at 630nm. N = 3. (B) Colony formation assay was plated and incubated for 14 days. Plates were fixed with ethanol and stained with geimsa. N = 3. Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using an independent *t*-test.

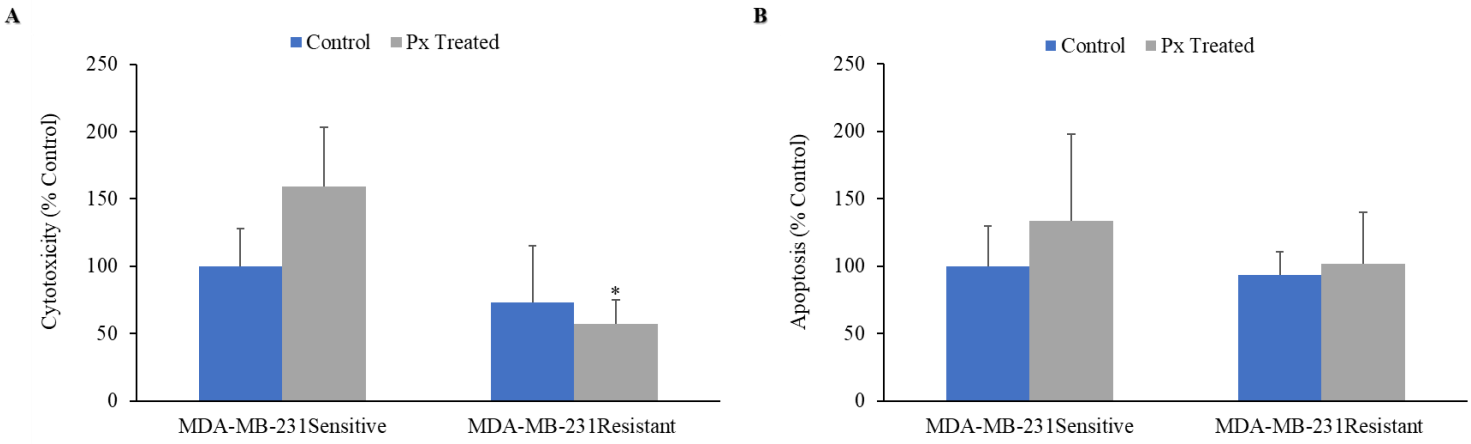
MDA-MB-231^{Resistant} Cells Express a Decrease in Cellular Death as Demonstrated by a Decrease in Cellular Cytotoxicity and Apoptosis

A lactate dehydrogenase (LDH) activity assay was conducted to investigate the cytotoxicity of paclitaxel on the MDA-MB-231^{Resistant} cells (Figure 2.4A). The MDA-MB-231^{Senstive} cells treated with paclitaxel (M = 21.17, SD = 5.84) demonstrated an increase in cytotoxicity (59.10 ± 43.92%, p = .29) compared to the MDA-MB-231^{Sensitive} control cells (M = 13.31, SD = 3.73), while the MDA-MB-231^{Resistant} cells (M = 8.85, SD = 6.88) expressed a slight decrease in cytotoxicity (26.53 ± 41.92%, p = .71) compared to the MDA-MB-231^{Sensitive} control

cells. A one-way analysis of the variance (ANOVA) with a Tukey's Post-Hoc test demonstrated that when the MDA-MB-231^{Resistant} cells are treated with paclitaxel (M = 7.65, SD = 2.32) they have a significant decrease in cytotoxicity to paclitaxel (101.60 ± 17.43%, p = .04) compared the MDA-MB-231^{Sensitive} cells treated with paclitaxel, F(3, 8) = 4.46, $p < .05$, $\eta 2p = .63$ (Table 2.S19).

Figure 2.4

MDA-MB-231^{Resistant} Cells Express a Decrease in Cellular Death as Demonstrated by a Decrease in Cellular Cytotoxicity and Apoptosis



Note. (A) Cytotoxicity assay was conducted with a CyQUANT^{TM} LDH Cytotoxicity Assay following manufacturer's protocol. Cells were treated with DMSO (control) or paclitaxel (px). Absorbance was measured at 492 nm and 680 nm. LDH activity was determined prior to calculation of percent cytotoxicity. N = 3. (B) The eBioscience^{TM} Annexin V Apoptosis Detection Kit FITC was used per manufacturer's instructions to assess apoptosis following treatment with paclitaxel (px). Flow cytometry was conducted, and data analysis was conducted using FlowJo software. N = 3.

Data are presented as means ± standard deviation. Differences between groups were compared using a one-way ANOVA with Tukey's post hoc test. * $p < .05$ vs MDA-MB-231^{Sensitive} px treated.

To investigate MDA-MB-231^{Resistant} cells ability to withstand paclitaxel induced apoptosis, an apoptosis assay was used (Figure 2.4B). MDA-MB-231^{Senstive} cells treated with paclitaxel (M = 8.28, SD = 3.98) demonstrated an increase in apoptosis (33.65 ± 64.23%, p = .75) compared to the MDA-MB-231^{Sensitive} control cells (M = 6.19, SD = 1.83), while the MDA-MB-231^{Resistant} cells (M = 5.78, SD = 1.06) and the MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 6.32, SD = 2.35) did not show a change in apoptosis compared to the MDA-MB-231^{Sensitive} control cells. MDA-MB-231^{Resistant} cells treated with paclitaxel did show a slight increase (8.57 ± 17.09%, p = .99) in apoptosis compared the MDA-MB-231^{Resistant} cells, though this change was insignificant. A one-way ANOVA demonstrated that there was no significant difference in apoptosis between groups, $F(3, 8) = 3.72$, $p > .05$, $\eta 2p = .18$ (Table 2.S20).

MDA-MB-231^{Resistant} Cells Express an Increase in Growth Potential as Demonstrated with Proliferation and Relative Population Doublings

To explore the phenotype of cellular growth within the MDA-MB-231^{Resistant} cells, proliferation and relative population doubling assays were conducted (Figure 2.5). A Methylene Blue Proliferation assay was performed to investigate changes in proliferation potential within the MDA-MB-231^{Resistant} cells. Cells were treated with paclitaxel for 48-hours to determine the short-term effect of paclitaxel on the cell's proliferation potential (Figure 2.5A). There was a slight decrease in proliferation (9.44 ± 15.96%, p = .73) when the MDA-MB-231^{Sensitive} cells are treated with paclitaxel (M = 0.93, SD = 0.16) compared to the MDA-MB-231^{Sensitive} control cells (M = 1.03, SD = 0.10), whereas the MDA-MB-231^{Resistant} cells (M = 1.11, SD = 0.09) show a

minute increase in proliferation (8.15 ± 8.31%, p = .81) compared to the MDA-MB-231^{Sensitive} control cells. When the MDA-MB-231^{Resistant} cells are treated with paclitaxel (M = 1.06, SD = 0.09) they demonstrate an increase in proliferation (13.20 ± 15.96%, p = .50) compared to the MDA-MB-231^{Sensitive} control cells treated with paclitaxel. A one-way ANOVA demonstrated that there was no significant difference in proliferation between groups, F(3, 8) = 1.37, $p > .05$, $\eta 2p$ = .34 (Table 2.S21).

To evaluate the long-term effects of paclitaxel on the MDA-MB-231^{Resistant} cell population a 5-day assay was conducted (Figure 2.5B). Unlike in the short-term, MDA-MB-231^{Sensitive} cells treated with paclitaxel (M = 0.32, SD = 0.07) demonstrated a significant decrease in proliferation (59.71 ± 8.71%, p < .001) compared to the MDA-MB-231^{Sensitive} control cells (M = 0.80, SD = 0.13). The MDA-MB-231^{Resistant} cells (M = 0.91, SD = 0.09) did not demonstrate a significant change in proliferation compared to the MDA-MB-231^{Sensitive} control cells (12.83 ± 11.27%, p = .58). A one-way ANOVA with a Tukey's Post-Hoc test demonstrated that there was a significant decrease in the proliferation potential (51.63 ± 11.27%, p = .003) of MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 0.39, SD = 0.08) compared to the MDA-MB-231^{Sensitive} control cells and a significant decrease (64.47 ± 10.31%, p < .001) compared to the MDA-MB-231^{Resistant} cells, F(3, 8) = 27.54, $p < .001$, $\eta 2p$ = .91 (Table 2.S22).

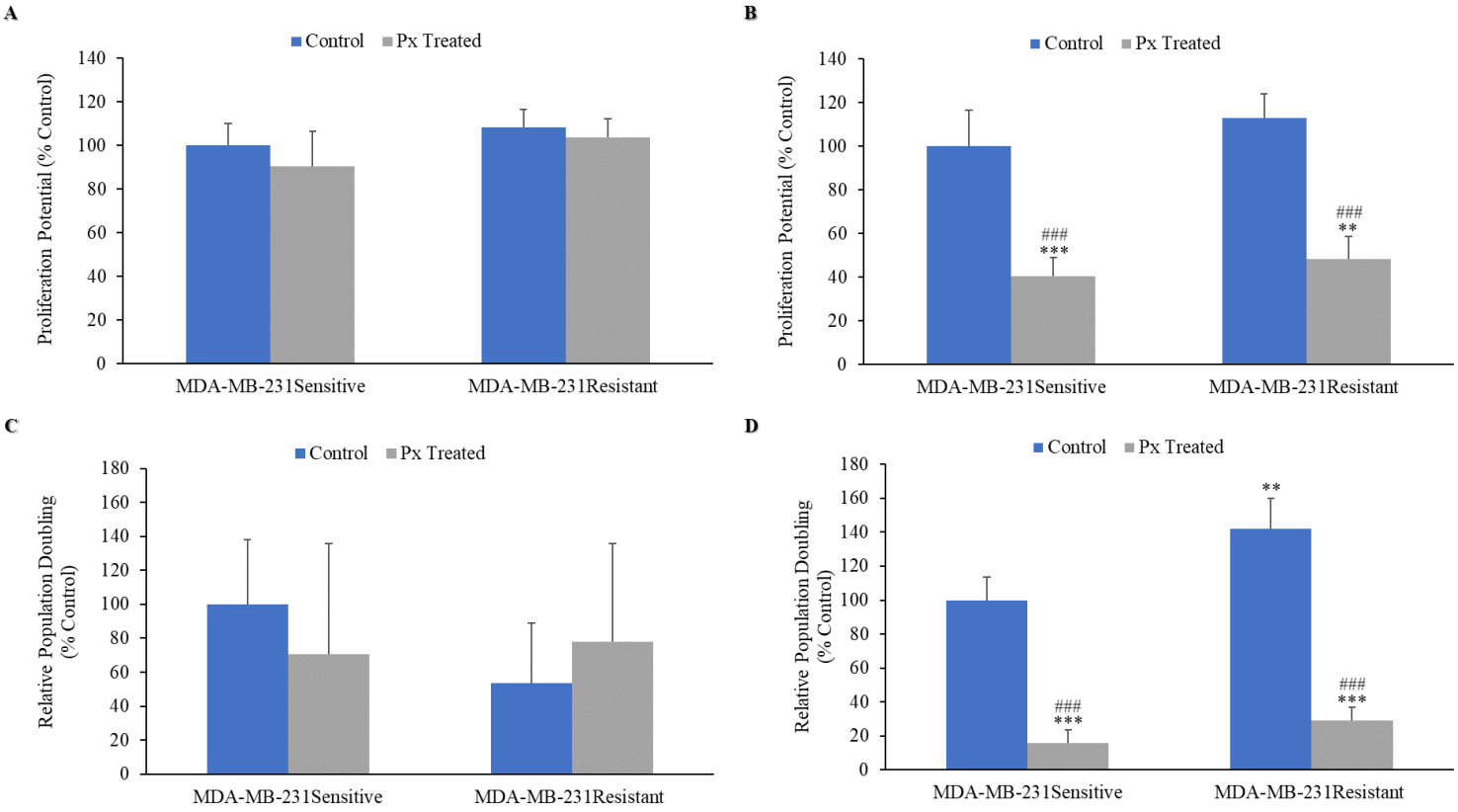
A trypan blue exclusion assay was conducted to investigate the MDA-MB-231^{Resistant} cells ability to increase in population size. A short-term 48-hour assay was conducted to explore the MDA-MB-231^{Resistant} cells ability to grow in a small amount of time (Figure 2.5C). MDA-MB-231^{Sensitive} cells treated with paclitaxel (M = 4.78, SD = 3.79) demonstrated a decrease in population doubling (29.50 ± 38.02%, p = .93) compared to the MDA-MB-231^{Sensitive} control cells (M = 6.14, SD = 2.16). The MDA-MB-231^{Resistant} cells (M = 4.53, SD = 2.79) also

demonstrated a decrease in population doubling (46.54 ± 35.35%, p = .87) compared to the MDA-MB-231^{Sensitive} control cells. Interestingly though, the MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 4.44, SD = 1.71) demonstrated an increase in population doubling (24.45 ± 57.63%, p = 1.0) compared to the MDA-MB-231^{Resistant} cells, however an ANOVA demonstrated that there was no significant difference between the different treatment groups, F (3, 8) = .25, $p > .05$, $\eta 2p = .087$ (Table 2.S23).

A long-term 5-day trypan blue assay was conducted to investigate the relative population doubling of the MDA-MB-231^{Resistant} cells (Figure 2.5D). MDA-MB-231^{Sensitive} cells treated with paclitaxel (M = 5.10, SD = 2.53) demonstrated a significant decrease in relative population doublings (84.31 ± 13.63%, p < .001) compared to the MDA-MB-231^{Sensitive} cells (M = 32.49, SD = 4.43) as well as a significant decrease (126.35 ± 7.79%, p < .001) compared to the MDA-MB-231^{Resistant} cells (M = 46.15, SD = 5.76), as expected. Interestingly, the MDA-MB-231^{Resistant} cells demonstrated a significant increase in relative population doubling (42.04 ± 17.71%, p = .014) compared to the MDA-MB-231^{Sensitive} cells. The MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 9.52, SD = 2.54) also showed a significant decrease in relative population doublings (70.69 ± 7.81%, p < .001) compared to the MDA-MB-231^{Sensitive} cells and to the MDA-MB-231^{Resistant} cells (112.74 ± 7.81%, p < .001). A one-way ANOVA with a Tukey's Post-Hoc test demonstrated that there was significant differences between the relative population doublings of the treatment groups, F(3, 8) = 68.79, $p < .001$, $\eta 2p = .96$ (Table 2.S24).

Figure 2.5

MDA-MB-231^{Resistant} Cells Express an Increase in Growth Potential as Demonstrated with Proliferation and Relative Population Doublings



Note: Proliferation potential following treatment with paclitaxel (px) was evaluated via a Methylene Blue Proliferation Assay. (A) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3. Relative population doubling was determined using a Trypan Blue Exclusion Assay following treatment with paclitaxel (Px). (C) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (D) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with Tukey's post hoc test. ** p <

0.01 vs MDA-MB-231^{Sensitive} control, *** p < 0.001 vs MDA-MB-231^{Sensitive} control, ### p < 0.001 vs MDA-MB-231^{Resistant} control.

MDA-MB-231^{Resistant} Cells Express a Change in Health as Demonstrated by Viability

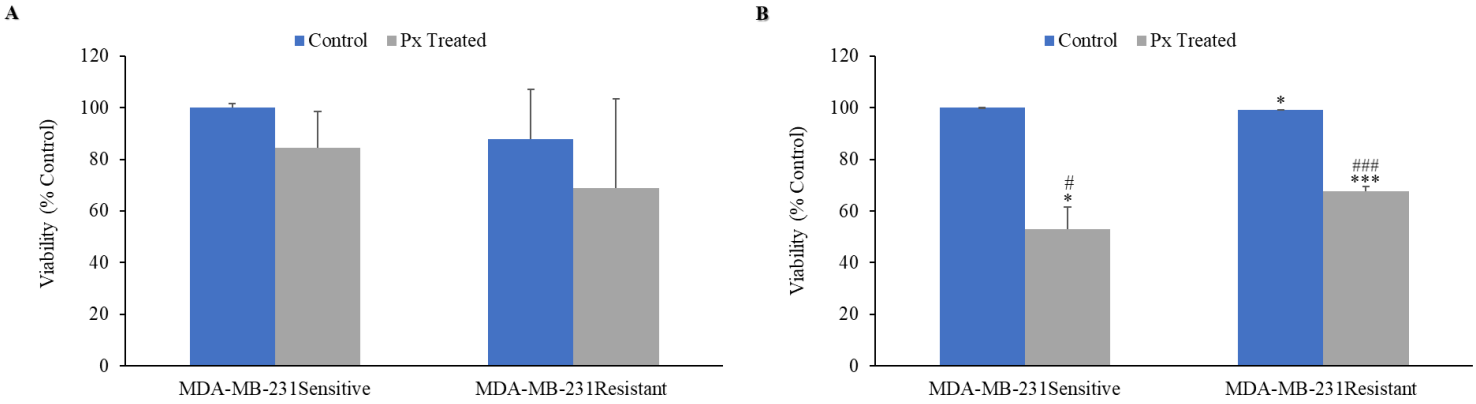
To explore the health of the MDA-MB-231^{Resistant} population, a trypan blue exclusion assay was conducted (Figure 2.6). A short-term 48-hour assay was conducted to explore the viability of the MDA-MB-231^{Resistant} cells, alone and in the presence of paclitaxel (Figure 2.6A). MDA-MB-231^{Sensitive} cells treated with paclitaxel (M = 82.44, SD = 18.97) demonstrated a slight decrease in viability (15.66 ± 14.31%, p = .44) compared to the MDA-MB-231^{Sensitive} control cells (M = 97.75, SD = 1.64). The MDA-MB-231^{Resistant} cells (M = 85.83, SD = 18.97) and the MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 67.19, SD = 33.97) also expressed a slight decrease in viability (12.19 ± 19.40%, p = .73 and 31.26 ± 34.75%, p = .54, respectively) compared to the MDA-MB-231^{Sensitive} control cells. However, a one-way ANOVA demonstrated that there was no significant difference between treatment groups in short-term viability, F(3, 3.38) = 1.69, $p > .05$, $\eta 2p = .29$ (Table 2.S25).

A 5-day trypan blue exclusion assay was performed to investigate the long-term effects of paclitaxel in the MDA-MB-231^{Resistant} cells (Figure 2.6B). MDA-MB-231^{Sensitive} cells treated with paclitaxel (M = 52.75, SD = 8.27) demonstrated a significant decrease in viability (46.90 ± 8.32%, p = .02) compared to the MDA-MB-231^{Sensitive} control cells (M = 99.33, SD = 0.14) and a significant decrease (46.06 ± 8.32%, p = .03) compared to the MDA-MB-231^{Resistant} cells (M = 98.5, SD = 0.00). The MDA-MB-231^{Resistant} cells expressed a minute significant decrease in viability (0.84 ± 0.00%, p = .03) compared to the MDA-MB-231^{Sensitive} control cells. A one-way ANOVA with a Games-Howell Post-Hoc demonstrated that compared to the MDA-MB-231^{Resistant} cells treated with paclitaxel (M = 67.11, SD = 1.92) had a significant decrease in

viability (32.44 ± 1.94%, p = .003) compared to the MDA-MB-231^{Sensitive} cells as well as a decrease in viability (31.60 ± 1.94%, p = .003) compared to MDA-MB-231^{Resistant} cells, F(3, 8) = 90.06, $p < .001$, $\eta 2p = .97$ (Table 2.S26).

Figure 2.6

MDA-MB-231^{Resistant} Cells do not Express a Change in Health as Demonstrated by Viability



Note: Viability was determined using a Trypan Blue Exclusion Assay following treatment with paclitaxel (px). (A) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, n = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with Games-Howell post hoc test. * p < 0.05 vs MDA-MB-231^{Sensitive} control, *** p < 0.001 vs MDA-MB-231^{Sensitive} control, # p < 0.05 vs MDA-MB-231^{Resistant} control, ### p < 0.001 vs MDA-MB-231^{Resistant} control.

MDA-MB-231^{Resistant} cells are Fundamentally Altered on a Genomic Level

To further investigate the MDA-MB-231^{Resistant} cell population, RNA-sequencing was performed on the MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} cell populations (Table 2.2). Preliminary comparison of the MDA-MB-231^{Sensitive} cells to the MDA-MB-231^{Resistant} cells demonstrated that there was a total of 2,169 genes significantly ($p < .05$) altered within the MDA-MB-231^{Resistant} cells. Of these genes, 1,159 were significantly upregulated while 1,010 were significantly downregulated.

Table 2.2

Gene changes within the MDA-MB-231^{Resistant} Population.

	$p < .05$
Total	2,169
Upregulated	1,159
Downregulated	1,010

Note. MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} cells were pelleted and flash frozen in liquid nitrogen. RNA was extracted using the RNAeasy Mini Kit Plus. RNA levels were quantified using a NanoDrop spectrophotometer and RNA quality was assessed using the TapeStation. Next generation sequencing using the Illumina HiSeq4000 sequencing system was performed at the Indiana University Center for Medical Genomics Core Facility, n=3. MDA-MB-231^{Resistant} cells were then compared to the MDA-MB-231^{Sensitive} cells to identify the number of genes with a significant difference in expression ($p < .05$). Listed here are the total number of genes along with the number of genes upregulated or down regulated.

Table 2.3

Common Microtubule-Associated Proteins Involved in Paclitaxel Resistance

Gene	Role in Microtubule Dynamics	Regulation in Paclitaxel Resistance	Sequencing				Citations
			Log_2Fold Change	P-Value	FDR	Up/ Down	
MAP2	Stabilization	Down	1.03	$1.32x10^{-5}$ ***	$6.20x10^{-5}$	Up	(Bauer et al., 2010)
EB1	Assembly and Stabilization	Down	0.25	0.13	$2.24x10^{-1}$	Up	(Luo et al., 2014; Thomas et al., 2015)
EB3	Assembly and Stabilization	Down	-1.18	$3.39x10^{-13}$ ***	$4.12x10^{-12}$	Down	(Gouveia et al., 2010; Schrøder et al., 2011; Yang et al., 2017)
SIK2	Destabilization	Up	0.18	0.19	0.29	Up	(Ahmed et al., 2010; Li et al., 2015; Liu et al., 2016; Zohrap et al., 2018)
KIF14	Destabilization	Up	1.59	$2.83x10^{-15}$ ***	$4.18x10^{-14}$	Up	(Arora et al., 2014; Corson & Gallie, 2006; Corson et al., 2007; Li et al., 2017; Madhavan et al., 2009; Qiu et al., 2017; Wang et al., 2016; Yang et al., 2019; Zhang et al., 2017)
MCAK	Destabilization	Up	0.98	$1.85x10^{-14}$ ***	$2.52x10^{-13}$	Up	(Ganguly et al., 2011; Sanhaji et al., 2011; Xie et al., 2016)

Note. MDA-MB-$231^{Sensitive}$ and MDA-MB-$231^{Resistant}$ cells were pelleted and flash frozen in liquid nitrogen. RNA was extracted using the RNAeasy Mini Kit Plus. RNA levels were quantified using a NanoDrop spectrophotometer and RNA quality was assessed using the TapeStation. Next generation sequencing using the Illumina HiSeq4000 sequencing system was performed at the Indiana University Center for Medical Genomics Core Facility, n=3. MDA-

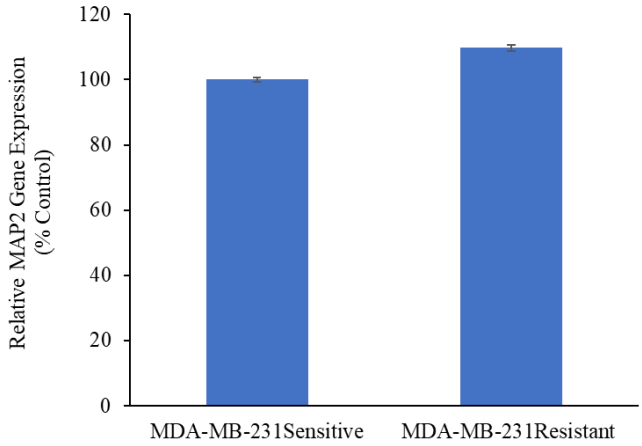
MB-231^{Resistant} cells were then compared to the MDA-MB-231^{Sensitive} cells to identify genes with a significant difference in expression. Listed here are common microtubule-associated proteins involved in paclitaxel resistance along with reported regulation, and sequencing results of the MDA-MB-231^{Resistant} cells.

*** $p < .001$ MDA-MB-231^{Resistant} versus the MDA-MB-231^{Sensitive}

With paclitaxel's known mechanism of action to be on the microtubules, common microtubule-associated proteins (MAPs) known to be associated with paclitaxel resistance are listed in Table 2.3. Preliminary sequencing results of EB1 showed an upregulation in the MDA-MB-231^{Resistant} cells compared to MDA-MB-231^{Senstive} cells, however, this was not significant. EB3 showed a significant ($p < .001$) downregulation within the MDA-MB-231^{Resistant} cells compared the MDA-MB-231^{Sensitive} cells. SIK2, KIF14, and MCAK, all demonstrated a significant ($p < .001$) upregulation within the MDA-MB-231^{Resistant} cells compared the MDA-MB-231^{Sensitive} cells.

Figure 2.7

MAP2 Expression is not Significantly Altered based on RT-PCR



Note. RNA was isolated from MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} cells, and the quantity was assessed. TaqMan gene expression assays were used to quantify the mRNA expression of MAP2 in triplicate. MDA-MB-231^{Resistant} groups were normalized to control expression levels. N = 3.

Interesting to note is MAP2 which demonstrated a significant ($p < .001$) upregulation within the MDA-MB-231^{Resistant} cells compared the MDA-MB-231^{Sensitive} cells which was contradictory to published work showing that it is typically downregulated in association with paclitaxel resistance (Bauer et al., 2010). To further investigate this gene, real-time polymerase chain reaction (RT-PCR) was completed (Figure 2.7). MAP2 gene expression was increased (9.8 ± 0.95%, p = .71) within the MDA-MB-231^{Resistant} cells compared to MDA-MB-231^{Sensitive} cells, however, an independent *t*-test demonstrated that this increase was not significant, $t(4) = 13.99$, $p = .71$ (Table 2.S27).

Discussion

Breast cancer remains one of the most essential cancers to study and although improvements in therapies and early detection exist, the number of individuals expected to succumb to advanced, metastatic disease each year has remained relatively constant (Siegel et al., 2021; Siegel et al., 2015, 2017, 2019). A key reason for the lack of significant improvement in outcomes for patients is the presence or development of resistance to therapeutic interventions (Shi & Sun, 2017). This resistance could be either innate, in which there is an inherent resistance present in the cells prior to treatment, or resistance could develop over time, known as acquired resistance (Sara M Maloney et al., 2020; Mansoori et al., 2017; Nikolaou et al., 2018; Catherine E Steding, 2016). While an understanding of innate resistance is important, it does not address the long-term consequences of therapeutic intervention. As such, work in our laboratory has emphasized evaluating long-term treatments that better recapitulate acquired chemoresistance against the microtubule-stabilizing drug, paclitaxel. Treatment with paclitaxel, as well as other members of the Taxane family, does not necessarily cause complete tumor regression even when improving survival rates thus, it generates a perfect environment for the development of resistance (Tabuchi et al., 2009). In fact, studies have shown paclitaxel effects can be significantly tempered by drug resistance (Goldblatt et al., 2009). The unique paclitaxel-resistant cell lines developed in our laboratory provide a critical model system for evaluating mechanisms of true, acquired chemoresistance.

To develop this cell line, MDA-MB-231 cells were treated with paclitaxel at concentrations of 0.0005 ng/mL, 0.005 ng/mL, 0.05 ng/mL, 0.5 ng/mL, 5 ng/mL, 50 ng/mL, 500 ng/mL, or 5,000 ng/mL of paclitaxel. In this chapter, I demonstrated that the MDA-MB-231 cells respond to paclitaxel in a dose-dependent manner. A short-term assay was completed to

investigate the cells initial response to paclitaxel. There was a decrease in proliferation as the concentration increased as expected, however, none of these decreases were significant. A long-term assay was completed to investigate the effects of paclitaxel on the cells in a more clinically relevant time span (Adams et al., 2019; Falchook et al., 2019; Schmid et al., 2020). In this longer-term assay, there was a dose-dependent decrease in proliferation as expected, with a significant decrease at a concentration of 0.5 ng/mL and continuing to the max concentration of 5,000 ng/mL paclitaxel. Although there was not a significant decrease in proliferation at a concentration of 0.005 ng/mL (5 pg/mL) of paclitaxel, this concentration was selected to initiate paclitaxel treatment as there was about a 50% decrease in proliferation. Cells were treated with this concentration every six days with an increase in dose every tenth treatment. Sporadically, short-term proliferation assays were completed to monitor the increase in resistance until the 60^{th} treatment. At this time, treatment was maintained at 1.6 ng/mL indefinitely and the cells were designated MDA-MB-$231^{Resistant}$.

As the cell line was created, it was observed that the MDA-MB-$231^{Resistant}$ cells expressed a larger cell morphology then the MDA-MB-$231^{Sensitive}$ cells which is the opposite of what Park et al. discovered in their laboratory (Jeong et al., 2016). It is important to note, that their treatments with paclitaxel consisted for 6 days, so this could be more of an innate resistance phenotype; while our cells have grown in paclitaxel for many years and our enlarged morphological phenotype could be more indicative of acquired paclitaxel resistance (Jeong et al., 2016).

To further investigate potential phenotype changes of the MDA-MB-$231^{Resistant}$ cells, I first looked for a potential metastatic change to the resistant cell line. One of the hallmarks of cancer cells is their ability to invade other tissues and, it is important to note, that the MDA-MB-

231^{Resistant} cell line is more aggressive (Hanahan & Weinberg, 2011). Although there was an increase in the MDA-MB-231^{Resistant} population's cell invasion and colony formation potential, these minute increases were not significant and therefore the MDA-MB-231^{Resistant} cells are not more invasive compared to their sensitive counterparts.

The next phenotype to investigate within the newly established MDA-MB-231^{Resistant} cell line was their ability to perish in the presence of paclitaxel. To investigate this phenomenon, a cytotoxicity assay was conducted because it has previously been established that paclitaxel can have a cytotoxic effect on cells (Ehrlichova et al., 2005; Önyüksel et al., 2009; Zasadil et al., 2014). As expected, the MDA-MB-231^{Sensitive} cells when treated with paclitaxel demonstrated an increase in percent cytotoxicity and the MDA-MB-231^{Resistant} cells treated with paclitaxel demonstrated a significant decrease in percent cytotoxicity. Thus, supporting the resistance of this cell line. Apoptosis in the presence of paclitaxel was also investigated as it has been established that paclitaxel is known to induce apoptosis in a sensitive cell line (Janczar et al., 2017; Paradiso et al., 2005; Sun et al., 2015; Xie et al., 2016). As expected, when the MDA-MB-231^{Sensitive} cells are treated with paclitaxel, there is an increase in the percentage of apoptosis occurring and the MDA-MB-231^{Resistant} cells treated with paclitaxel have a decrease in the percentage of apoptosis occurring compared to the MDA-MB-231^{Senesitive} paclitaxel treated cells. Unfortunately, none of these apoptotic changes are significant, as a significant decrease within the MDA-MB-231^{Resistant} cells treated with paclitaxel would be promising especially since Lev et al. in 2017 reported that short-term paclitaxel treatment can sensitize TNBC cells to apoptosis (Panayotopoulou et al., 2017). It is important to note though, that they report short-term treatment while the cells in our laboratory have experienced paclitaxel long-term and it is not unexpected as Sprouse and Herbert reported similar results (Sprouse & Herbert, 2014).

With the creation of the MDA-MB-231^{Resistant} cell line it is imperative to know, for future experimenting, if they grow differently than their counterparts, MDA-MB-231^{Sensitive} cell line. As expected in the short-term there was not a significant change in growth between the MDA-MB-231^{Sensitive} and the MDA-MB-231^{Resistant} cell lines and the same is true when both cell lines were treated with paclitaxel; these results are supported by data from the Herbert laboratory (Sprouse & Herbert, 2014). However, in the long-term there are changes between the two cell lines. For proliferation potential, there is not a significant difference between the MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} cells; however, both cell lines experience a significant decrease in proliferation when treated with paclitaxel. The same is true with paclitaxel treatment of the cell lines for relative population doubling; although it is interesting to note that the MDA-MB-231^{Resistant} cells are significantly more fruitful in their population doublings and this is similar to findings published by other laboratories (Jeong et al., 2016).

In conjunction with this potential increase in cell growth with the MDA-MB-231^{Resistant} cells, it was interesting to note if they are still as healthy as the MDA-MB-231^{Sensisitve} cells. In the short-term, there is not a significant difference in viability between the two cell lines even when treated with paclitaxel, yet, in the long-term we do see significant changes. The MDA-MB-231^{Resistant} cells experienced a minute significant decrease in viability compared to the MDA-MB-231^{Sensitive} cells and a more significant decrease when both cell lines are treated with paclitaxel.

With the establishment that the MDA-MB-231^{Resistant} cells exhibit no changes in the phenotypic expression of metastatic ability, cellular death, short-term cellular growth and health, the genotypic changes of the MDA-MB-231^{Resistant} can be investigated. With next generation sequencing it was found that the MDA-MB-231^{Resistant} cells had a total of 2,169 genes that were

significantly altered; of those 1,159 are upregulated and 1,010 are downregulated. To begin delving into these genes, I first looked at genes known to affect microtubules and have been reported to be altered with paclitaxel resistance. Of those common genes, two were found to be insignificantly altered, EB1 and SIK2. Three genes, EB3, KIF14, and MCAK, were confirmed to be significantly upregulated within our MDA-MB-231^{Resistant} cells. One of the genes, a microtubule-associated protein functioning in stabilization, MAP2, was significantly upregulated in our model system; however, it has previously been reported to be downregulated within paclitaxel resistance (Bauer et al., 2010). To further investigate this discrepancy a real-time polymerase chain reaction (RT-PCR) was performed on the cells looking at the gene expression of MAP2 and this demonstrated that there was not a significant change in MAP2 gene expression within the MDA-MB-231^{Resistant} cells at this level though further changes could be investigated. KIF14, a microtubule-associated motor protein that functions in destabilization of the microtubule, demonstrated a significant upregulation in the MDA-MB-231^{Resistant} cells compared the MDA-MB-231^{Sensitive} cells and had the greatest increase in $\log_2$fold change (Arora et al., 2014; Corson & Gallie, 2006; Li et al., 2017; Madhavan et al., 2009; Qiu et al., 2017; Thériault, Basavarajappa, et al., 2014; Wang et al., 2016). With this increase in a destabilization microtubule protein, it could be a mechanism for acquired paclitaxel resistance.

CHAPTER 3

THE MICROTUBULE-ASSOCIATED PROTEIN, KIF14, CONTRIBUTES TO THE ACQUIRED CHEMORESISTANCE OF PACLITAXEL IN THE BREAST CANCER CELL LINE, MDA-MB-231

Abstract

Chemoresistance remains the greatest cause of therapeutic failure in cancer treatment. Development of chemoresistance for compounds that often only suppress growth and do not eliminate tumors has been shown to correlate with more severe clinical phenotypes. Paclitaxel, a microtubule stabilizing compound, has been shown to be of particular importance in the study of chemoresistance as its therapeutic efficacy has been correlated to resistance. Here, I demonstrate that there is a significant increase in gene expression of a microtubule-associated protein, KIF14, within a paclitaxel resistant triple-negative breast cancer (TNBC) cell line. KIF14 is a member of the kinesin 3 superfamily of microtubule motor proteins that function in destabilization of the microtubule. Thus, a proposed mechanism of acquired paclitaxel resistance is that resistant cells increase destabilization proteins, like KIF14, to counteract the stabilization effects of paclitaxel. If KIF14 functions in the mechanism of chemoresistance then when KIF14 expression is reduced, there will be an increase in sensitivity of the cells to paclitaxel. Here, I show that reducing expression of KIF14 within a TNBC cell line causes a reduction in cellular growth including proliferation potential and colony formation abilities. I also demonstrate that when

treated with paclitaxel, the TNBC cells are more responsive to paclitaxel treatment. When KIF14 expression is also reduced within resistant cells, they express a reduction in growth capabilities and are more responsive to paclitaxel treatment. This information thus provides a deeper understanding of KIF14's role in the mechanism of chemoresistance.

Introduction

The development of resistance to paclitaxel's microtubule stabilizing activity is closely associated with the fact that microtubules are diverse in name and in function. This diversity is the result of several factors that act upon them including microtubule-associated proteins (MAPs). MAPs regulate microtubules by binding to multiple different locations on the microtubules, for example MAPs can bind to the end of the microtubule to promote destabilization of the microtubule (Borys et al., 2020; Shi & Sun, 2017). If changes in the MAPs occurred, it can cause a deregulation of microtubules that can lead to resistance to chemotherapeutic agents like paclitaxel (Rodrigues-Ferreira et al., 2020; Shi & Sun, 2017). Preliminary RNA sequencing (RNAseq) analysis from Chapter 2 "Characterization of an Acquired Chemoresistant Breast Cancer Cell Line" identified upregulation of the MAP, KIF14 (Table 3.1).

Table 3.1

KIF14 Gene Expression

Gene	Log_2Fold Change in Resistant Cells	P-Value	False Discovery Rate
KIF14	1.59	$2.83x10^{-15}$	$4.18x10^{-14}$

Note. MDA-MB-$231^{Sensitive}$ and MDA-MB-$231^{Resistant}$ cells were pelleted, and flash frozen in liquid nitrogen. RNA was extracted using the RNAeasy Mini Kit Plus. RNA levels were

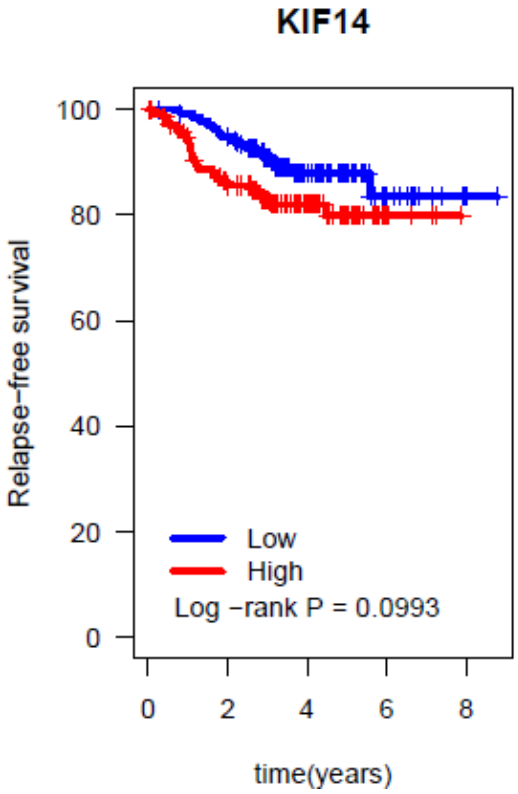
quantified using a NanoDrop spectrophotometer and RNA quality was assessed using the TapeStation. Next generation sequencing using the Illumina HiSeq4000 sequencing system was performed at the Indiana University Center for Medical Genomics Core Facility, N=3. MDA-MB-231^{Resistant} cells were then compared to the MDA-MB-231^{Sensitive} cells to identify genes with a significant difference in expression ($p < .001$). Listed here is the expression level change in the microtubule-associated protein, KIF14.

KIF14 is a member of the kinesin 3 superfamily of microtubule motor proteins that functions in vesicle transport, chromosome segregation, mitotic spindle formation, and cytokinesis (Basavarajappa & Corson, 2012; Gruneberg et al., 2006; Huang et al., 2015). KIF14 also functions in destabilization of microtubules thus promoting depolymerization of the microtubule (Arora et al., 2014; van der Vaart et al., 2009). The destabilization effect of KIF14 could be an avenue for acquired paclitaxel chemoresistance. If a destabilization protein like KIF14 is upregulated within a resistant cell line then the cells can balance the stabilization of paclitaxel and still proliferate. Within the MDA-MB-231^{Resistant} cells, KIF14 was found to be upregulated when compared to the sensitive cells, MDA-MB-231^{Sensitive} (Table 3.1), which is supported by findings within previous labs (Corson & Gallie, 2006; Singel et al., 2013; Singel et al., 2014; Wang et al., 2018) that found with knock-down of KIF14, breast cancer cells are sensitized to docetaxel. This phenomenon will be explored further with paclitaxel in our cells since paclitaxel and docetaxel differ in key consequences and can exhibit different patterns of sensitivity and resistance in breast cancer cells (Sara M Maloney et al., 2020; Verweij et al., 1994).

To further investigate KIF14's potential role in the mechanism of chemoresistance, gene expression data of TNBC patients under paclitaxel treatment were searched from public domains with assistance from our collaborators at Indiana University Center for Computational Biology & Bioinformatics. A total of 360 TNBC patients with RNA sequencing data were discovered. Of these, 276 had paclitaxel treatment records (Jiang et al., 2019). From this data, a Kaplan Meier survival curve was generated (Figure 3.1), the results of which suggests an inverse correlation between an increased expression of KIF14 and the survival of patients leading to a potential oncogenic role of KIF14. This data, in addition to the transcriptome analysis, suggests a role for KIF14 in the mechanism of chemoresistance.

Figure 3.1

Kaplan Meier Survival Curve of KIF14



Note. Gene expression data of triple-negative breast cancer patients under Paclitaxel treatment were searched from public domain including 360 triple-negative breast cancer patients with RNA sequencing data; of these, 276 had paclitaxel treatment records (Jiang, Y-Z. 2019). Raw data was obtained from NCBI Sequence Read Archive under accession of SRP157974. The RNA-seq data was downloaded and converted to fastq via SRA-tools 2.12 and then aligned to GRCh38 reference genome using STAR 2.7.2. Counts of reads that map to a single gene level were estimated with featureCounts and further normalized to FPKM value as gene expression value. High and Low were created using the median value with high expression being greater than the mean while low expression was lower than the mean.

The first part of this chapter will explore the relation of KIF14 within the TNBC cell line, MDA-MB-231. It will investigate the function of KIF14 within the basic cellular phenotypes of cancer cells including: cell invasion potential, colony formation ability, and key measures of cellular growth. Then KIF14's function will be evaluated in these cells in relation to the presence of paclitaxel. If KIF14 is required for the cells to balance the stabilization effect of paclitaxel, then with reduced expression of KIF14 the cells will be more sensitive to treatment with paclitaxel.

The second section of this chapter will explore the function of KIF14 in acquired paclitaxel resistance. First, KIF14's function will be evaluated in maintaining normal phenotypes of the resistant cells. If KIF14 is necessary for normal function of the resistant cells, then with the reduced expression of KIF14 there will be a disappearance of basic phenotypes like: invasion potential, colony formation ability, and key measures of growth. Secondly, there will be an exploration of KIF14's function in the mechanism of acquired paclitaxel resistance. If KIF14

functions in the mechanism of chemoresistance, then when KIF14 expression is reduced there will be an increase in sensitivity of the resistant cells to paclitaxel.

Here, I show that reducing expression of KIF14 within a TNBC cell line results in the reduction of the cell's ability grow including proliferation potential and colony formation abilities. I also demonstrate that when treated with paclitaxel, the TNBC cells are more responsive to the chemotherapy treatment. When KIF14 expression is also reduced within the resistant cells, they express a reduction in growth capabilities and are more responsive to chemotherapeutic treatment. This information therefore provides a deeper understanding of KIF14's role in the mechanism of chemoresistance.

Materials and Methods

Culture Conditions

The triple-negative breast cancer cell line (TNBC), MDA-MB-231, was maintained using traditional means (Steding et al., 2011). Cells were cultured in Nunc tissue culture flasks and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Cosmic Calf Serum (CCS) (Gibco and Fisher Scientific). Cells were maintained at 37℃ and 5% CO_2 at sub-confluent levels and passaged once cells reached a confluency between 75% - 95% via treatment with 0.25% Trypsin EDTA (Gibco).

Generation of Acquired Chemoresistant Cell Lines

MDA-MB-231 cells were passaged into three groups and treated with either 1x Phospho-Buffered Saline (PBS), MDA-MB-231, Dimethyl Sulfoxide (DMSO), MDA-MB-231^{Sensitive}, or paclitaxel, MDA-MB-231^{Resistant} (Table 3.1) weekly over a period of several years.

Table 3.2

Treatment Groups

Name	Maintained Treatment Condition
MDA-MB-231	1x PBS
MDA-MB-231^{Sensitive}	0.01% DMSO
MDA-MB-231^{Resistant}	1.6 ng/mL Paclitaxel

Note. Table expressing treatment conditions of cell line. MDA-MB-231 cells were passaged into three groups and treated with either 1x Phospho-Buffered Saline (PBS), Dimethyl Sulfoxide (DMSO), or paclitaxel. Once treatments were initiated, treated cells were maintained as isolated cell lines designated as: MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant}. Cells were maintained with a concentration of 0.01% DMSO or 1.6 ng/mL of paclitaxel, respectively, weekly over the period of several years to maintain a resistant population.

RNA-Sequencing

MDA-MB-231^{Sensitive}, and MDA-MB-231^{Resistant} cells were pelleted, and flash frozen in liquid nitrogen. RNA was extracted using the RNAeasy Mini Kit Plus (Qiagen). RNA levels were quantified using a NanoDrop spectrophotometer (ThermoFisher) and RNA quality was assessed using the TapeStation (Agilent Technologies). Next generation sequencing using the Illumina HiSeq4000 sequencing system was performed at the Indiana University Center for Medical Genomics Core Facility (Breese & Liu, 2013; Dobin et al., 2013; Liao et al., 2014; Robinson et al., 2010).

siRNA- mediated Transient Transfection of KIF14

Transfection was performed using SilencerTM Selected Pre-Designed siRNA for KIF14 and Lipofectamine 3000 according to the recommended protocol provided (Invitrogen). Briefly,

cells were plated subconfluently a day before transfection and on transfection day siRNA was diluted in Opti-MEM Reduced Serum Medium (Invitrogen).

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Cells were harvested at 24-, 48-, 72-, 96- hours, 5-, and 6-days, post transfection with KIF14 siRNA. RNA was isolated using the PureLink RNA Mini Kit (Invitrogen) and the quantity of RNA was assessed using the NanoDrop™ One Spectrophotometer system (Thermo Scientific). TaqMan gene expression assays were used to quantify the mRNA expression of KIF14 (Hs00978236_m1). Quantification of gene expression was performed in triplicate in a 50 uL volume in 96- well plates on Applied Biosystems® QuantStudio® 3 Real-Time PCR System using a TaqMan RNA-to-Ct 1-step kit (Applied Biosystems). KIF14 knockdown groups were normalized to the control to determine percent change in expression levels (Guan & Yang, 2008; Pfaffl, 2001; Tsai & Wiltbank, 1996). These results determined the timeline the following experiments were conducted (Figure 3.2).

Figure 3.2

Timeline of Knockdown and Experimentation

<table>
<tr><th></th><th>Long ago & Continuous</th><th>Start of Transfection</th><th>24-hrs Post Transfection</th><th>48-hrs Post Transfection</th><th>96-hrs Post Transfection</th><th>7 Days Post Transfection</th><th>15 Days Post Transfection</th></tr>
<tr><td>MDA-MB-231</td><td>1xPBS</td><td rowspan="3">KIF14 Control</td><td rowspan="6">Plate Assays:
Cell Invasion
Cytotoxicity
Colony Formation
Proliferation
Relative Population Doubling
Viability</td><td rowspan="6">Treat Assays:
Cytotoxicity
Proliferation
Relative Population Doubling
Viability</td><td rowspan="6">Fix Short-term Assays:
Cell Invasion
Cytotoxicity
Proliferation
Relative Population Doubling
Viability</td><td rowspan="6">Fix Long-Term Assays:
Proliferation
Relative Population Doubling
Viability</td><td rowspan="6">Fix Colony Formation Assay</td></tr>
<tr><td>MDA-MB-231^{Sensitive}</td><td>DMSO</td></tr>
<tr><td>MDA-MB-231^{Resistant}</td><td>Px</td></tr>
<tr><td>MDA-MB-231 KIF14 KD</td><td>1xPBS</td><td rowspan="3">KIF14 siRNA</td></tr>
<tr><td>MDA-MB-231^{Sensitive} KIF14 KD</td><td>DMSO</td></tr>
<tr><td>MDA-MB-231^{Resistant} KIF14 KD</td><td>Px</td></tr>
</table>

Note: Timeline of culture conditions and experimentation start and end times relative to the start of transfection. Cells were maintained in either 1x PBS, DMSO, or paclitaxel (Px) at the start of transfection, cells were then either treated with KIF14 control or KIF14 siRNA. 24- hours (hrs) post transfection cell invasion, cytotoxicity, colony formation, proliferation, relative population doubling, and viability assays were plated. 48- hours following transfection cytotoxicity,

proliferation, relative population doubling, and viability assays were treated with either 0.01% DMSO or 10 ng/mL of Paclitaxel. 96- hours post transfection short-term assays (cell invasion, cytotoxicity, proliferation, relative population doubling, and viability) were fixed. 7 days post transfection long-term assays (proliferation, relative population doubling, and viability) were fixed. At 15 days post transfection colony formation assays were fixed.

Cell Invasion Assay

24- hours following transfection, cells were seeded in a 6-well plate with DMEM to be serum starved overnight at 37℃ and 5% CO_2. They were then placed within a Transwell™ Multiple Well Plate with Permeable Polycarbonate Membrane Inserts (Corning) with the use of a .25% Trypsin. Cells were incubated at 37℃ and 5% CO_2 for 48- hours at which point the DMEM was removed from the transwells. The transwells were then placed in 0.5% solution of Methylene Blue for 30 minutes while on a plate shaker. The transwells were rinsed with Milli-Q water and allowed to dry overnight at room temperature. The transwells were placed in a 0.5 M Hydrochloric acid (HCL) solution on a shaker for 30 minutes then the HCL was placed in triplicate 96- wells and absorbance was read at 630 nm using a spectrophotometer (BioTeK).

Cytotoxicity Assay

Following transfection, cells were assessed for cytotoxicity using CyQUANT™ LDH (lactate dehydrogenase) Cytotoxicity Assay following manufacturer's protocol (Invitrogen). Briefly, cells were seeded in a 96- well plate and incubated overnight at 37℃ and 5% CO_2. They were then treated with 10 ng/mL of Paclitaxel or 0.1% DMSO for compound LDH release, or Milli-Q water for spontaneous LDH release and incubated overnight at 37℃ and 5% CO_2. Wells serving as the maximum LDH release received 10x Lysis Buffer and the plate was incubated at 37℃ and 5% CO_2 for 45 minutes. Each sample was transferred to a 96- well plate in duplicate wells, Reaction Mixture was added, and the plate was incubated at room temperature for 30

minutes in the dark. Stop Solution was added to each well and the absorbance was measured at 492 nm and 680 nm using a spectrophotometer. LDH activity was then determined by subtracting the 680 nm absorbance value from the 492 nm absorbance value before calculation of percent cytotoxicity. Percent cytotoxicity was calculated by the following equation:

$$Percent\ Cytotoxicity = \left[\frac{Compound\ treated\ LDH\ activity - Spontaneous\ LDH\ activity}{Maximum\ LDH\ activity - Spontaneous\ LDH\ activity}\right] \times 100$$

Compound treated LDH activity was either those treated with DMSO as the control or paclitaxel. Transfected and paclitaxel treated cells were then normalized to the control.

Colony Formation Assay

24-hours following transfection with KIF14 siRNA, cells were seeded at 200 cells/mL in triplicate within 6-well plates and incubated overnight at 37℃ and 5% CO^2 for 14 days. Plates were then fixed with 70% Ethanol and stained with 5% Geimsa for 1 hour at room temperature. Plates were rinsed with RO water and allowed to air dry, at which point stained colonies were counted.

Apoptosis Assay

The eBioscienceTM Annexin V Apoptosis Detection Kit FITC (Invitrogen) was used per manufacturer's instructions to assess apoptosis following transfection with KIF14 siRNA and treatment with paclitaxel. 24-hours following transfection cells were treated with 10 ng/mL of Paclitaxel or 0.1% DMSO (control). 48-hours following transfection cells were harvested, rinsed, and stained with Annexin V and Propidium Iodide. Flow cytometry was conducted on BD AccuriTM C6 Plus (BD) and data analysis was conducted using FlowJo software (Tree Star, Inc).

Proliferation Assay

Proliferation potential following transfection with KIF14 siRNA and treatment with paclitaxel was evaluated via a Methylene Blue Proliferation Assay. 24- hours following transfection with KIF14 siRNA, cells were plated at a density of $1x10^5$ cells/well (short-term) or $1x10^4$ cells/well (long-term), then incubated at 37℃ and 5% CO_2 for 48 hours (short-term) or 5 days (long-term). Cells were treated with 10 ng/mL of Paclitaxel or 0.1% DMSO 48- hours following transfection. Plates were fixed with 100% methanol after final incubation time. Adherent cells were stained with 0.5% Methylene Blue and allowed to air dry. Treatment with 0.5 M HCL was used to redistribute stain into solution and absorbance was evaluated at 630 nm using a spectrophotometer (BioTek).

Trypan Blue Exclusion Assay

Relative population doubling and cellular viability was determined using a Trypan Blue Exclusion Assay. 24- hours following transfection with KIF14 siRNA, cells were plated at a density of $5x10^4$ cells/well (short-term) or $2.5x10^4$ cells/well (long-term) and treated with 10 ng/mL paclitaxel diluted in DMSO, or 0.1% DMSO. Cells were then incubated at 37℃ and 5% CO_2 for 48 hours (short-term) or 5 days (long-term). Cells were harvested using 0.25% Trypsin EDTA and the resulting cell population was evaluated using an automated cell counter (BIO-rad). Relative population doubling was calculated by the following equation and then normalized to control cells.

$$\frac{\text{Number of Live Cells}}{\text{Number of Plated Cells}}$$

Viability of cells was calculated by the following equation and was normalized to the control cells.

$$\frac{\text{Number of Live Cells}}{\text{Total Number of Cells}} * 100\%$$

Kaplan Meier Survival Curve

My collaborators, including Dr. Yunlong Liu and Chuanpeng Dong of Indiana University, searched gene expression data of TNBC patients under paclitaxel treatment from public domain, including 360 TNBC patients with RNA sequencing data; of these, 276 had paclitaxel treatment records (Jiang et al., 2019). RNA sequencing data of these patients came from primary tumor tissue and from blood samples (Jiang et al., 2019). Raw data was obtained from NCBI Sequence Read Archive, under accession of SRP157974. The RNA-seq data was downloaded and converted to fastq via SRA-tools 2.12 and then aligned to GRCh38 reference genome using STAR 2.7.2. Counts of reads that map to a single gene level were estimated with featureCounts and further normalized to FPKM value as gene expression value. High and low were created using the median value with high expression being greater than the mean while low expression was lower than the mean.

Statistics

Data was expressed as the means ± the standard deviation. IBM SPSS Statistics Version 27 was used for statistical analysis. For two-group comparisons, normally distributed data with homogeneity of variance, used an independent *t*-test. Data comparison between multiple groups was performed using one-way analysis of variance (ANOVA) (Brown, 2005; Kim, 2017). A Levene's test of homogeneity was used; if Levene's statistic was not significant, then homogeneity of variance was assumed and a Tukey's post hoc test was conducted to determine individual group statistics (Abdi & Williams, 2010; Glass, 1966). If Levene's statistic was significant, then homogeneity of variance was not assumed, and a Welch's test of homogeneity was conducted with a Games-Howell post hoc test. *p*-values of less than .05 were considered significant (Sauder & DeMars, 2019).

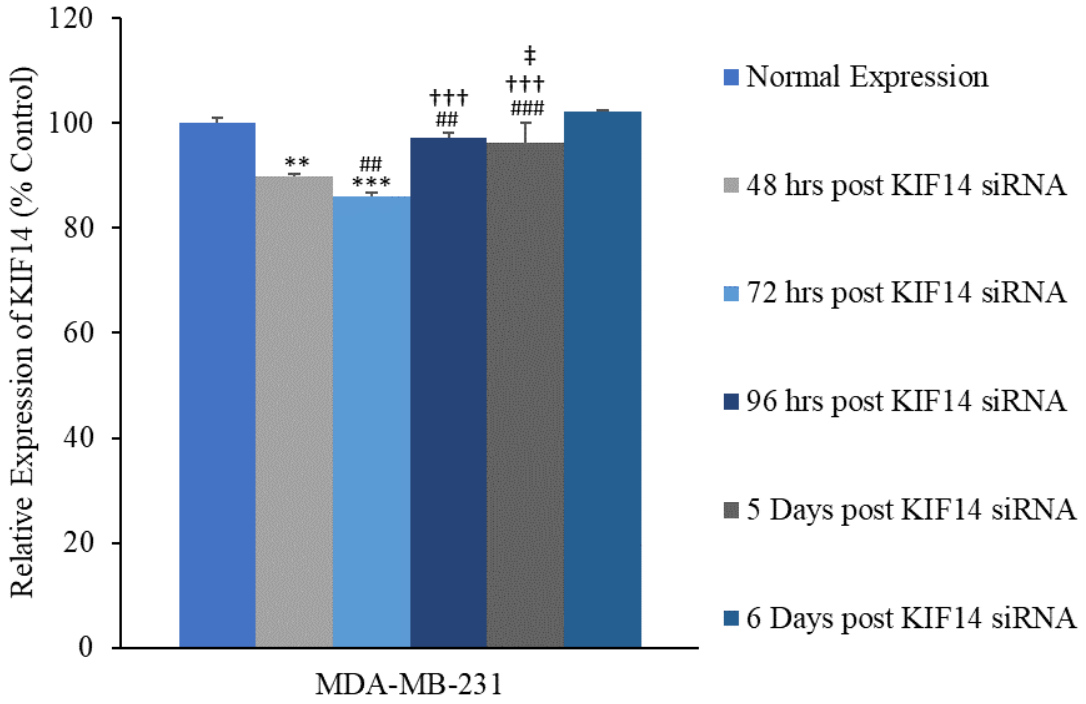
Results

KIF14 Expression was Maximally Reduced 72 hours Following Treatment with KIF14 siRNA

Reverse transcriptase polymerase chain reaction (RT-PCR) was used to determine the maximum reduction in KIF14 expression following treatment with KIF14 small interfering RNA (siRNA) within the triple-negative breast cancer (TNBC) cell line, MDA-MB-231 (Figure 3.3). Expression of KIF14 was evaluated 48- , 72- , 96- hours, 5- , and 6- days following transfection. A one-way analysis of variance (ANOVA) confirmed that there was a significant decrease in gene expression, $F(5, 12) = 40.04$, $p < .001$, $\eta^2_p = .94$ (Table 3.S1). A Tukey's post hoc test demonstrated that at 48- hours post transfection ($M = 29.55$, $SD = 0.14$) expression of KIF14 also demonstrated a significant decrease (10.16 ± 0.52 %; $p = .002$) when compared to the control ($M = 26.82$, $SD = 0.24$) (Table 3.S1). 72- hours following transfection, there was a significant decrease (3.835 ± 0.67%; $p = .01$) in gene expression of KIF14 ($M = 30.58$, $SD = 0.18$) compared to expression at 48-hours and a significant decrease (14 ± 0.67%; $p < .001$) compared to the control. At 96- hours following transfection ($M = 27.56$, $SD = 0.23$) KIF14 expression was not significantly different from the control, however, there was a significant increase in KIF14 expression (7.41 ± 0.85%; $p = .003$) compared to expression levels at 48- hours and (11.24 ± 0.85%; $p < .001$) 72- hours. At 6 days following transfection ($M = 26.21$, $SD = 0.06$), expression levels were not significantly altered from the control, however there was still a significant increase in expression from 48- hours (12.43 ± 0.23%; $p < .001$) and 72- hours (16.26 ± 0.23%; $p < .001$).

Figure 3.3

KIF14 Expression was Maximally Reduced 72 Hours following Treatment with KIF14 siRNA



Note. Following KIF14 siRNA transfection, at 24, 48, 72, 96 hours, and 5-6 days RNA was isolated, and the quantity was assessed. TaqMan gene expression assays were used to quantify the mRNA expression of KIF14 in triplicate. KIF14 knockdown groups were normalized to control expression levels. N = 3.

Data are presented as means ± standard deviation. Differences between groups were compared using a one-way ANOVA with a Games-Howell post hoc test. ** $p < .01$ vs Normal Expression, *** $p < .001$ vs Normal Expression, ## $p < .01$ vs 48 hours post KIF14 siRNA Treated, ### $p < .001$ vs 48 hours post KIF14 siRNA Treated, ††† $p < .001$ vs 72 hours post KIF14 siRNA Treated, ‡ $p < .05$ vs 96 hours post KIF14 siRNA Treated.

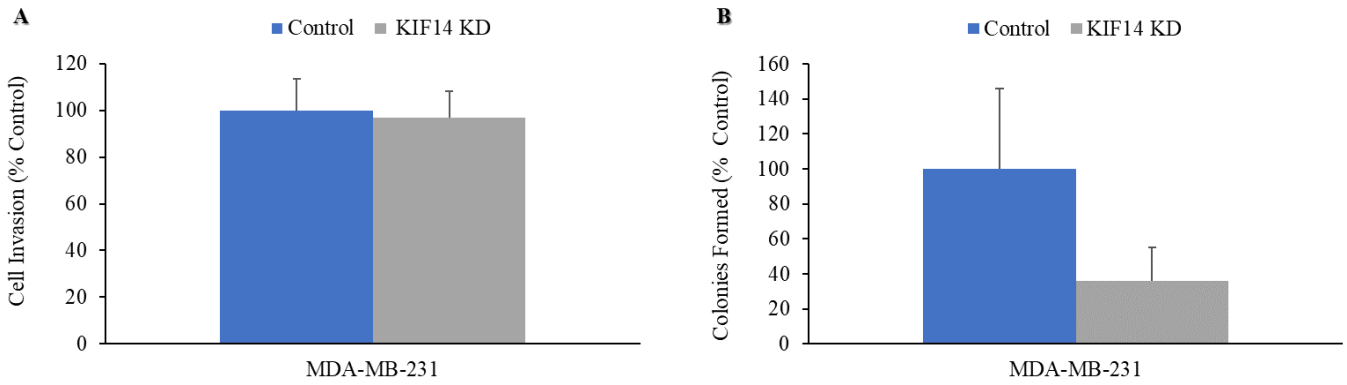
KIF14 Expression does not Alter Metastatic Potential as Demonstrated by Cell Invasion Potential and Colony Formation Ability within a TNBC Cell Line

To investigate KIF14's function in metastatic potential a cell invasion and colony formation assay was conducted. A cell invasion assay was performed following the knockdown of KIF14 ($KIF14^{KD}$) (Figure 3.4A). Initial analysis showed decrease (3.05 ± 11.43%, p = .83) in cell invasion potential; however, an independent samples t-test was conducted and determined that the MDA-MB-231 cells (M = .14, SD = 0.019) did not differ significantly, t (4) = .30, p = .83, from the MDA-MB-231 $KIF14^{KD}$ cells (M = 0.13, SD = 0.016) (Table 3.S2).

A colony formation assay was conducted following $KIF14^{KD}$ and resulted in a decrease in colonies formed (64.21 ± 45.82%, p = .19) in MDA-MB-231 $KIF14^{KD}$ cells (M = 46.33, SD = 24.97) when compared to MDA-MB-231 cells (M = 129.44, SD = 59.32) (Figure 3.4B). An independent samples t-test demonstrated that there was not a significant change between the two groups t (4) = 2.24, p = .19 (Table 3.S3).

Figure 3.4

Knockdown of KIF14 does not Alter Metastatic Potential as Demonstrated by Cell Invasion Potential and Colony Formation Ability within a TNBC Cell Line



Note. (A) Cells were serum starved for 24 hours a day after transfection with KIF14 siRNA (KIF14 KD). Cell invasion assay was plated using Transwell™ Multiple Well Plate. Methylene

blue and HCL were used to stain and de-stain transwells then absorbance was read at 630 nm. (B) Colony formation assay was plated 24-hours following transfection with KIF14 siRNA (KIF14 KD) and incubated for 14 days. Plates were fixed with ethanol and stained with geimsa. N = 3.

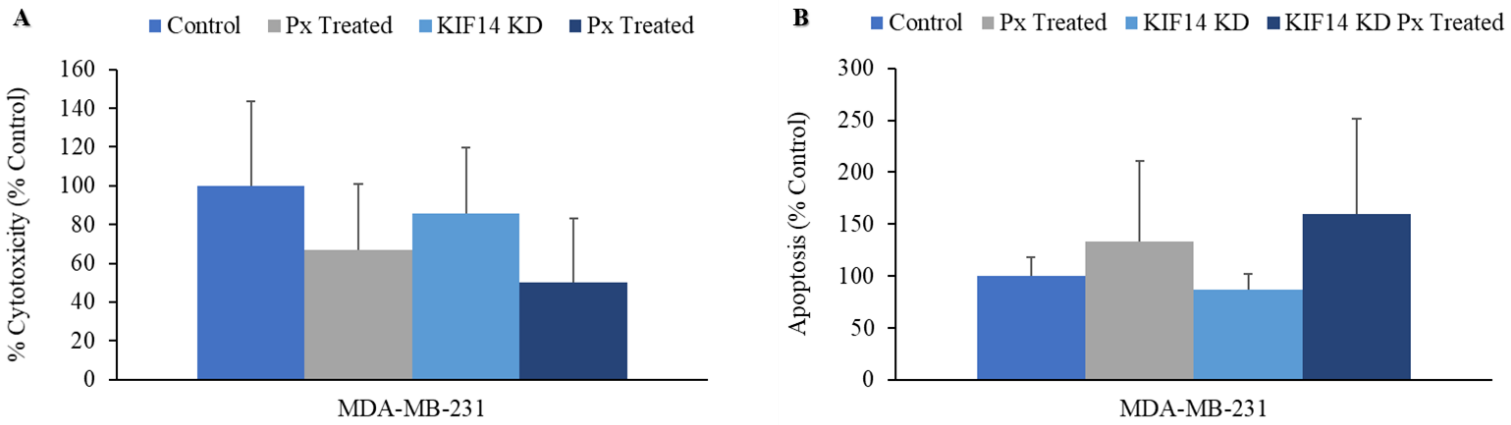
Data are presented as means ± standard deviation, n = 3 independent experiments. Differences between groups were compared using an independent *t*-test.

KIF14 Expression does not Alter Cellular Death Potential in the Presence of Paclitaxel within a TNBC Cell Line

To investigate KIF14's function in protecting against cellular death, a lactate dehydrogenase (LDH) activity and apoptosis assay was conducted following $KIF14^{KD}$ with the treatment of paclitaxel. LDH activity assay was used to determine the percent cytotoxicity in the cells in the presence of paclitaxel (Figure 3.5A). Although there was a decrease in percent cytotoxicity (14.6 ± 34.51%, $p = .99$) following $KIF14^{KD}$ ($M = 23.94$, $SD = 9.67$) compared to MDA-MB-231 ($M = 28.03$, $SD = 27.09$), a one-way ANOVA exposed that there was not a significant difference with $KIF14^{KD}$ with the treatment of paclitaxel, $F(3, 8) = .138$, $p > .05$, $\eta^2_p = 0.49$ (Table 3.S4). A decrease in cytotoxicity (49.67 ± 32.61%, $p = 1$) occurred following paclitaxel treatment within MDA-MB-231^{KD} ($M = 26.24$, $SD = 21.99$) when compared to MDA-MB-231, however, a Tukey's post hoc test demonstrated this was not a significant change.

Figure 3.5

KIF14 Expression does not Alter Cellular Death Potential in the Presence of Paclitaxel as Demonstrated by Cytotoxicity and Apoptosis within a TNBC Cell Line



Note. (A) Cytotoxicity assay was conducted CyQUANT™ LDH Cytotoxicity Assay following manufacturer's protocol 24-hours following transfection with KIF14 siRNA (KIF14 KD). Cells were treated with DMSO (control) or paclitaxel (Px). Absorbance was measured at 492 nm and 680 nm. LDH activity was determined prior to calculation of percent cytotoxicity. (B) The eBioscienceTM Annexin V Apoptosis Detection Kit FITC was used per manufacturer's instructions to assess apoptosis following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (Px). Flow cytometry was conducted, and data analysis was conducted using FlowJo software. N = 3.

Data are presented as means ± standard deviation. Differences between groups were compared using a one-way ANOVA with a Tukey's post hoc test.

An apoptosis assay was conducted to investigate the effects of KIF14 gene expression changes on paclitaxel induced apoptosis (Figure 3.5B). $KIF14^{KD}$ (M = 11.72, SD = 8.13) had reduced apoptosis (13.07 ± 15.08%, p = .97) compared to the control (M = 8.11, SD = 4.36). An ANOVA indicated that there was not a significant difference in apoptosis between treatment

groups, F (3, 20) = 1.848, p >.05, $\eta 2p$ = .22 (Table 3.S5). KIF14^{KD} with paclitaxel treatment (M = 22.71, SD = 17.68) increased apoptosis (59.86 ± 91.9%, p = .59) compared to the control, however this was not a significant difference in apoptosis as indicated with a Tukey's post-hoc test.

KIF14 Expression Correlates with Cellular Growth as Demonstrated with Proliferation and Relative Population Doubling Following Treatment with Paclitaxel in a TNBC Cell Line

To investigate KIF14's function in cellular growth, a methylene blue proliferation and trypan blue exclusion assay was conducted. A methylene blue proliferation assay was performed following KIF14^{KD} with treatment of paclitaxel to determine the effects of KIF14 expression on proliferation potential (Figure 3.6 A & B). A 48-hour, short-term, assay was conducted to ascertain the effects of KIF14 expression with paclitaxel treatment (Figure 3.6A). There was a slight decrease in proliferation (17.57 ± 2.06%, p = .14) in the KIF14^{KD} paclitaxel treated cells (M = 0.90, SD = 0.022) compared to the control (M = 1.09, SD = 0.10). ANOVA demonstrated that there was not a significant change among groups in proliferation, F (3, 8) = 2.45, p > .05, $\eta 2p$ = .48 (Table 3.S6).

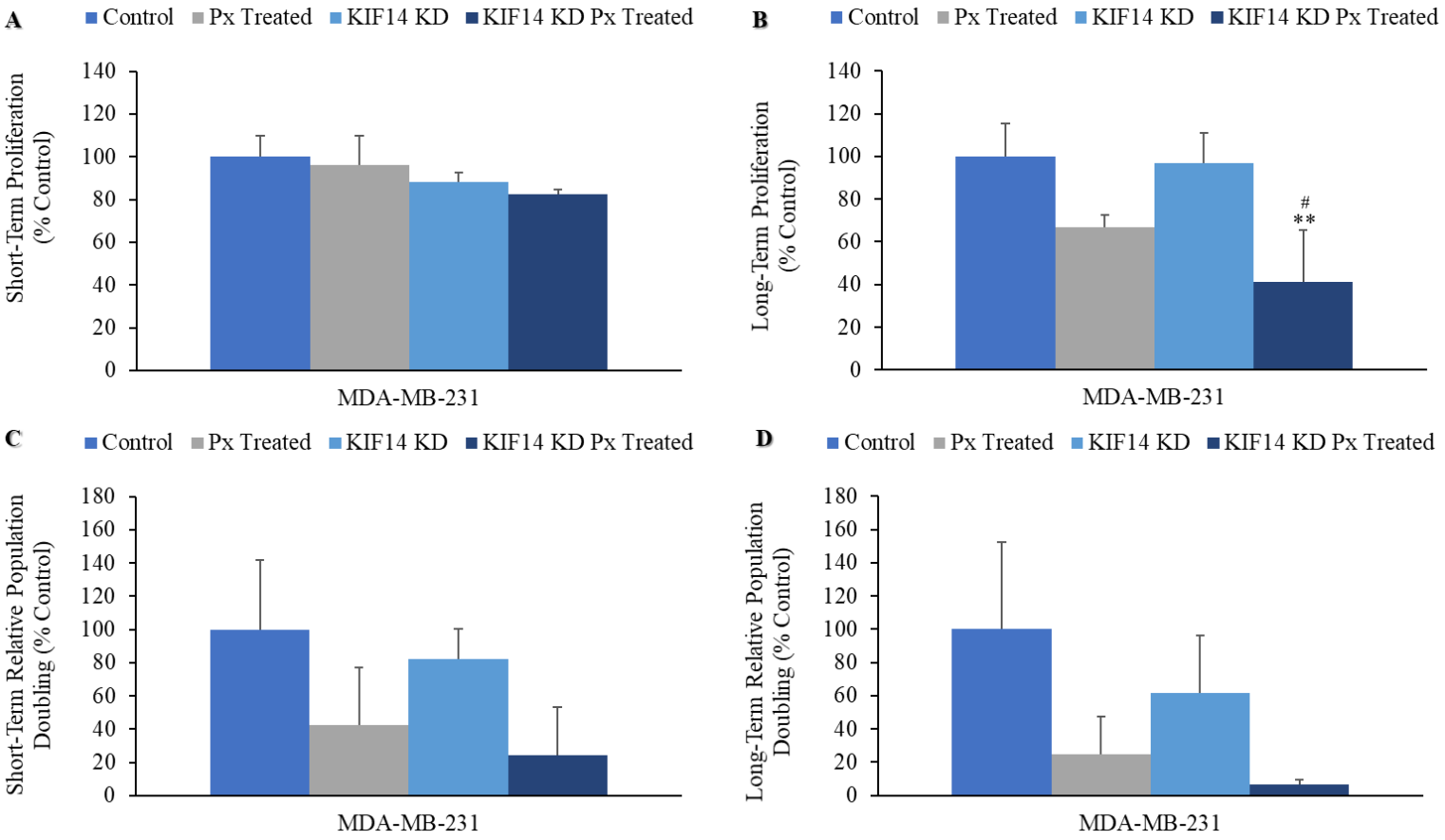
A 5-day assay was conducted to investigate the effects of KIF14 expression with paclitaxel treatment in a longer setting (Figure 3.6B). Completion of a one-way ANOVA demonstrated that there was a significant change among groups in proliferation, F (3, 8) = 8.66, p < .01, $\eta 2p$ =.76 (Table 3.S7). A Tukey's post hoc test demonstrated that there was a significant decrease in proliferation (25.73 ± 24.33%, p = .01) in the KIF14^{KD} paclitaxel treated cells (M = 0.36, SD = 0.21) compared to the control (M = 0.87, SD = 0.13). A significant decrease (55.59 ± 24.33%, p = .013) also occurred in proliferation of the KIF14^{KD} paclitaxel treated cells compared to the KIF14^{KD} cells (M = 0.84, SD = 0.12).

A trypan blue exclusion assay was conducted following KIF14^{KD} with treatment of paclitaxel to establish the effects of KIF14 expression on relative population doubling potential (Figure 3.6 C & D). A 48-hour, short-term, assay resulted in a decrease (75.40 ± 63.44%, p = .81) in proliferation within the KIF14^{KD} paclitaxel treated cells (M = 4.71, SD = 4.89) compared to the control (M = 7.71, SD = 3.23) (Figure 3.6C); however, completion of a one-way ANOVA demonstrated that there was not a significant change between treatment groups, F (3, 3.79) = .34 p > .05, $\eta 2p$ =.17 (Table 3.S8).

A 5-day, long-term, assay was conducted to investigate the effects of KIF14 expression with paclitaxel treatment (Figure 3.6D) on the relative population doubling of cells. Completion of a one-way ANOVA demonstrated that there was a not significant change among groups in relative population doubling, F (3, 3.34) = 3.79, p > .05 $\eta 2p$ =.57 (Table 3.S9). Even with a decrease (94.20 ± 1.37%, p = .30) in relative population doubling in the KIF14^{KD} paclitaxel treated cells (M = 2.82, SD = 0.54) compared to MDA-MB-231 (M = 45.40, SD = 29.40), a Games-Howell post hoc test verified that there was a not significant decrease in relative population doubling (Table 3.S9).

Figure 3.6

KIF14 Expression Correlates with Cellular Growth as Demonstrated with Proliferation and Relative Population Doubling within a TNBC cell line



Note. (A & B) Proliferation potential following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (px) was evaluated via a Methylene Blue Proliferation Assay. (A) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. (C & D) Relative population doubling was determined using a Trypan Blue Exclusion Assay following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (Px). (C) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (D) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, n = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with a Tukey's post hoc test. ** p < .01 vs control, ## p < .05 vs KIF14 KD.

Viability of Cells is Reduced Following the Reduction of KIF14 Expression with Paclitaxel Treatment within a TNBC Cell Line

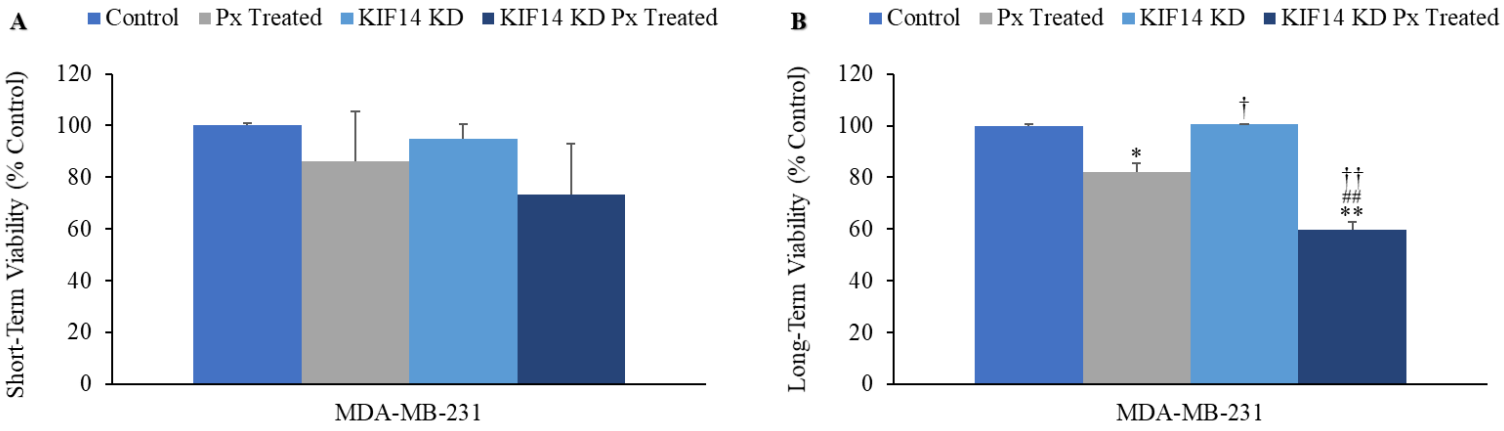
A trypan blue exclusion assay was performed following the KIF14^{KD} with the treatment with paclitaxel to determine the effects of KIF14's expression on viability (Figure 3.7). A 48-hour, short-term, assay was conducted to explore the effects of KIF14's expression with paclitaxel treatment on viability (Figure 3.7A). Completion of a one-way ANOVA demonstrated that there was a not a significant change between treatment groups, F (3, 3.39) = 2.28, $p > .05$, $\eta 2p$ =.44 (Table 3.S10). A Games-Howell post hoc test demonstrated that there was not a significant decrease in viability (26.72 ± 19.57%, $p = .32$) in the KIF14^{KD} paclitaxel treated cells ($M = 72.61$, $SD = 19.39$) compared to MDA-MB-231 control cells ($M = 99.08$, $SD = 0.80$) (Table 3.S10).

A 5-day, long-term, assay was conducted to explore the effects of KIF14's expression with paclitaxel treatment on viability (Figure 3.7B). Completion of a one-way ANOVA demonstrated that there was a significant change between treatment groups, F (3, 3.73) = 153.56, $p < .01$, $\eta 2p$ =.99 (Table 3.S11). A Games-Howell post hoc test demonstrated that there was a significant decrease (18.14 ± 19.57%, $p = .029$) in viability in the MDA-MB-231 with paclitaxel treatment ($M = 80.5$, $SD = 3.53$) compared to MDA-MB-231 control cells ($M = 98.33$, $SD = 0.52$). KIF14^{KD} with paclitaxel treatment ($M = 58.58$, $SD = 2.96$) had a significant decrease (40.42 ± 3.01%, $p = .004$) in viability compared to the MDA-MB-231 control cells. There was also a significant decrease (22.29 ± 3.59%, $p = .005$) in viability in KIF14^{KD} paclitaxel treated

cells compared to MDA-MB-231 cells treated with paclitaxel. KIF14^{KD} paclitaxel treated also had a significant decrease in viability (40.84 ± 0.25%, p = .004) compared to the KIF14^{KD} cells (M = 98.75, SD = 0.25).

Figure 3.7

Viability of Cells is Reduced Following the Reduction of KIF14 Expression with Paclitaxel Treatment within a TNBC cell line



Note: Viability was determined using a Trypan Blue Exclusion Assay following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (Px). (A) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with a Games-Howell post hoc test. * p < .05 vs control, ** p < .01 vs control, ## p < .01 vs KIF14 KD, †† p < .01 vs Px Treated.

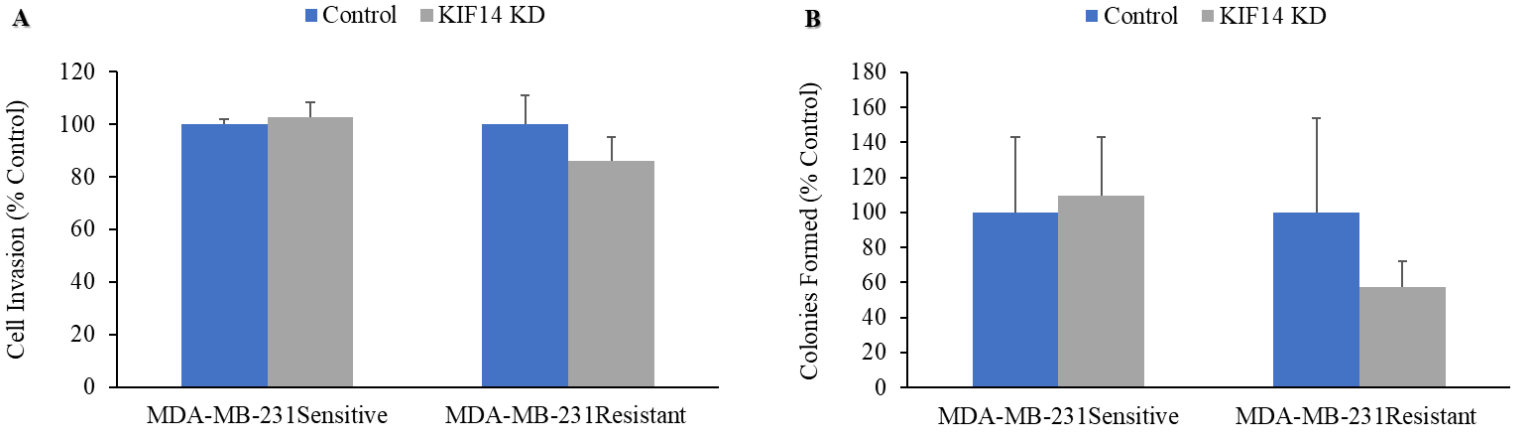
Expression of KIF14 does not Correlate with Metastatic Ability as Demonstrated with Cell Invasion Potential and Colony Formation Ability within a Paclitaxel Resistant TNBC Cell Line

To investigate KIF14's function in metastatic ability a cell invasion and colony formation assay was conducted. A cell invasion assay was performed to investigate the effects of KIF14 expression on a paclitaxel resistant TNBC cell line, MDA-MB-231^{Resistant}, on cell invasion potential (Figure 3.8A). Although there was a decrease (13.77 ± 10.89%, p = .58) in cell invasion potential with KIF14^{KD} in MDA-MB-231^{Resistant} cells (M = 0.12, SD = 0.01) completion of an independent samples t-test demonstrated that there was not a significant change between KIF14^{KD} and control MDA-MB-231^{Resistant} cells (M = 0.14, SD = 0.02), t (4) = 1.69, p = .58 (Table 3.S12). Interesting to note, MDA-MB-231^{Sensitive} cells demonstrated a minute increase in cell invasion (2.77 ± 1.80%, p = .11) although completion of an independent samples t-test demonstrated that there was not a significant change between KIF14^{KD} and control MDA-MB-231^{Sensitive} (Table 3.S13).

A colony formation assay was performed to investigate the effects of KIF14^{KD} within a paclitaxel resistant TNBC cell line (Figure 3.8B). There was a decrease (42.58 ± 14.87%, p = .068) in the formation of colonies with KIF14^{KD} in the MDA-MB-231^{Resistant} cells (M = 65.33, SD = 16.92) compared to control MDA-MB-231^{Resistant} cells (M = 113.78, SD = 61.62), nevertheless completion of an independent samples t-test demonstrated that there was not a significant change between them, t (4) = 1.31, p = .068 (Table 3.S14). Of note, MDA-MB-231^{Sensitive} KIF14^{KD} cells (M = 69.89, SD = 30.11) demonstrated a minute increase (9.38 ± 33.66%, p = .59) in number of colonies formed, however this was not significant as demonstrated by an independent samples t-test, t (4) = - 0.30, p = .59 (Table 3.S15).

Figure 3.8

Expression of KIF14 does not Alter Metastatic Ability as Demonstrated with Cell Invasion Potential and Colony Formation Ability



Note. (A) Cells were serum starved for 24hrs a day after transfection with KIF14 siRNA (KIF14 KD). Cell invasion assay was plated using Transwell™ Multiple Well Plate. Methylene blue and HCL was used to stain and de-stain transwells then absorbance was read at 630 nm. (B) Colony formation assay was plated 24-hours following transfection with KIF14 siRNA (KIF14 KD) and incubated for 14 days. Plates were fixed with ethanol and stained with geimsa. N = 3.
Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with a Tukey's post hoc test.

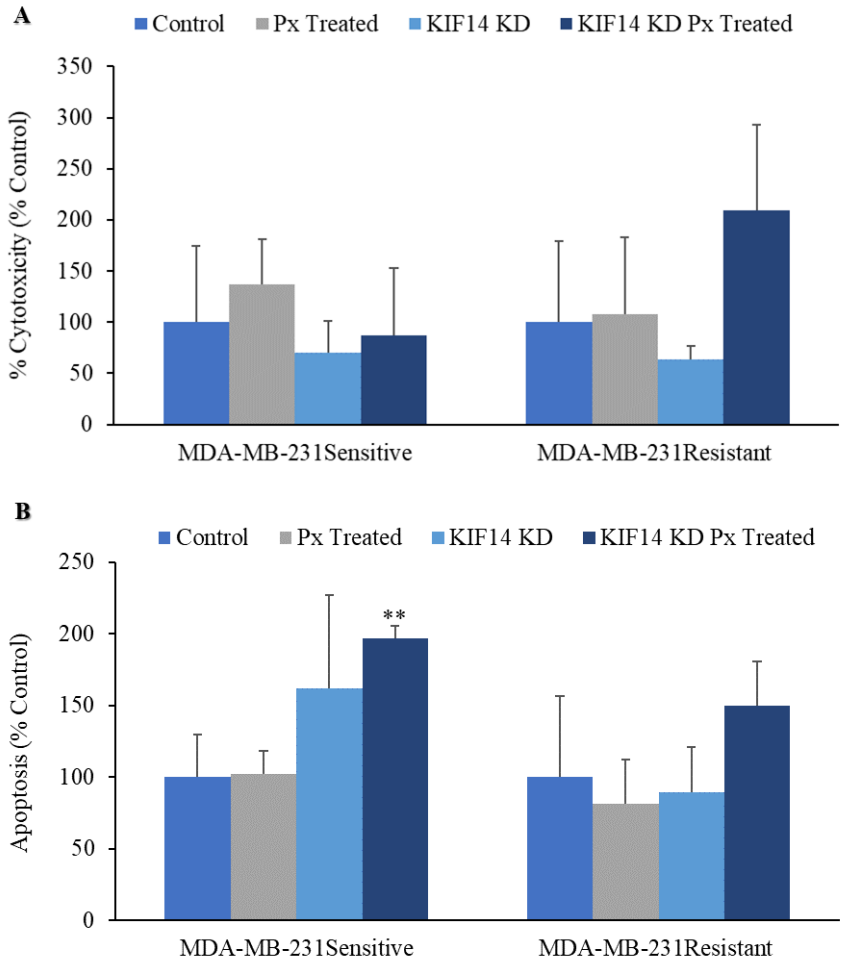
Expression of KIF14 Could Correlate with Cellular Death in the Presence of Paclitaxel as Determined by Cytotoxicity and Apoptosis

To investigate KIF14's function in connection to cellular death in the presence of paclitaxel, a LDH activity and apoptosis assay was conducted following $KIF14^{KD}$ with the treatment of paclitaxel. LDH activity assay was used to determine the percent cytotoxicity in the cells in the presence of paclitaxel following $KIF14^{KD}$ (Figure 3.9A).

Completion of a one-way ANOVA demonstrated that there was not a significant change in the cytotoxicity of paclitaxel following $KIF14^{KD}$ within the MDA-MB-231, $F(3, 8) = 0.57$, $p > .05$, $\eta 2p = 0.18$ (Table 3.S15). Although there was an increase ($48.47 \pm 74.67\%$, $p = .77$) in cytotoxicity in the $MDA\text{-}MB\text{-}231^{Resistant}$ $KIF14^{KD}$ paclitaxel treated cells ($M = 39.68$, $SD = 27.78$) compared to the $MDA\text{-}MB\text{-}231^{Resistant}$ cells ($M = 34.63$, $SD = 47.31$) a Tukey's post hoc demonstrated that it was not significant. Following $KIF14^{KD}$ in the $MDA\text{-}MB\text{-}231^{Resistant}$ cells ($M = 34.63$, $SD = 47.31$) there was a non-significant decrease ($55.45 \pm 14.32\%$, $p = .83$) in cytotoxicity compared to the $MDA\text{-}MB\text{-}231^{Resistant}$ cells. There was also a non-significant decrease ($12.71 \pm 71.60\%$, $p = 1$) in cytotoxicity of the $MDA\text{-}MB\text{-}231^{Resistant}$ cells treated with paclitaxel ($M = 14.37$, $SD = 11.79$) compared to the $MDA\text{-}MB\text{-}231^{Resistant}$ control cells. Within the $MDA\text{-}MB\text{-}231^{Sensitive}$ cells an ANOVA demonstrated that there were no significant differences between treatment groups, $F(3, 8) = 0.61$, $p > .05$, $\eta 2p = 0.19$ (Table 3.S15).

Figure 3.9

Expression of KIF14 Could Correlate with Cellular Death in the Presence of Paclitaxel in Cellular Death as determined by Cytotoxicity and Apoptosis



Note. (A) Cytotoxicity assay was conducted CyQUANT™ LDH Cytotoxicity Assay following manufacturer's protocol 24-hours following transfection with KIF14 siRNA (KIF14 KD). Cells were treated with DMSO (control) or paclitaxel (Px). Absorbance was measured at 492 nm and 680 nm. LDH activity was determined prior to calculation of percent cytotoxicity. (B) The eBioscience™ Annexin V Apoptosis Detection Kit FITC was used per manufacturer's instructions to assess apoptosis following transfection with KIF14 siRNA (KIF14 KD) and

treatment with paclitaxel (px). Flow cytometry was conducted, and data analysis was conducted using FlowJo software. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with a Tukey's and Games-Howell post hoc test. ** $p < .01$.

An apoptosis assay was conducted to determine the effects of $KIF14^{KD}$ within the MDA-MB-$231^{Resistant}$ cells on paclitaxel induced apoptosis (Figure 3.9B). Completion of a one-way ANOVA demonstrated that there was not a significant change in paclitaxel induced apoptosis following $KIF14^{KD}$ within the MDA-MB-$231^{Resistant}$ cells, $F (3, 8) = 2.13$, $p > .05$, $\eta 2p = 0.44$ (Table 3.S18). A Tukey's post hoc test demonstrated that there was not a significant increase (68.35 ± 30.37%, $p = .16$) in apoptosis within the MDA-MB-$231^{Resistant}$ $KIF14^{KD}$ paclitaxel treated ($M = 13.63$, $SD = 3.89$) compared to the MDA-MB-$231^{Resistant}$ paclitaxel treated cells, ($M = 6.32$, $SD = 2.35$). There was also not a significant decrease (10.17 ± 56.73%, $p = .97$) in apoptosis with MDA-MB-$231^{Resistant}$ $KIF14^{KD}$ cells ($M = 9.08$, $SD = 4.09$) compared to the MDA-MB-$231^{Resistant}$ control cells ($M = 7.72$, $SD = 4.38$). As expected, the MDA-MB-$231^{Resistant}$ paclitaxel treated cells did not result in a significant decrease (18.23 ± 30.30%, $p = .97$) in apoptosis compared to the MDA-MB-$231^{Resistant}$ control cells. MDA-MB-$231^{Resistant}$ $KIF14^{KD}$ paclitaxel treated cells did not result in a significant increase in apoptosis (50.12 ± 56.73%, $p = .29$) compared to the MDA-MB-$231^{Resistant}$ control cells. Interestingly, completion of a one-way ANOVA determined that there was a significant difference among treatment groups in the MDA-MB-$231^{Sensitive}$ cells, $F (3, 3.92) = 24.72$, $p < .01$, $\eta 2p = 0.61$. A Games-Howell post-hoc test demonstrated that there was a significant increase (94.24 ± 8.77%, $p = .008$) in apoptosis in

the MDA-MB-231^{Sensitive} KIF14^{KD} paclitaxel treated cells compared to MDA-MB-231^{Sensitive} paclitaxel treated cells (Table 3.S19).

Paclitaxel Treatment Following KIF14^{KD} Results in a Decrease in Cellular Growth as Measured by Proliferation and Relative Population Doublings

To investigate KIF14's function in cellular growth, a methylene blue proliferation and trypan blue exclusion assay was conducted on the MDA-MB-231^{Resistant} cells. A methylene blue proliferation assay was performed following KIF14^{KD} with treatment of paclitaxel to determine the effects of KIF14 expression on proliferation potential (Figure 3.10 A & B). To ascertain the short-term effects of paclitaxel treatment following KIF14^{KD} in the MDA-MB-231^{Resistant} cells, a 48-hour assay was executed (Figure 3.10 A). Completion of a one-way ANOVA demonstrated that there was a significant change in proliferation following KIF14^{KD} within the MDA-MB-231^{Resistant} cells, F (3, 8) = 6.36, $p < .05$, $\eta 2p = 0.71$ (Table 2.S20). A Tukey's post hoc test revealed that there was a significant decrease (24.20 ± 9.44%, $p = .015$) in proliferation within the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells ($M = 0.84$, $SD = 0.10$) compared the MDA-MB-231^{Resistant} control cells ($M = 1.11$, $SD = 0.09$). There was also a significant decrease (20.14 ± 7.71%, $p = .039$) in proliferation in the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells compared to the MDA-MB-231^{Resistant} paclitaxel treated cells ($M = 1.06$, $SD = 0.09$). A non-significant decrease (11.09 ± 2.19%, $p = .31$) in proliferation occurred in the MDA-MB-231^{Resistant} KIF14^{KD} cells ($M = 0.99$, $SD = 0.02$) compared to the MDA-MB-231^{Resistant} control cells. As expected, MDA-MB-231^{Resistant} cells demonstrated an insignificant reduction in proliferation (4.06 ± 7.71%, $p = .90$) compared to the MDA-MB-231^{Resistant} control cells. Within the MDA-MB-231^{Sensitive} cells a one-way ANOVA demonstrated that there were no significant differences between treatment groups, F (3, 8) = 1.00, $p > .05$, $\eta 2p = 0.27$ (Table 3.S21).

The long-term effects of paclitaxel treatment following KIF14^{KD} in the MDA-MB-231^{Resistant} cells was determined using a 5- day assay (Figure 3.10 B). Completion of a one-way ANOVA demonstrated that there was a significant change in proliferation following KIF14^{KD} within the MDA-MB-231^{Resistant} cells, F (3, 8) = 29.72, $p < .001$, $\eta 2p = 0.92$ (Table 3.S22). A Tukey's post hoc test demonstrated that there was a significant decrease (75.26 ± 12.45%, $p < .001$) in proliferation within the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells ($M = 0.22$, $SD = 0.11$) compared to the proliferation of the MDA-MB-231^{Resistant} control cells, ($M = 0.91$, $SD = 0.09$), as well as a significant decrease (75.94 ± 16.73%, $p < .001$) in proliferation compared to the MDA-MB-231 KIF14^{KD} cells ($M = 0.91$, $SD = 0.15$). MDA-MB-231^{Resistant} paclitaxel treated cells ($M = 0.39$, $SD = 0.08$) exhibited a significant reduction in proliferation (57.14 ± 9.14%, $p = .002$) compared to the MDA-MB-231^{Resistant} cells. MDA-MB-231^{Resistant} KIF14^{KD} cells did not result in a significant increase in proliferation (0.67 ± 16.73%, $p = 1$) compared to the MDA-MB-231^{Resistant} cells. There was an inconsequential reduction in proliferation (18.12 ± 12.45%, $p = .35$) between the MDA-MB-231^{Resistant} paclitaxel treated cells compared to the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells.

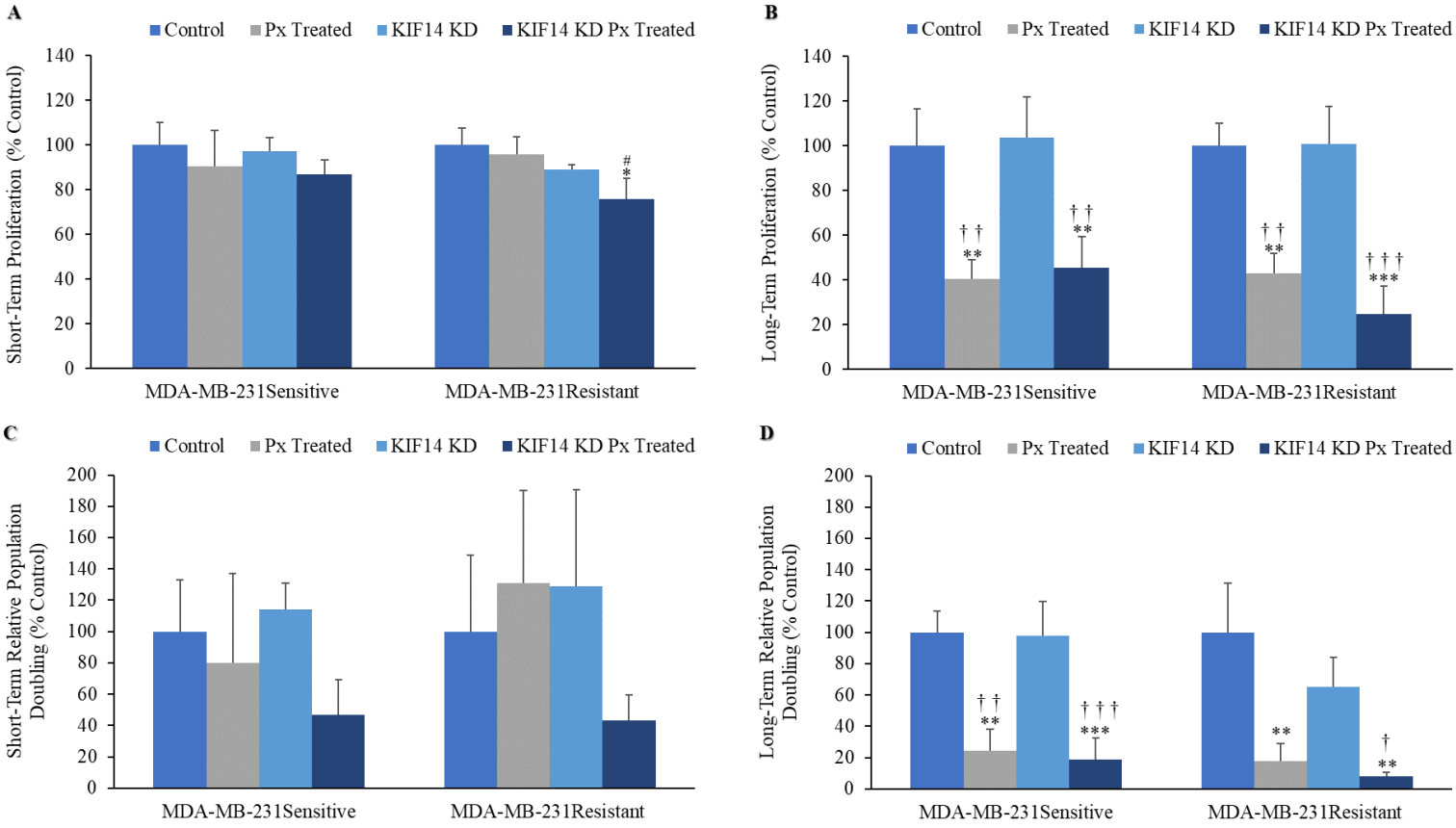
Completion of an ANOVA determined a significant difference among treatment groups within the MDA-MB-231^{Sensitive} cells, F (3, 8) = 16.26, $p < .001$, $\eta 2p = 0.86$ (Table 3.S23). A Tukey's post hoc test revealed a significant decrease (54.51 ± 13.77%, $p = .008$) in proliferation within the MDA-MB-231^{Sensitive} KIF14^{KD} paclitaxel treated cells ($M = 0.37$, $SD = 0.11$) compared to the MDA-MB-231^{Sensitive} control cells ($M = 0.80$, $SD = 0.13$) along with a significant decrease (58.28 ± 18.12%, $p = .005$) in proliferation compared the MDA-MB-231^{Sensitive} KIF14^{KD} cells ($M = 0.83$, $SD = 0.15$). As expected, there was also a significant

decrease (59.71 ± 8.71%, p = .005) in proliferation in the MDA-MB-231^{Sensitive} paclitaxel treated cells (M = 0.32, SD = 0.07) compared to the MDA-MB-231^{Sensitive} control cells.

A trypan blue exclusion assay was conducted to establish the effects of KIF14 expression with paclitaxel treatment in the MDA-MB-231^{Resitant} cells in relation to relative population doubling (Figure 3.10 C & D). To investigate these effects in the short-term a 48- hour assay was conducted (Figure 3.10 C) and completion of a one-way ANOVA demonstrated that there was not a significant change between treatment groups in the MDA-MB-231^{Resistant} cells, F (3, 8) = 2.01, p > .05, $\eta 2p$ = 0.43 (Table 3.S24). An insignificant reduction (56.54 ± 15.98%, p = .54) in relative population doubling occurred in the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells (M = 2.44, SD = .90) compared to MDA-MB-231^{Resistant} control cells (M = 5.62, SD = 2.74). As anticipated, there was an inconsequential increase (30.85 ± 58.99%, p = .87) in relative population doublings in the MDA-MB-231^{Resistant} paclitaxel treated cells (M = 5.62, SD = 2.74) compared to MDA-MB-231^{Resistant} control cells. Within the MDA-MB-231^{Sensitive} cells a one-way ANOVA determined that there were no significant differences between treatment groups, F (3, 8) = 2.01, p > .05, $\eta 2p$ = 0.43 (Table 3.S25).

Figure 3.10

Paclitaxel Treatment Following KIF14 Knockdown Results in a Decrease in Cellular Growth as Measured by Proliferation and Relative Population Doubling



Note. (A & B) Proliferation potential following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (px) was evaluated via a Methylene Blue Proliferation Assay. (A) Short-term assay, cells were incubated for 48- hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. (C & D) Relative population doubling was determined using a trypan blue exclusion assay following transfection with KIF14 siRNA and treatment with paclitaxel. (C) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (D) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with Tukey's post hoc test. ** $p < .01$ vs Control, *** $p < .001$ vs Control, † $p < .05$ vs KIF14 KD, †† $p < .01$ vs KIF14 KD, ††† $p < .001$ vs KIF14 KD.

A 5- day assay was conducted to investigate the effects of KIF14 expression with paclitaxel treatment in a longer setting (Figure 3.10 D) on the relative population doubling of the MDA-MB-231^{Resistant} cells. Completion of a one-way ANOVA demonstrated that there was a significant change among groups in relative population doubling, $F (3, 8) = 14.97$, $p < .001$, $\eta 2p = 0.85$ (Table 3.S26). A Tukey's post hoc test demonstrated that there was a significant decrease (82.17 ± 10.91%, $p = .003$) in relative population doubling within the MDA-MB-231^{Resistant} paclitaxel treated cells ($M = 7.80$, $SD = 4.78$) compared to the MDA-MB-231^{Resistant} control cells ($M = 43.77$, $SD = 13.78$). There is a significant reduction in relative population doubling (92.14 ± 2.63%, $p = .002$) in the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells ($M = 3.44$, $SD = 1.15$) compared to the MDA-MB-231^{Resistant} control cells as well as a significant decrease (57.22 ± 18.91%, $p = .027$) compared to the MDA-MB-231^{Resistant} KIF14^{KD} cells ($M = 28.49$, $SD = 8.28$). KIF14^{KD} in the MDA-MB-231^{Resistant} cells resulted in a non-significant reduction (34.91 ± 31.47%, $p = .20$) in relative population doubling compared to the MDA-MB-231^{Resistant} cells.

Completion of a one-way ANOVA revealed a significant difference among treatment groups within the MDA-MB-231^{Sensitive} cells, $F (3, 8) = 23.24$, $p < .001$, $\eta 2p = 0.90$ (Table 3.S27). A Tukey's post hoc test revealed a significant decrease (81.20 ± 13.48%, $p = .001$) in relative population doubling within the MDA-MB-231^{Sensitive} KIF14^{KD} paclitaxel treated cells ($M = 6.11$, $SD = 4.38$) compared to the MDA-MB-231^{Sensitive} control cells ($M = 32.49$, $SD = 4.43$)

along with a significant decrease (79.23 ± 21.91%, $p = .001$) in relative population doubling compared the MDA-MB-231^{Sensitive} KIF14^{KD} cells ($M = 28.49$, $SD = 8.28$). As expected, there was also a significant decrease (75.77 ± 13.75%, $p = .002$) in relative population doubling in the MDA-MB-231^{Sensitive} paclitaxel treated cells ($M = 7.87$, $SD = 4.47$) compared to the MDA-MB-231^{Sensitive} control cells.

KIF14 Expression with Paclitaxel Treatment is Correlated to a Decrease in Cell Viability within a Paclitaxel Resistant TNBC Cell Line

A trypan blue exclusion assay was conducted to ascertain change in cellular viability within the MDA-MB-231^{Resistant} cells with KIF14^{KD} and paclitaxel treatment (Figure 3.11). A 48-hour, short-term, assay was completed following KIF14^{KD} with paclitaxel treatment (Figure 3.11A) and an ANOVA test revealed that there was a significant change between treatment groups within the MDA-MB-231^{Resistant} cells, $F (3, 8) = 11.81$, $p < .01$, $\eta 2p = 0.82$ (Table 3.S28). MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells ($M = 67.89$, $SD = 10.22$) demonstrated a significant decrease (30.37 ± 2.04%, $p = .003$) in viability compared to MDA-MB-231^{Resistant} control cells ($M = 97.50$, $SD = 1.98$) as well as a significant decrease (25.84 ± 5.86%, $p = .007$) compared to the MDA-MB-231^{Resistant} KIF14^{KD} cells ($M = 93.08$, $SD = 5.71$). There was also a significant decrease (20.74 ± 10.48%, $p = .023$) in the MDA-MB-231^{Resistant} KIF14^{KD} paclitaxel treated cells compared to the MDA-MB-231^{Resistant} paclitaxel treated cells ($M = 88.11$, $SD = 35.72$). As expected, there was an insignificant decrease (9.63 ± 5.86%, $p = .36$) in viability of the MDA-MB-231^{Resistant} paclitaxel treated cells compared to the MDA-MB-231^{Resistant} cells. Within the MDA-MB-231^{Sensitive} cells, an ANOVA revealed that there were no significant differences between treatment groups, $F (3, 3.68) = 2.45$, $p > .05$, $\eta 2p = 0.43$ (Table 3.S29).

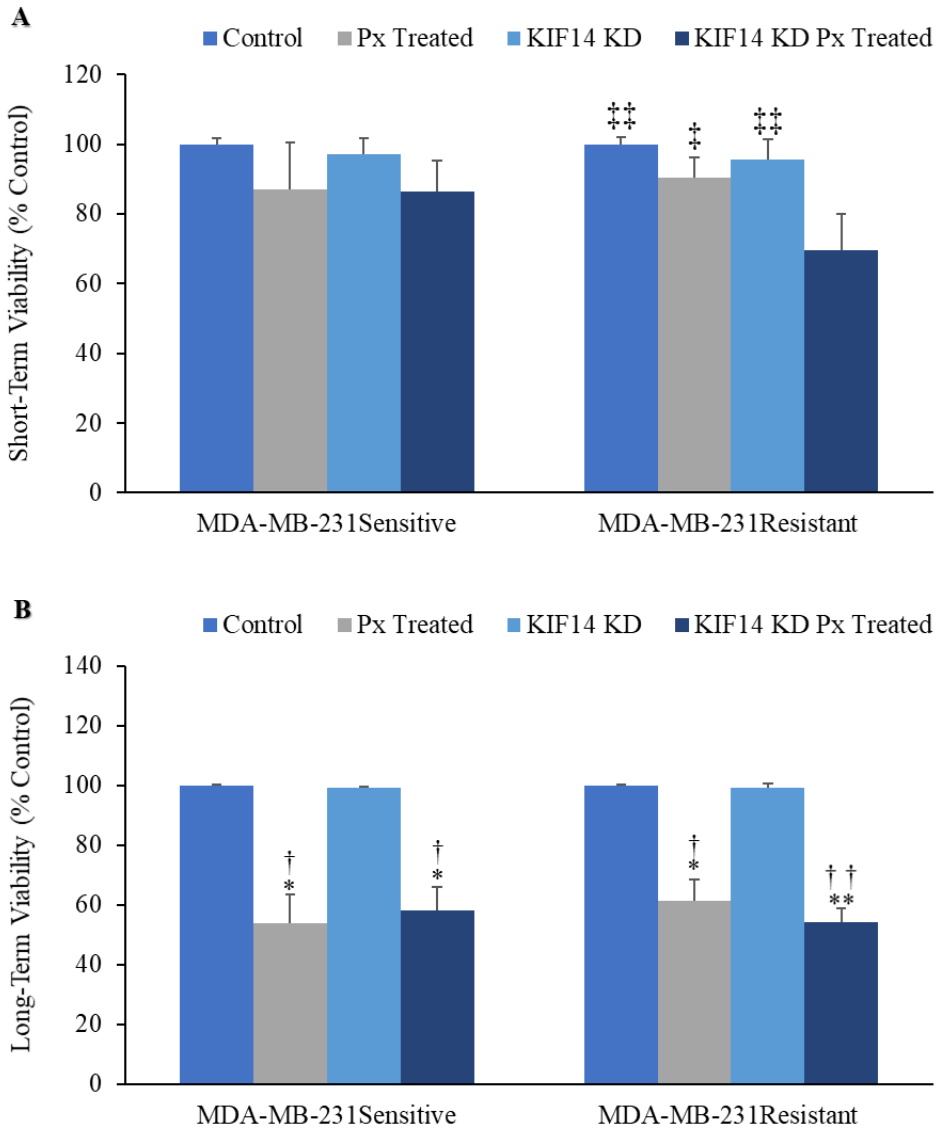
A 5-day, long-term, assay was conducted to ascertain the effects of KIF14 expression with paclitaxel treatment (Figure 3.11 B) on the cellular viability of cells. A one-way ANOVA

proved that there was a significant change among treatment groups in viability for the MDA-MB-231^{Resistant} cells, F (3, 3.48) = 89.18, $p < .001$, $\eta 2p = 0.97$ (Table 3.S30). A Games-Howell post hoc test verified that there was a significant decrease (45.78 ± 0.39%, $p = .008$) in cellular viability following KIF14^{KD} with treatment of paclitaxel in the MDA-MB-231^{Resistant} cells (M = 53.58, SD = 4.63) compared to the MDA-MB-231^{Resistant} control cells (M = 98.83, SD = 0.38) as well as a significant decrease (44.94 ± 1.54%, $p = .005$) compared to the MDA-MB-231^{Resistant} KIF14^{KD} cells (M = 98.00, SD = 1.52). Paclitaxel treatment in the MDA-MB-231^{Resistant} cells (M = 60.58, SD = 7.13) resulted in a significant reduction (38.70 ± 7.21%, $p = .028$) in cellular viability compared to the MDA-MB-231^{Resistant} control cells.

Completion of an ANOVA ascertained a significant difference among treatment groups within the MDA-MB-231^{Sensitive} cells, F (3, 3.60) = 38.19, $p < .01$, $\eta 2p = 0.95$ (Table 3.S31). A Games-Howell post hoc test revealed a significant decrease (42.03 ± 0.15%, $p = .030$) in cellular viability within the MDA-MB-231^{Sensitive} KIF14^{KD} paclitaxel treated cells (M = 57.58, SD = 8.06) compared to the MDA-MB-231^{Sensitive} control cells (M = 99.33, SD = 0.14) along with a significant decrease (41.36 ± 0.38%, $p = .031$) in cellular viability compared the MDA-MB-231^{Sensitive} KIF14^{KD} cells (M = 98.67, SD = 0.38). As expected, there was also a significant decrease (46.06 ± 9.58%, $p = .035$) in cellular viability in the MDA-MB-231^{Sensitive} paclitaxel treated cells (M = 53.58, SD = 9.52) compared to the MDA-MB-231^{Sensitive} control cells.

Figure 3.11

KIF14 Expression with Paclitaxel Treatment is Correlated to a Decrease in Viability Within the MDA-MB-231^{Resistant} Cells



Note. Viability was determined using a Trypan Blue Exclusion Assay following transfection with KIF14 siRNA (KIF14 KD) and treatment with paclitaxel (Px). (A) Short-term assay, cells were incubated for 48 hours following treatment with paclitaxel. (B) Long-term assay, cells were incubated for 5 days following treatment with paclitaxel. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with a Tukey's and Games- Howell post hoc test. * $p < .05$ vs Control, ** $p < .01$ vs Control, † $p < .05$ vs KIF14 KD, †† $p < .01$ vs KIF14 KD, ‡ $p < .05$ vs KIF14 KD Px Treated, ‡‡ $p < .01$ vs KIF14 KD Px Treated.

Discussion

Kinesin family member 14, KIF14, is a member of the kinesin-3 super family of microtubule-associated motor proteins (MAPs) (Nomura et al., 1994). KIF14 operates in a variety of cellular activities including but not limited to mitotic spindle formation, cytokinesis, vesicle transport, and chromosome segregation (Basavarajappa & Corson, 2012; Gruneberg et al., 2006; Huang et al., 2015). KIF14 accomplishes these functions by causing depolymerization of the microtubules (Arora et al., 2014; van der Vaart et al., 2009). KIF14 has been deemed a potential oncogene due to its upregulation in a variety of cancers including: hepatocellular carcinoma (HCC), human malignant astrocytomas, medulloblastoma, retinoblastoma, lung adenocarcinoma, ovarian, colorectal, prostate, cervical, and most important to this study, breast cancer (Corson & Gallie, 2006; Ehrlichova et al., 2013; Huang et al., 2015; Hung et al., 2013; Li et al., 2017; Madhavan et al., 2009; Qiu et al., 2017; Schiewek et al., 2018; Thériault, Basavarajappa, et al., 2014; Thériault, Cybulska, et al., 2014; Wang et al., 2018; Xiao et al., 2021; Yang et al., 2013; Zhang et al., 2017). The work of Singel et. al. used the Cancer Genome Atlas to discover that KIF14 is expressed in 92% of all triple-negative breast cancers (TNBC), thus demonstrating a need to study KIF14 within TNBC (Singel et al., 2013).

Work in our laboratory has focused on the mechanism of paclitaxel chemoresistance within a TNBC cell line, MDA-MB-231, and I found that KIF14 is significantly upregulated

within our resistant cell line, MDA-MB-231^{Resistant}, when compared to the MDA-MB-231^{Sensititve} cells. This is of interest as KIF14 is naturally overexpressed in breast cancer (Corson & Gallie, 2006; Singel et al., 2013; Singel et al., 2014). Decreased KIF14 expression has also been correlated with chemo-sensitization to docetaxel (Singel et al., 2013; Singel et al., 2014). Our laboratory is focused on the function of KIF14 in the mechanism of chemoresistance with paclitaxel in a TNBC cell line. This chapter delved into those functions with the use of transient knockdown of KIF14 with the use of short interfering RNA (siRNA) in both a paclitaxel sensitive cell line and a resistant cell line.

The work of Shay et al. achieved a transient knockdown of 50% in MDA-MB-231 cells with KIF14 siRNA and demonstrated that the cells could be sensitized to docetaxel (Singel et al., 2013). Conformation of siRNA knockdown of KIF14 in our cell line demonstrated only a 14% knockdown. Results in this study could have achieved greater significance with an increase in the knockdown of KIF14 (KIF14^{KD}).

In this chapter, I demonstrated that with the KIF14^{KD} within the MDA-MB-231 cells did not result in a significant change in metastatic ability as measured with cell invasion potential and colony formation ability. I did show a decrease in the number of colonies formed with KIF14^{KD} however this decrease was not significant due to a large standard deviation. There is a possibility that with increased KIF14^{KD} there could be a significant decrease in metastatic ability as demonstrated by previous studies (Ahmed et al., 2012; Corson et al., 2007; Hung et al., 2013; Li et al., 2017; Yang et al., 2013).

In my cell line KIF14^{KD} did not alter cellular death potential as measured by percent cytotoxicity and apoptosis in the presence of paclitaxel. This could be due to the fact that there was only a 14% decrease in KIF14 expression and there is a possibility that with an increase in

$KIF14^{KD}$ that we could see a greater impact on cellular death potential in the presence of paclitaxel. KIF14 has been demonstrated to induces apoptosis within HCC, glioblastoma, medulloblastoma, and prostate cancer (Huang et al., 2015; Li et al., 2017; Yang et al., 2013; Zhang et al., 2017) and there is a possibility that with a greater alteration in KIF14 expression, I could see the induction of apoptosis as well.

In terms of cellular growth $KIF14^{KD}$ with paclitaxel treatment did demonstrate a significant decrease in proliferation in a long-term setting, however, this was not echoed in the short term or in relative population doublings. Previous studies have demonstrated a correlation between cellular growth and KIF14 expression with the treatment of a chemotherapeutic agent, however, with only a 14% reduction in KIF14 expression in my cell line that was not seen in this study (Hung et al., 2013; Li et al., 2017; Thériault, Cybulska, et al., 2014; Xu et al., 2014; Yang et al., 2013; Zhang et al., 2017). There is the possibility that with a greater $KIF14^{KD}$ treatment with paclitaxel could show significant changes in cellular growth potential. Although I did not see as significant as a decrease in cellular growth, I did demonstrate a significant decrease in viability of the cells with paclitaxel treatment following $KIF14^{KD}$ potentially suggesting a role for KIF14 in maintain healthy cells in the presence of paclitaxel.

A novel aspect of this study was the exploration of KIF14 within a paclitaxel chemoresistant TNBC cell line. Within the MDA-MB-$231^{Resistant}$ cells I demonstrated a slight decrease in cell invasion potential and colony formation ability, potentially suggesting a role for KIF14 in metastatic ability in chemoresistance. However, with only a 14% $KIF14^{KD}$ there was not a significant decrease, there is the possibility that with an increase in the reduction of KIF14 expression that we could see a more significant alteration in metastatic ability in chemoresistance.

Although not significant I did see an increase in cellular death in the presence of paclitaxel following $KIF14^{KD}$ within the MDA-MB-$231^{Resistant}$ cells potentially suggesting a role for KIF14 in the cell's survival potential following treatment with paclitaxel. Again, with a greater reduction in KIF14's expression than 14% there is the possibility that this increase in cellular death potential could be one of significance.

Even though there was only a 14% reduction in KIF14 expression with my cells, I did see a significant decrease in cellular growth potential as measured by proliferation and relative population doublings in the presence of paclitaxel in the MDA-MB-$231^{Resistant}$ cells. As well as a significant decrease in viability within the MDA-MB-$231^{Resistant}$ $KIF14^{KD}$ cells treated with paclitaxel. With this significant decrease with such a minute decrease in expression of KIF14 there is potential evidence for KIF14 functioning in the mechanism of chemoresistance within breast cancer. Further exploration with into KIF14 is necessary to fully identify KIF14's function in the mechanism of chemoresistance.

CHAPTER 4

EPIGALLOCATECHIN-3 GALLATE AS A COMBINATORIAL TO PACLITAXEL IN PACLITAXEL-RESISTANT BREAST CANCER CELLS

Abstract

Chemoresistance represents the biggest hurdle in the quest for effective therapeutics against breast cancer. The effectiveness of chemotherapeutic agents such as the microtubule stabilizing drug, paclitaxel, is significantly impeded by the acquisition of resistance. Investigation into compounds that can augment an existing therapy or sensitize resistant cells remains of critical importance in the ongoing fight against cancer. Adjuvant therapies that can incorporate safe, alternative approaches and could be capable of sensitizing cells to chemotherapeutic agents stand as the ideal neoadjuvant therapeutic. Exploration of dietary supplements as a means of enhancing the promising effects of chemotherapies has a long history with many compounds being actively utilized in clinic. The active ingredient of green tea, (-)-epigallocatechin-3-gallate (EGCG), stands as a potential adjuvant therapy even against resistant tumor cells. This study sought to explore the potential for ECGC to impact paclitaxel-resistant cells and serve as an adjuvant therapeutic against breast cancer. Here, I find that ECGC can suppress the growth of paclitaxel-resistant hormone responsive breast cancer cells in a dose-dependent manner. I also found that EGCG has the potential to decrease the viability of paclitaxel-resistant triple negative breast cancer cells when treated with both EGCG and paclitaxel, thus demonstrating the potential for

this compound. This information thus supports further investigation into the molecular mechanisms of EGCG activity in paclitaxel-resistant cells and the potential as EGCG as a combinatorial to chemotherapy.

Introduction

Breast cancer is a heterogeneous disease that is one of the most diagnosed cancers in American women (Siegel et al., 2020). This year alone, there will be an estimated of 276,000 new cases that will be diagnosed and approximately 42,000 individuals will die to either breast cancer or complications related to the disease (Siegel et al., 2020). A common treatment for breast cancer is the use of the chemotherapeutic agent paclitaxel, however one of the most significant hindrances of its efficacy is the development of chemoresistance (S. M. Maloney et al., 2020). Remarkably, every chemotherapeutic evaluated thus far has shown the capacity for the development of resistance (C. E. Steding, 2016). Given this tendency for the development of chemoresistance, investigation into safe supplemental therapies capable of reversing resistance remains of critical importance. One growing category of investigations involves focusing on dietary changes or dietary supplements that could provide beneficial outcomes for those undergoing chemotherapy. Dietary supplements and combination therapies have been shown to be capable of sensitizing resistant cells to paclitaxel and stand as promising neoadjuvant therapies (Attia et al., 2020; Liu et al., 2020; Ramadass et al., 2015).

Green tea is one of the most widely consumed beverages worldwide and is one example of a possible enhancement to current therapeutics (Baliga et al., 2005; Gan et al., 2018). Its wide distribution and long-term use provide a plethora of information regarding its safety and consequences. Green tea contains a polyphenol known as (-)-epigallocatechin-3-gallate (EGCG) that constitutes roughly 25% of green tea and has demonstrated chemo-preventive effects,

including inhibition of cellular growth, inducing apoptosis, and cell cycle arrest (Cabrera et al., 2006; Dufresne & Farnworth, 2001; Gianfredi et al., 2018; Khan et al., 2006; Lecumberri et al., 2013; Mohan et al., 2011; Stearns & Wang, 2011; Tang et al., 2012). Given that EGCG has been shown to exert these chemo-preventive effects without affecting healthy cells, it stands as a promising addition to a chemotherapeutic regiment (Chen et al., 1998). Studies investigating the potential for EGCG have shown that it can function to enhance the effects of paclitaxel and eliminate cancerous prostate tumors (Fujiki et al., 2018; Luo et al., 2010).

Although previous studies have shown the potential for ECGC to function in combination with paclitaxel, the ability of it to function in chemoresistance remains poorly defined. Utilizing cells generated to exhibit true, acquired chemoresistance, this study investigated the effects of EGCG on paclitaxel-resistant cells. Our laboratory has emphasized resistance against paclitaxel and has demonstrated the ability to generate cells exhibiting high levels of acquired resistance over time, as demonstrated in Chapter 2, “Characterization of an Acquired Chemoresistant Breast Cancer Cell Line.” By generating chemotherapeutic resistant breast cancer cell lines via incrementally increasing doses of a chemotherapeutic reagent, we have created a model system that can be utilized to define previously unidentified mechanisms of resistance. Using well characterized cell lines, we can evaluate changes to a far greater degree. Although chemoresistance to paclitaxel has been evaluated in the past, most of the model systems have utilized cells that have been maintained in paclitaxel for only brief periods of time, ranging from a couple of treatments prior to experimentation or even one treatment a week for a couple of months (Jeong et al., 2016; Kenicer et al., 2014; Sprouse & Herbert, 2014; Werner et al., 2014). With cells exposed to paclitaxel weekly over a long period of time, this better recapitulates the

initial therapeutic suppressions that would be observed in clinic and ensures that the cells retain a strong level of resistance throughout the culture process.

Here, I show that long-term acquired paclitaxel-resistant breast cancer cells demonstrate a dose-dependent response to EGCG and the combination of EGCG with paclitaxel results in triple-negative breast cancer cells demonstrating a reduced viability compared to paclitaxel treatment alone. This information thus supports further investigation into the molecular mechanisms of EGCG activity in paclitaxel-resistant cells and the potential as EGCG as an adjuvant to chemotherapy.

Materials and Methods

Culture Conditions

The triple-negative breast cancer cell line (TNBC), MDA-MB-231, and the hormone responsive breast cancer cell line, MCF7, was maintained using traditional means (Steding et al., 2011). Briefly, cells were cultured in Nunc tissue culture flasks and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Cosmic Calf Serum (CCS) (Gibco and Fisher Scientific). Cells were maintained at 37℃ and 5% CO_2 at sub-confluent levels and passaged once cells reached a confluency between 75% - 95% via treatment with 0.25% Trypsin EDTA (Gibco).

Table 4.1

Treatment Groups.

Name		Maintained Treatment Condition
MDA-MB-231^{Sensitive}	MCF7^{Sensitive}	0.01% DMSO
MDA-MB-231^{Resistant}	MCF7^{Resistant}	1.6 ng/mL Paclitaxel

Note. Table expressing treatment conditions of cell line. MDA-MB-231 cells were passaged into two groups and treated with Dimethyl Sulfoxide (DMSO) or paclitaxel. Once treatments were

initiated, treated cells were maintained as isolated cell lines designated as: MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant}. Cells were maintained with a concentration of 0.01% DMSO or 1.6 ng/mL of paclitaxel, respectively, weekly over the period of several years to maintain a resistant population.

Generation of Acquired Chemoresistant Cell Lines

MDA-MB-231 and MCF7 cells were passaged into groups and treated with either Dimethyl Sulfoxide (DMSO), MDA-MB-231^{Sensitive}/MCF7^{Sensisitve}, or 1.6 ng/mL paclitaxel diluted in DMSO, MDA-MB-231^{Resistant}/MCF7^{Resistant} weekly over a period of several years (Table 4.1).

Proliferation Assay

Proliferation potential following treatment with Epigallocatechin-3 Gallate (EGCG) was evaluated via a Methylene Blue Proliferation Assay. Cells were plated at a density of $1x10^5$ cells/well then treated with 12.5 ug/mL, 25 ug/mL, 50 ug/mL, 100 ug/mL, or 200 ug/mL of EGCG diluted in 0.1% Ethanol (EtOH) or 0.1% EtOH for control and incubated at 37℃ and 5% CO_2 for 48 hours at which time they were fixed with 100% methanol. Adherent cells were stained with 0.5% Methylene Blue and allowed to air dry. Treatment with 0.5 M HCL was used to redistribute stain into solution and absorbance was evaluated at 630nm using a spectrophotometer (BioTek).

Trypan Blue Exclusion Assay

Relative population doubling and cellular viability was determined using a Trypan Blue Exclusion Assay. Cells were plated at a density of $5x10^4$ cells/well and treated with 50 ug/mL EGCG diluted in EtOH, 10 ng/mL Px diluted in DMSO, or 0.1% DMSO or EtOH, or a combination of both. Cells were then incubated at 37℃ and 5% CO_2 for 48 hours. Cells were harvested using 0.25% Trypsin EDTA and the resulting cell population was evaluated using an

automated cell counter (BIO-rad). Relative population doubling was calculated by the following equation and then normalized to control cells.

$$\frac{\text{Number of Live Cells}}{50{,}000}$$

Viability of cells was calculated by the following equation and was normalized to the control cells.

$$\frac{\text{Number of Live Cells}}{\text{Total Number of Cells}} * 100\%$$

Statistics

Data was expressed as the means ± the standard deviation. IBM SPSS Statistics Version 27 was used for statistical analysis. Data comparison between multiple groups was performed using one-way analysis of variance (ANOVA) (Brown, 2005; Kim, 2017). A Levene's test of homogeneity was used, if Levene's statistic was not significant, then homogeneity of variance was assumed, and a Tukey's post hoc test was conducted (Abdi & Williams, 2010; Glass, 1966). If Levene's statistic was significant, then homogeneity of variance was not assumed, and a Welch's test of homogeneity was conducted with a Games-Howell post hoc test. *p*-values of less than .05 were considered significant (Sauder & DeMars, 2019).

Results

Paclitaxel Resistant Breast Cancer Cells Respond in a Dose-Dependent Manner to EGCG

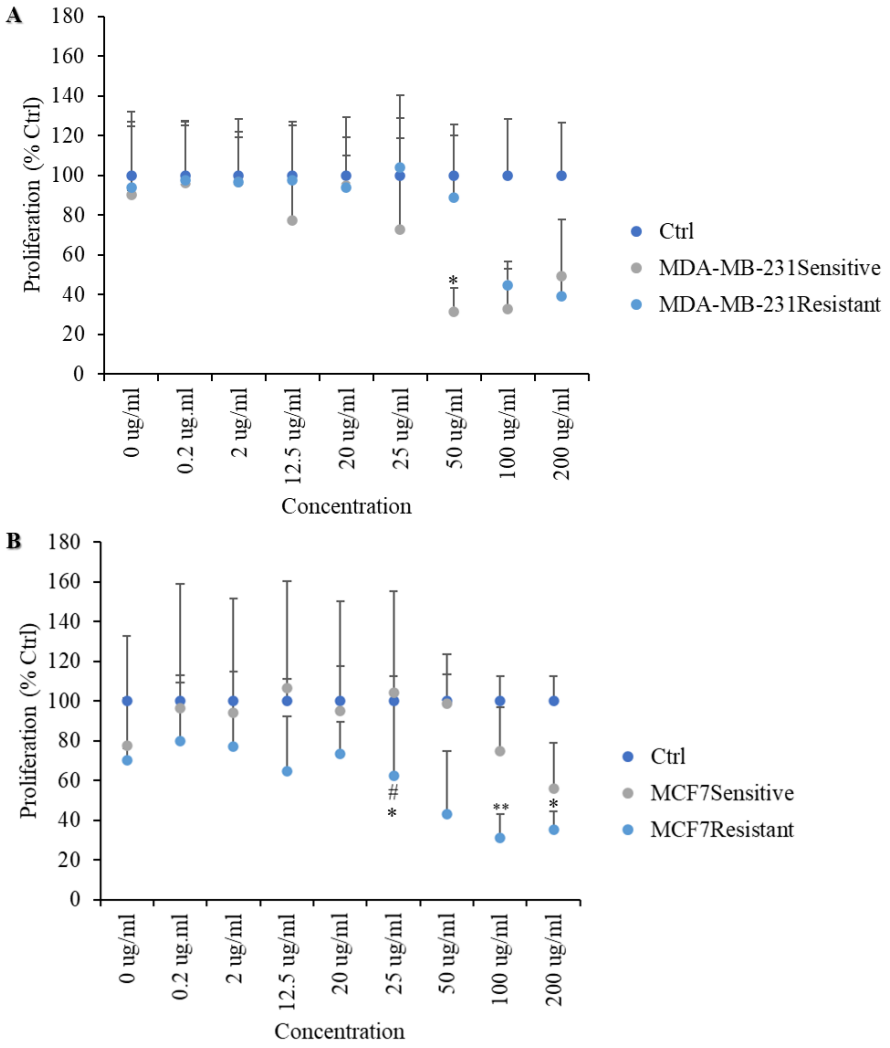
A methylene blue proliferation assay was performed with treatment of EGCG at concentrations of 12.5 ug/ml, 25 ug/mL, 50 ug/mL, 100 ug/mL, and 200 ug/mL to determine the optimal concentration of EGCG for further experimentations (Figure 4.1). The effect of EGCG on a TNBC cell line was conducted on the MDA-MB-231 cells (Figure 4.1A). The greatest decrease in proliferation (68.63 ± 12.17%, $p = .13$) with treatment of EGCG occurred at a concentration of 50 ug/mL EGCG within the MDA-MB-231$^{\text{Sensitive}}$ cells ($M = 0.20$, $SD = 0.08$)

compared to the control (M = 0.64, SD = 0.25), however at this concentration the MDA-MB-231^{Resistant} cells (M = 0.58, SD = 0.21) did not express a significant decrease in proliferation at that level (11.22 ± 31.35%, p = .98). The greatest decrease in proliferation (60.90 ± 9.35%, p = .12) with treatment of EGCG within the MDA-MB-231^{Resistant} cells (M = 0.25, SD = 0.06) occurred at a concentration of 200 ug/mL EGCG compared to the control (M = 0.59, SD = 0.22), interestingly, the MDA-MB-231^{Sensitive} cells (M = 0.29, SD = 0.17) at this concentration did not illustrate as much of a decrease in proliferation (50.60 ± 28.29%, p = .98), indicating this could be a characteristic of the resistant cells, however the decreases seen in proliferation in both MDA-MB-231^{Resistant} and MDA-MB-231^{Sensitive} cells were not of significance. At a concentration of 100 ug/mL EGCG MDA-MB-231^{Sensitive} cells (M = 0.20, SD = 0.12) showed a significant decrease in proliferation (67.24 ± 28.55%, p = .04), however there was not a significant decrease in proliferation (55.40 ± 20.21%, p = .11) in the MDA-MB-231^{Resistant} cells (M = 0.29, SD = 0.08) compared to the control (M = 0.66, SD = 0.19). Curiously, the MDA-MB-231^{Sensitive} cells (M = 0.13, SD = 0.08) experienced a slight decrease in proliferation (22.52 ± 27.25%, p = .99) compared to the control (M = 0.69, SD = 0.31) that the MDA-MB-231^{Resistant} cells (M = 0.72, SD = 0.20) did not experience (2.44 ± 19.48%, p = .83) at a concentration of 12.5 ug/mL EGCG, however this change was not significant. The MDA-MB-231^{Sensitive} cells (M = 0.49, SD = 0.31) demonstrated a non-significant decrease in proliferation (27.31 ± 29.07%, p = .83) at a concentration of 25 ug/mL EGCG compared to the control (M = 0.67, SD = 0.29), however the MDA-MB-231^{Resistant} cells (M = 0.73, SD = 0.26) demonstrated a slight insignificant increase in proliferation (3.83 ± 46.29%, p = .99) compared to the control. One-way analysis of the variance (ANOVA) with a Tukey's post-hoc test demonstrated that there was only a significant change in

proliferation at a concentration of 100 ug/mL EGCG, F (3, 8) = 5.49, $p < 0.05$, η2p = .67 (Tables 4.S1 - 6).

Figure 4.1

Paclitaxel Resistant Breast Cancer Cells Respond in a Dose-Dependent Manner to EGCG



Note. Proliferation potential following treatment with Epigallocatechin-3 Gallate (EGCG) at concentrations of 0 ug/ml, 12.5 ug/mL, 25 ug/mL, 50 ug/mL, 100 ug/mL, and 200 ug/mL were

evaluated via a Methylene Blue Proliferation Assay. (A) MDA-MB-231 cells. (B) MCF7 Cells. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between groups at each concentration were compared using a one-way ANOVA with Tukey's post hoc test. * $p < .05$ vs Ctrl, ** $p < .01$ vs Ctrl, # $p < .05$ vs Sensitive.

The breast cancer cell line, MCF7, was used to investigate the effects of EGCG in a hormone responsive breast cancer cell line (Figure 4.1B). Interesting to note, the $MCF7^{Sensitive}$ cells experienced an increase in proliferation (6.70 ± 53.37%, $p = .97$) at 12.5 ug/mL EGCG ($M = 1.30$, $SD = 0.28$) compared to the control ($M = 1.22$, $SD = 0.18$) and a smaller increase (3.97 ± 51.09%, $p = .99$) at 25 ug/ml EGCG ($M = 1.26$, $SD = 0.22$) compared to the control ($M = 1.21$, $SD = 0.21$), however neither of these increases were significant. The $MCF7^{Resistant}$ cells ($M = 0.75$, $SD = 0.62$) demonstrated a slight insignificant decrease in proliferation (35.26 ± 27.60%, $p = .66$) compared to the control at a concentration of 12.5 ug/mL EGCG and this trend continued (37.69 ± 36.66%, $p = .30$) at a concentration of 25 ug/mL EGCG ($M = 0.72$, $SD = 0.59$) compared to the control. One-way ANOVA with a Tukey's post-hoc test demonstrated a significant decrease (68.99 ± 12.03%, $p = .005$) in proliferation at an EGCG concentration of 100 ug/mL in the $MCF7^{Resistant}$ cells ($M = 0.36$, $SD = 0.26$) compared to the control ($M = 1.12$, $SD = 0.17$), $F (3, 8) = 11.44$, $p < .01$, $\eta 2p = .81$. Although there was a decrease in proliferation (25.25 ± 22.25%, $p = .33$) within the $MCF7^{Sensitive}$ cells ($M = 0.83$, $SD = 0.16$) at the same concentration, it was not significant compared to the control. $MCF7^{Resistant}$ cells ($M = 0.50$, $SD = 0.29$) demonstrated a significant decrease in proliferation (56.82 ± 31.36%, $p = .017$) at 50 ug/mL compared to the control ($M = 1.13$, $SD = 0.15$) and a significant decrease in proliferation (43.75

± 12.03%, p = .019) compared the MCF7^{Sensitive} cells (M = 1.13, SD = 0.15), however there was not a significant decrease in proliferation (1.35 ± 24.89%, p = 1.0) in the MCF7^{Sensitive} cells compared to the control, F (3, 8) = 8.00, p < 0.01, η2p = .75. The MCF7^{Resistant} cells (M = 0.39, SD = 0.26) also experienced a significant decrease in proliferation (64.79 ± 9.35%, p = .013) compared to the control (M = 1.11, SD = 0.19) at a concentration of 200 ug/mL EGCG, F (3, 8) = 8.95, p < 0.01, η2p = .77. The MCF7^{Sensitive} cells (M = 0.62, SD = 0.23) also decreased in proliferation (44.09 ± 23.12%, p > .05) at that concentration, however the decrease was not significant (Figure 4.S7 - 12).

Co-treatment of Epigallocatechin-3 Gallate and Paclitaxel Results in a Decrease in Relative Population Doubling

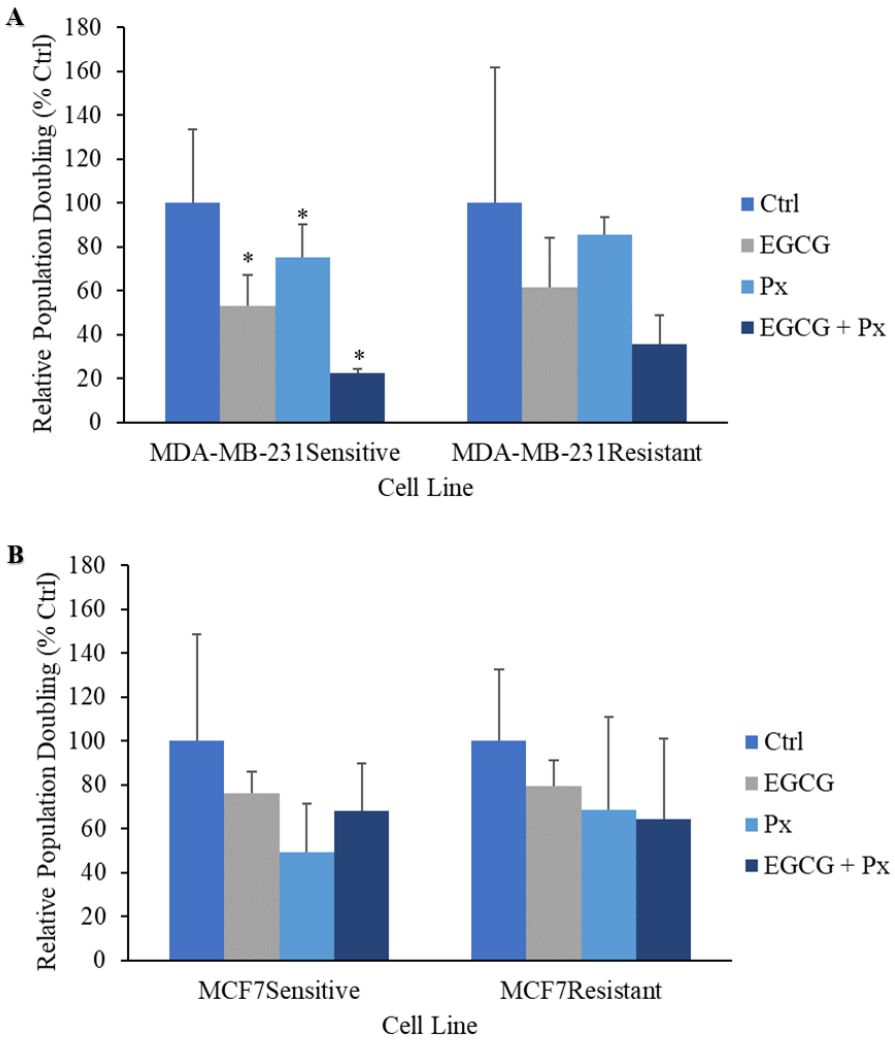
A trypan blue exclusion assay was conducted following treatment with 50 ug/mL EGCG and 10 ng/ml paclitaxel to determine the effects of EGCG and combinatorial treatment with paclitaxel on relative population doubling (Figure 4.2). When treated with EGCG the MDA-MB-231^{Sensitive} cells (M = 1.92, SD = 1.58) demonstrated a significant decrease (47.12 ± 14.17%, p = .023) in relative population doubling compared to the control (M = 5.91, SD = 1.99) and when treated with paclitaxel alone, the MDA-MB-231^{Sensitive} cells (M = 2.13, SD = 0.56) had a significant decrease in population doubling (24.61 ± 14.65%, p = .031) as well (Figure 4.2A). When treated with both EGCG and paclitaxel the MDA-MB-231^{Sensitive} cells (M = 1.32, SD = 0.11) expressed a significant decrease in relative population doubling (77.75 ± 1.84%, p = .011) compared to the control, however this decrease was not significant compared to either treatment individually even with a decrease compared to EGCG alone (30.63 ± 14.17%, p = .94) and paclitaxel alone (53.14 ± 14.65%, p = .87). A one-way ANOVA with a Tukey's post-hoc test demonstrated a significant decrease in relative population doubling between groups, F (3, 8) = 7.70, p < 0.01, η2p = .74 (Table 4.S13).

In the MDA-MB-231^{Resistant} cells there was a decrease in relative population doubling (38.59 ± 22.89%, p = .53) compared to the control (M = 4.26, SD = 3.28) when treated with EGCG (M = 1.22, SD = 0.45) (Figure 4.2A), as well as a non-significant decrease in population doubling (14.56 ± 8.15%, p = .73) with paclitaxel treatment alone (M = 2.20, SD = 0.21) compared to control. Following treatment with both EGCG and paclitaxel the MDA-MB-231^{Resistant} cells (M = 1.31, SD = 0.48) decreased in relative population (64.31 ± 13.06%, p = .54) compared to the control, EGCG alone (25.72 ± 22.89%, p = 1.0), and paclitaxel alone (49.75 ± 8.15%, p = .18). However, completion of a one-way ANOVA with a Games-Howell post-hoc test demonstrated that there was not a significant decrease in relative population doubling among treatment groups, F (3, 3.9) = 4.73, $p > 0.05$, $\eta 2p$ = .44 (Table 3.S14).

MCF7^{Sensitive} cells treated with EGCG (M = 1.24, SD = 0.16) demonstrated a decrease in relative population doubling (23.68 ± 9.63%, p = .46) compared to control (M = 2.22, SD = 1.08) (Figure 3.2B). When treated with paclitaxel alone (M = 2.28, SD = 1.01) there was also an insignificant decrease in population doubling (50.59 ± 21.87%, p = 1.0) compared to control. MCF7^{Senstive} cells treated with both EGCG and paclitaxel together (M = 1.51, SD = 0.48) expressed a decrease in relative population (32.03 ± 21.59%, p = .69) compared to both the control and EGCG treatment alone (8.35 ± 9.63%, p = .97); however, compared to paclitaxel treatment alone there was an increase in relative population doubling (18.56 ± 21.87%, p = .64), potentially suggesting EGCG protects against the effects of paclitaxel, though this change was proven insignificant, with an ANOVA demonstrating that there was not a significant decrease in relative population doubling among treatment groups, F (3, 8) = 1.31, $p > 0.05$, $\eta 2p$ = .33 (Table 4.S15).

Figure 4.2

Co-treatment of Epigallocatechin-3 Gallate and Paclitaxel Results in a Decrease in Relative Population Doubling



Note. Relative population doubling was determined using a Trypan Blue Exclusion Assay following treatment with Epigallocatechin-3 Gallate (EGCG) and paclitaxel (Px). (A) MDA-MB-231 cells. (B) MCF7 Cells. N = 3.

Data are presented as means ± standard deviation, N = 3 independent experiments. Differences between treatment groups were compared per cell line using a one-way ANOVA with Tukey's and Games-Howell post hoc test. * $p < .05$ vs Ctrl.

EGCG treatment on $MCF7^{Resistant}$ cells ($M = 1.13$, $SD = 0.17$) demonstrated a non-significant decrease in population doubling (20.58 ± 11.71%, $p = .64$) compared to the control ($M = 1.86$, $SD = 0.61$). Treatment with paclitaxel alone ($M = 1.84$, $SD = 1.13$) also demonstrated an insignificant decrease in relative population doubling (31.26± 42.26%, $p = 1.0$) compared to the control (Figure 4.2B). Co-treatment with both EGCG and paclitaxel together on the $MCF7^{Resistant}$ cells ($M = 1.20$, $SD = 0.68$) demonstrated slight insignificant decreases in relative population doublings, compared to the control (35.60 ± 36.51%, $p = .70$), EGCG treatment alone (15.02 ± 11.71%, $p = 1.0$), and paclitaxel treatment alone (4.34 ± 42.26%, $p = .71$). Despite these decreases a one-way ANOVA demonstrated that there was not a significant difference in relative population doubling among treatment groups, $F (3, 8) = 0.88$, $p > 0.05$, $\eta 2p = .248$ (Table 4.S16).

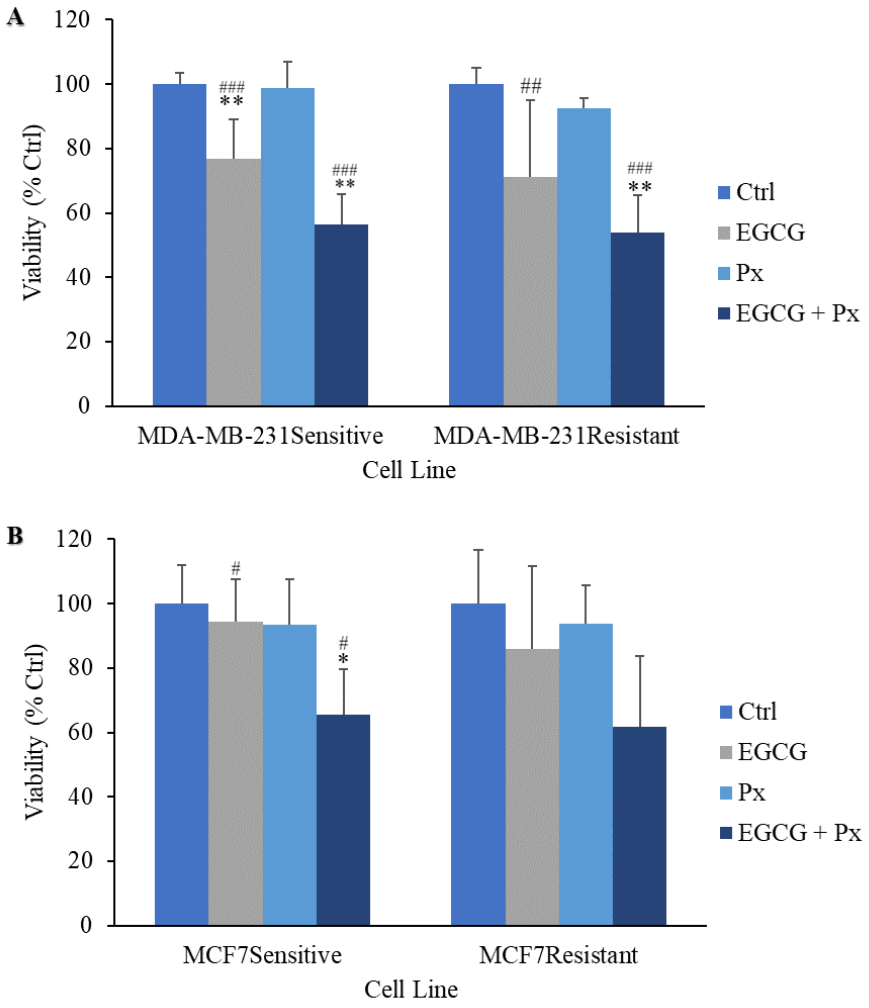
Co-treatment of Epigallocatechin-3 Gallate and Paclitaxel Results in a Decrease in Viability in Chemoresistant Breast Cancer Cells

A trypan blue exclusion assay was conducted following treatment with 50 ug/mL EGCG and 10 ng/ml paclitaxel to determine the effects of EGCG and combinatorial treatment with paclitaxel on viability (Figure 4.3). $MDA\text{-}MB\text{-}231^{Sensitive}$ cells treated with EGCG ($M = 50.03$, $SD = 7.32$) results in a significant decrease in cellular viability (23.15 ± 3.66%, $p < .001$) compared to control ($M = 93.33$, $SD = 3.41$) as well as a significant decrease (21.83 ± 8.36%, $p = .002$) compared to $MDA\text{-}MB\text{-}231^{Sensisitve}$ cells treated with paclitaxel ($M = 81.50$, $SD = 6.91$) (Figure 3.3A). Co-treatment with EGCG and paclitaxel in the $MDA\text{-}MB\text{-}231^{Sensitive}$ cells ($M = 52.50$, $SD = 8.95$) results in a significant decrease in cell viability compared to the control (43.75

± 9.59%, $p < .001$) and to those treated with paclitaxel alone (42.44 ± 9.59%, $p = .004$). Co-treatment with EGCG and paclitaxel in the MDA-MB-231^{Sensitive} cells also resulted in a non-significant decrease in viability (20.60 ± 12.16%, $p = .97$) compared to EGCG treatment alone. One-way ANOVA with Tukey's post-hoc test determined that there was a significant difference between treatment groups, $F (3, 8) = 28.61$, $p < 0.001$, $\eta 2p = .915$ (Table 4.S17).

Figure 4.3

Co-treatment of Epigallocatechin-3 Gallate and Paclitaxel Results in a Decrease in Viability in Chemoresistant Breast Cancer Cells



Note. Viability was determined using a Trypan Blue Exclusion Assay following treatment with Epigallocatechin-3 Gallate (EGCG) and treatment with paclitaxel (Px). (A) MDA-MB-231 cells. (B) MCF7 Cells. N = 3.

Data are presented as means ± standard deviation, n = 3 independent experiments. Differences between groups were compared using a one-way ANOVA with Tukey's post hoc test. * $p < .05$ ** $p < .01$ vs. Px, ## $p < .01$ vs Ctrl, ### $p < .001$ vs Ctrl.

Treatment with EGCG in the MDA-MB-231^{Resistant} cells (M = 58.11, SD = 10.77) significantly reduced cellular viability (28.87 ± 5.00%, p = .004) compared to the control (M = 93.53, SD = 4.67), whereas treatment with paclitaxel (M = 76.58, SD = 6.11) non-significantly decreased viability (7.61 ± 3.15%, p = .15) compared to the control (Figure 4.3A). MDA-MB-231^{Resistant} cells treated with both EGCG and paclitaxel (M = 50.53, SD = 10.86) demonstrated a significant decrease in cellular viability (45.98 ± 11.61%, $p < .001$) compared to the control and a significant decrease (28.39 ± 3.15%, p = .024) when compared to paclitaxel treatment alone, potentially suggesting EGCG could enhance the effects of paclitaxel on resistant TNBC cells. A decrease was also observed in cellular viability (17.11 ± 23.76%, p = .71) of the co-treatment cells compared to EGCG treated alone, however, this was not a significant decrease. Completion of a one-way ANOVA with a Tukey's post-hoc test demonstrated that there was significant change among treatment groups within the MDA-MB-231^{Resistant} cells, $F(3, 8) = 15.24$, $p < 0.001$, $\eta 2p = .851$ (Table 4.S18).

MCF7^{Sensitive} cells treated with EGCG (M = 58.67, SD = 8.03) demonstrated a significant decrease in cellular viability (5.50 ± 12.94%, $p < .05$) compared to the control (M = 88.42, SD = 10.61), while treatment with paclitaxel (M = 87.75, SD = 13.00) decreased viability (6.4 ± 13.87%, p = 1.0) a non-significant amount (Figure 3.3B). Treatment with both EGCG and paclitaxel in the MCF7^{Sensitive} cells (M = 57.92, SD = 12.41) demonstrated a significant decrease in viability (28.10 ± 14.04%, p = .046) compared to treatment with paclitaxel alone and a

significant decrease (34.50 ± 12.00%, p = .041) compared to the control. However, there was not a significant decrease in viability (28.99 ± 12.94%, p = 1.0) in the co-treatment $MCF7^{Sensitive}$ cells when compared to EGCG treatment alone. One-way ANOVA with a Tukey's post-hoc test demonstrated that there was a significant difference among treatment groups, F (3, 8) = 7.10, p < 0.05, η2p = .727 (Table 4.S19).

Treatment with EGCG within the $MCF7^{Resistant}$ cells (M = 58.75, SD = 17.54) demonstrated an insignificant decrease in cellular viability (13.92 ± 16.70%, p = .16) when compared to the control (M = 89.50, SD = 14.94). Treatment with paclitaxel (M = 76.00, SD = 9.5) also demonstrated a slightly insignificant decrease in viability (6.08 ± 11.74%, p = .73) compared to the control (Figure 4.3B). Co-treatment with EGCG and paclitaxel in the $MCF7^{Reistant}$ cells (M = 55.33, SD = 19.50) demonstrated a decrease in cellular viability compared to the control (38.17 ± 11.74%, p = .11), EGCG treatment alone (24.26 ± 21.79%, p = .99), and paclitaxel treatment alone (32.10 ± 11.74%, p = .43). However, completion of a one-way ANOVA with a Tukey's post-hoc test demonstrated that there was no significant difference between treatment groups, F (3, 8) = 3.027, p > 0.05, η2p = .532 (Table 4.S20).

Discussion

Green tea represents a promising avenue of investigation as it remains a dietary staple for many individuals as the second most consumed beverage (Yuan et al., 2011). To investigate the potential benefits of this beverage as an combinatorial to paclitaxel, this chapter emphasized Epigallocatechin-3-gallate (EGCG), a major catechin located in the tea (Khan et al., 2006). Green tea and specifically EGCG in the tea, is among the best studied for its potential health benefits, such as those related to cancer (Khan et al., 2006; Mukhtar & Ahmad, 2000). EGCG has been studied for its potential role in cancer prevention and cancer therapy, including

reducing cancer therapy side effects and sensitization to radio- and chemotherapy (Lecumberri et al., 2013; Suganuma et al., 2011). With work in our laboratory focusing on acquired, long-term chemoresistance, EGCG is an attractive target for a potential combinatorial to paclitaxel in both a triple-negative breast cancer (TNBC) and a hormone responsive breast cancer cell line, MDA-MB-231 and MCF7, respectively, and its potential to enhance the effects of paclitaxel in resistant populations.

In this chapter, I demonstrated that both cell lines respond in a dose-dependent manner to treatment with EGCG. There was a significant decrease in proliferation at a concentration of 50 ug/mL of EGCG within the MDA-MB-231^{Sensitive} cells, however, there was not a significant decrease in proliferation within the MDA-MB-231^{Resistant} cells. The results of the MDA-MB-231 cells are similar to that found by Katiyar et al. in 2005 with MDA-MB-468 cells however, these results are contradictory to data found by Richter et al. in 2018 that MDA-MB-231 cells do not experience a difference in proliferation at concentrations of 10ug/mL to 180ug/mL (Roy et al., 2005; Schröder et al., 2019) (Table 4.2). This could be due to the minute differences between the MDA-MB-231 cells and the MDA-MB-468 cells, as the MDA-MB-468 are a basal cell line whereas the MDA-MB-231 are a claudin-low cell line.

Table 4.2

Dose-Response to EGCG Treatment in Comparison to Literature

Cell Line	Dose-Response with EGCG	Support	Opposition
MDA-MB-231^{Sensitive}	Significant Decrease	(Roy et al., 2005)	(Schröder et al., 2019)
MDA-MB-231^{Resistant}	Decrease		
MCF7^{Sensitive}	Decrease		(Schröder et al., 2019)
MCF7^{Resistant}	Significant Decrease	(Schröder et al., 2019)	

Note. Table summarizing dose-response results in relation to current known literature.

Interestingly, the hormone responsive cells, MCF7, experience the opposite phenomenon that was demonstrated in the MDA-MB-231 cell line. The MCF7^{Resistant} cells experience a significant decrease in proliferation at a concentration of 50, 100, and 200 ug/mL EGCG, that the MCF7^{Sensitive} cells did not experience, thus suggesting a potential for combinatorial therapeutic with EGCG and paclitaxel (Table 4.2). Richter *et al.* found a significant decrease in proliferation in the MCF7 cells at concentrations of 90 - 180 ug/mL, however, my results for the MCF7^{Sensitive} cells did not demonstrate the same significant decrease (Schröder et al., 2019). Interestingly, the MCF7^{Resistant} cells did express a significant decrease in proliferation, as supported by previous findings (Schröder et al., 2019). EGCG has also been demonstrated to decrease proliferation in numerous cancers including bladder, liver, and nasopharyngeal (Fang et al., 2015; Luo et al., 2017; Sun et al., 2019; Sur & Panda, 2017; Xu et al., 2019). With a significant difference in proliferation at 50ug/mL in both cell lines this concentration was used to investigate the cells response to EGCG in both terms of cell growth and viability.

EGCG has been found to decrease cell growth in several cancers including bladder, prostate, epidermoid carcinoma, inflammatory breast, and in the breast cancer cell lines MDA-

MB-231 and MCF7 (Ahmad et al., 2000; Luo et al., 2017; Mineva et al., 2013; Paschka et al., 1998; Tyagi et al., 2015; Xu et al., 2019). In support of this the MDA-MB-231^{Sensitive} cells demonstrated a significant decrease in relative population growth with treatment of EGCG, however the MCF7 cells did not show this same significant decrease nor did either of the resistant cell lines (Table 4.3). This would suggest that EGCG would not be successful as a combinatorial with paclitaxel in the resistant cells to manage cell growth.

Although cells grow in number, it does not necessarily mean that they are healthy cells. Interestingly, conflicting reports on the effect of EGCG on viability on breast cancer have been published. Richter et al., Katiyar et al., and Lisanti et el., demonstrated that there was a significant decrease in viability in MDA-MB-231 and MCF7 cells, however, Chatterjee *et al.*, did not see the same effects on MCF7 cells (Bonuccelli et al., 2018; Roy et al., 2005; Schröder et al., 2019; Sen et al., 2009). I found that my data supports a decrease in viability upon treatment with EGCG as the MDA-MB-231 cells had a significant decrease in cell viability with treatment of EGCG in both the MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} (Table 4.4). There was also a significant difference with treatment of EGCG within the MCF7^{Sensitive} cells, unfortunately this significance was not expressed in the MCF7^{Resistant} cells. Also, there was a significant decrease in viability in both the MDA-MB-231^{Sensitive} and MDA-MB-231^{Resistant} cells when treated with a combination of EGCG and paclitaxel which supports Madhan *et al*. whom also found that treatment with both results in a decrease in viability (Ramadass et al., 2015) This data would suggest EGCG could be a combinatorial to paclitaxel treatment in terms of cell viability.

Table 4.3

Cell Growth with EGCG Treatment in Comparison to Literature

Cell Line	Cell Growth with EGCG	Support	Opposition
MDA-MB-231^{Sensitive}	Significant Decrease	(Tyagi et al., 2015)	
MDA-MB-231^{Resistant}	Decrease		
MCF7^{Sensitive}	Decrease		(Tyagi et al., 2015)
MCF7^{Resistant}	Decrease		

Note. Table summarizing cell growth results in relation to current known literature.

The data presented in this chapter could potentially support the use of EGCG as a combinatorial to paclitaxel treatment within paclitaxel resistant TNBC, however further study within this unique population would have to confirm, as EGCG and paclitaxel did not result in a significant decrease in growth but did result in a significant decrease in viability. It would be interesting to explore the effects of EGCG in combination with paclitaxel in inducing apoptosis as EGCG has been found to increase apoptosis in multiple cancers including, breast, bladder, lung, and prostate (Gupta et al., 2003; Luo et al., 2017; Meeran et al., 2011; Oya et al., 2017; Paschka et al., 1998; Ramadass et al., 2015; Roy et al., 2005). Taken together these findings could potentially support a role for EGCG as an combinatorial with paclitaxel within chemoresistance.

Table 4.4

Viability with EGCG Treatment in Comparison to Literature

Cell Line	Cell Growth with EGCG	Support	Opposition
MDA-MB-231^{Sensitive}	Significant Decrease	(Bonuccelli et al., 2018; Roy et al., 2005; Schröder et al., 2019)	
MDA-MB-231^{Resistant}	Decrease	(Bonuccelli et al., 2018)	
MCF7^{Sensitive}	Decrease	(Bonuccelli et al., 2018; Roy et al., 2005; Schröder et al., 2019)	(Sen et al., 2009)
MCF7^{Resistant}	Decrease		

Note. Table summarizing cell viability results in relation to current known literature

CHAPTER 5

CONCLUDING REMARKS

The heterogenous nature of breast cancer makes it one of the most essential cancers to study as it is the second leading cancer death among women (Siegel et al., 2021). Even with continuous improvements in early detection and therapies, the relative number of individuals expected to succumb to advanced, metastatic disease has remained relatively constant (Siegel et al., 2021; Siegel et al., 2015, 2017). A major reason for the lack of significant improvement in outcomes for breast cancer patients is the presence or development of resistance to therapeutic interventions (Nedeljković & Damjanović, 2019). Resistance can be present from the beginning (innate) or develop as a result of treatment (acquired) (Catherine E Steding, 2016). Acquired chemoresistance occurs when there is an accumulation of changes within the breast cancer that enables it to be resistant to chemotherapy (Luqmani, 2005). Although an understanding of innate resistance is valuable, it does not address the long-term consequences of therapeutic intervention. As such, work in our laboratory has emphasized evaluating long-term treatments that better recapitulate acquired chemoresistance against the microtubule-stabilizing drug, paclitaxel.

Paclitaxel, a common chemotherapeutic agent utilized in breast cancer therapy, functions by binding to the β-tubulin of microtubules thus resulting in stabilization of the breast cancer cells. With microtubules needing a constant state of instability to function appropriately the stabilization effect of paclitaxel is devastating to the cancer cells and results in their termination. Treatment with paclitaxel has demonstrated the ability to increase survival rates in patients,

though not necessarily cause complete tumor regression, thus creating a perfect environment for the development of resistance (Alves et al., 2018; Goldblatt et al., 2009; Gradishar et al., 2009; Stage et al., 2018; Tabuchi et al., 2009).

To adequately investigate acquired resistance to paclitaxel it is necessary to generate a breast cancer cell line resistant to paclitaxel. Although chemoresistance to paclitaxel has been previously evaluated, most model systems utilize cells that have been maintained in paclitaxel for only brief periods of time, however the short-term treatments of these cells could be more indicative of innate resistance (Dumontet et al., 1996; Guo et al., 2004; Han et al., 2018; Jeong et al., 2016; Lian et al., 2019; Liu et al., 2017; Sha et al., 2016; Sprouse & Herbert, 2014). It is estimated that creating an acquired chemoresistant cell line could take anywhere from three to eighteen months (McDermott et al., 2014). The extended treatments utilized in our laboratory are unique as they have been maintained in paclitaxel for years. Here, I generated a triple-negative breast cancer (TNBC) cell line, MDA-MB-231, resistant to paclitaxel to adequately investigate the mechanisms of acquired paclitaxel resistance.

With the generation of MDA-MB-231^{Resistant} cells, I evaluated them for potential phenotypic alterations including changes in cell morphology, metastatic ability, cellular death, cell growth, and viability. I demonstrated that with the addition of the resistant phenotype these cells express an increase in cellular morphology and a minute increase in growth and viability, however they are not different from their sensitive counterparts in metastatic ability and cellular death; therefore, suggesting that besides being resistant to paclitaxel the resistant cells did not gain any additional abilities (Table 5.1). To further investigate potential variations within the resistant cells genotypic changes were evaluated with the use of next-generation sequencing technology identifying several genes significantly altered with the addition of chemoresistance.

The identification of these genes and their functions within chemoresistance is the beginning of understanding the mechanism of acquired chemoresistance.

To begin delving into the mechanism of acquired paclitaxel resistance it is imperative to start with the known mechanism of action of paclitaxel, the microtubules. Microtubules function in a variety of tasks with the aid of microtubule-associated proteins (MAPs). MAPs, like MAP2 and MCAK, have previously been indicative of chemoresistance (Bauer et al., 2010; Xie et al., 2016), however, I identified a MAP previously not associated with acquired resistance to paclitaxel, KIF14. KIF14 is a motor protein that functions in the destabilization of microtubules, thus could be a candidate for the mechanism of acquired chemoresistance. In this proposed mechanism of chemoresistance paclitaxel is acting as a stabilizer to the microtubules consequently preventing them from destabilization. To overcome the stabilization occurring with paclitaxel the cells upregulate KIF14 to destabilize the microtubules, therefore overcoming the stabilization that paclitaxel is providing. Based on the preliminary sequencing data presented in Chapter 2 “Characterization of an Acquired Chemoresistant Breast Cancer Cell Line” I hypothesized that the upregulation of KIF14 contributes paclitaxel resistance the cells have acquired.

To investigate the potential for KIF14 as a mechanism of chemoresistance I knocked-down expression of KIF14 ($KIF14^{KD}$) in both MDA-MB-$231^{Sensitive}$ and MDA-MB-$231^{Resistant}$ cells. I identified that with the minute $KIF14^{KD}$ of only 14% that the cells still retained normal cellular phenotypes as measured by metastatic ability, cellular death, cell growth, and viability (Table 5.1). However, even at this minute decrease in KIF14 expression, treatment with paclitaxel in the resistant cells still demonstrated a potential for re-sensitization for chemotherapy with a decrease in cell growth and viability. Further investigation into the expression of KIF14

could identify it as a mechanism of chemoresistance within TNBC and is a potential avenue for future research. With such a minute reduction in KIF14 expression I did not see significantly altered phenotypes of the cells in the presence of paclitaxel for metastatic ability and cellular death, however there was significant decreases in cell growth and viability, thus my hypothesis cannot be rejected, yet further work needs to be completed to support it.

Not only is identification of markers in chemoresistance necessary, but potential alternative therapeutic options are also a necessity as well. Green tea is one of the most widely consumed beverages worldwide and could serve as a possible enhancement to current therapies like paclitaxel (Baliga et al., 2005; Gan et al., 2018). The active ingredient in green tea is a polyphenol known as (-)-epigallocatechin-3-gallate (EGCG) that has demonstrated chemo-preventive effects like inhibiting cancer growth (Cabrera et al., 2006; Tyagi et al., 2015). I hypothesize that EGCG treatment in conjunction with paclitaxel treatment could result in a decrease in growth potential of the breast cancer cells.

I identified that a hormone sensitive paclitaxel resistant breast cancer cell line, $MCF7^{Resistant}$, experience dose-dependent changes in growth with treatment of EGCG. Potentially suggesting EGCG as an adjuvant therapy to paclitaxel. Although further investigations did not demonstrate a significant decrease in growth within the resistant cell populations when treated with EGCG or a combination of EGCG and paclitaxel, there was a decrease in the cell's viability when treated with a combination of EGCG and paclitaxel (Table 5.1). Although this does not support my hypothesis, it does not reject it either. With a significant decrease in viability with co-treatment there could be a possibility for future investigations into the combinatorial treatment of EGCG and paclitaxel in patients with resistance to paclitaxel.

Overall, my investigations into the mechanism of chemoresistance with the use of a long-term acquired paclitaxel cell line deepens our understanding of acquired chemoresistance. I identified a potential candidate gene as a mechanism for chemoresistance and further investigation into the function of this gene could broaden our understanding of acquired chemoresistance. I also demonstrated the potential use for combinatorial therapeutics with EGCG.

Future Directions

The generation of an acquired chemoresistant TNBC cell line opens the possibility into a wide range of further investigation into the mechanisms of acquired chemoresistance. With the identification of over 2,000 genes that are significantly altered within the MDA-MB-231^{Resistant} cells it is possible to begin identifying novel mechanisms of chemoresistance. A first step in this process would be investigating the gene ontology for these changes. From there we can identify which genes are known to participate in mechanisms of chemoresistance like those in the signaling pathways of PI3k/AKT/mTOR or RAS/MAPK/ERK. Those that do not fit into known mechanisms can then be the first investigations into novel mechanisms of chemoresistance.

This study barely scratched the surface of KIF14's function in the mechanism of acquired chemoresistance. With a minute reduction in expression of KIF14 within the MDA-MB-231^{Resistant} cells I was able to demonstrate a significant decrease in cellular growth in the presence of paclitaxel. This data could suggest a possible role for KIF14 in the mechanism of chemoresistance. To further investigate this potential achieving a greater increase in KIF14^{KD} is necessary, however it is not the only path. To continue investigating KIF14's role it would be necessary to overexpress KIF14 within a sensitive cell line to examine if resistance to paclitaxel can be induced.

Although much is known about KIF14, its explicit role in microtubule dynamics and chemoresistance has yet to be elucidated. Previous studies have identified KIF14 in chemoresistance however, they have not fully described KIF14's function in chemoresistance in relation to microtubule dynamics (Singel et al., 2014; Wang et al., 2018). To investigate KIF14's role in microtubule dynamics and chemoresistance it would be necessary to explore KIF14's interaction with microtubules in the presence of paclitaxel. KIF14 is a known destabilizing protein and if its upregulation within the MDA-MB-231^{Resistant} cells is functioning in the destabilization of the protein, then if KIF14's expression is reduced, there would be an increase in stabilization in the presence of paclitaxel. With continuous investigation into novel gene changes in an acquired chemoresistant cell line there is the potential to open new areas of translational research and future clinical investigations.

Table 5.1

Summary of Results

Experimental Condition	Changed Phenotypes	Constant Phenotypes
Paclitaxel Resistance	• Enlarged morphology • Increase in Long-term Growth and Viability	• Metastatic Ability • Cellular Death
$KIF14^{KD}$ Sensitive Cells		• Metastatic Ability • Cellular Death • Growth and Viability
$KIF14^{KD}$ Resistant Cells	• Long-Term Cell Growth	• Metastatic Ability • Cellular Death • Growth and Viability
$KIF14^{KD}$ Resistant Cells Response to Paclitaxel	• Cell Growth and Viability	• Metastatic Ability • Cellular Death
TNBC Cells Treated with EGCG	• Cell Growth and Viability	
Hormone Responsive Cells Treated with EGCG	• Cell Growth and Viability	
Resistant TNBC cells Treated with EGCG	• Cell Viability	• Cell Growth
Resistant Hormone Responsive Cells Treated with EGCG		• Cell Growth and Viability

REFERENCES

Abdi, H., & Williams, L. J. (2010). Newman-Keuls test and Tukey test. *Encyclopedia of research design*, 1-11.

Adams, S., Diamond, J. R., Hamilton, E., Pohlmann, P. R., Tolaney, S. M., Chang, C.-W., . . . Molinero, L. (2019). Atezolizumab plus nab-paclitaxel in the treatment of metastatic triple-negative breast cancer with 2-year survival follow-up: a phase 1b clinical trial. *JAMA oncology*, *5*(3), 334-342.

Ahmad, N., Cheng, P., & Mukhtar, H. (2000). Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate. *Biochemical and biophysical research communications*, *275*(2), 328-334.

Ahmed, A. A., Lu, Z., Jennings, N. B., Etemadmoghadam, D., Capalbo, L., Jacamo, R. O., . . . Lopez-Berestein, G. (2010). SIK2 is a centrosome kinase required for bipolar mitotic spindle formation that provides a potential target for therapy in ovarian cancer. *Cancer cell*, *18*(2), 109-121.

Ahmed, S. M., Thériault, B. L., Uppalapati, M., Chiu, C. W., Gallie, B. L., Sidhu, S. S., & Angers, S. (2012). KIF14 negatively regulates Rap1a–Radil signaling during breast cancer progression. *Journal of Cell Biology*, *199*(6), 951-967.

Alves, R. C., Fernandes, R. P., Eloy, J. O., Salgado, H. R. N., & Chorilli, M. (2018). Characteristics, properties and analytical methods of paclitaxel: a review. *Critical reviews in analytical chemistry*, *48*(2), 110-118.

Anderson, W. F., Chatterjee, N., Ershler, W. B., & Brawley, O. W. (2002). Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. *Breast cancer research and treatment*, *76*(1), 27-36.

Arora, K., Talje, L., Asenjo, A. B., Andersen, P., Atchia, K., Joshi, M., . . . Kwok, B. H. (2014). KIF14 binds tightly to microtubules and adopts a rigor-like conformation. *Journal of Molecular Biology*, *426*(17), 2997-3015.

Attia, Y. M., El-Kersh, D. M., Ammar, R. A., Adel, A., Khalil, A., Walid, H., . . . Elmazar, M. M. (2020). Inhibition of aldehyde dehydrogenase-1 and p-glycoprotein-mediated multidrug resistance by curcumin and vitamin D3 increases sensitivity to paclitaxel in breast cancer. *Chem Biol Interact*, *315*, 108865. https://doi.org/10.1016/j.cbi.2019.108865

Ayers, M., Symmans, W., Stec, J., Damokosh, A., Clark, E., Hess, K., . . . Ibrahim, N. (2004). Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *Journal of clinical oncology*, *22*(12), 2284-2293.

Baliga, M. S., Meleth, S., & Katiyar, S. K. (2005). Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4T1 cells in vitro and in vivo systems. *Clinical Cancer Research*, *11*(5), 1918-1927.

Basavarajappa, H. D., & Corson, T. W. (2012). KIF14 as an oncogene in retinoblastoma: a target for novel therapeutics? *Future medicinal chemistry*, *4*(17), 2149-2152.

Bauer, J. A., Chakravarthy, A. B., Rosenbluth, J. M., Mi, D., Seeley, E. H., Granja-Ingram, N. D. M., . . . Meszoely, I. M. (2010). Identification of markers of taxane sensitivity using proteomic and genomic analyses of breast tumors from patients receiving neoadjuvant paclitaxel and radiation. *Clinical Cancer Research*, *16*(2), 681-690.

Benbow, S. J., Cook, B. M., Reifert, J., Wozniak, K. M., Slusher, B. S., Littlefield, B. A., . . . Feinstein, S. C. (2016). Effects of paclitaxel and eribulin in mouse sciatic nerve: a microtubule-based rationale for the differential induction of chemotherapy-induced peripheral neuropathy. *Neurotoxicity research*, *29*(2), 299-313.

Benbow, S. J., Wozniak, K. M., Kulesh, B., Savage, A., Slusher, B. S., Littlefield, B. A., . . . Feinstein, S. C. (2017). Microtubule-targeting agents eribulin and paclitaxel differentially affect neuronal cell bodies in chemotherapy-induced peripheral neuropathy. *Neurotoxicity research*, *32*(1), 151-162.

Bonuccelli, G., Sotgia, F., & Lisanti, M. P. (2018). Matcha green tea (MGT) inhibits the propagation of cancer stem cells (CSCs), by targeting mitochondrial metabolism, glycolysis and multiple cell signalling pathways. *Aging (Albany NY)*, *10*(8), 1867.

Borys, F., Joachimiak, E., Krawczyk, H., & Fabczak, H. (2020). Intrinsic and extrinsic factors affecting Microtubule dynamics in normal and cancer cells. *Molecules*, *25*(16), 3705.

Breese, M. R., & Liu, Y. (2013). NGSUtils: a software suite for analyzing and manipulating next-generation sequencing datasets. *Bioinformatics*, *29*(4), 494-496.

Brown, A. M. (2005). A new software for carrying out one-way ANOVA post hoc tests. *Computer methods and programs in biomedicine*, *79*(1), 89-95.

Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea—a review. *Journal of the American College of Nutrition*, *25*(2), 79-99.

Cailleau, R., Olive, M., & Cruciger, Q. V. (1978). Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In vitro*, *14*(11), 911-915.

Carey, L. A., Dees, E. C., Sawyer, L., Gatti, L., Moore, D. T., Collichio, F., . . . Perou, C. M. (2007). The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clinical Cancer Research*, *13*(8), 2329-2334.

Chen, Z. P., Schell, J. B., Ho, C.-T., & Chen, K. Y. (1998). Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. *Cancer letters*, *129*(2), 173-179.

Cleator, S., Heller, W., & Coombes, R. C. (2007). Triple-negative breast cancer: therapeutic options. *The lancet oncology*, *8*(3), 235-244.

Cohen, M. H., Gootenberg, J., Keegan, P., & Pazdur, R. (2007). FDA Drug Approval Summary: Bevacizumab (Avastin®) Plus Carboplatin and Paclitaxel as First-Line Treatment of Advanced/Metastatic Recurrent Nonsquamous Non-Small Cell Lung Cancer. *The oncologist*, *12*(6), 713-718.

Corson, T. W., & Gallie, B. L. (2006). KIF14 mRNA expression is a predictor of grade and outcome in breast cancer. *International journal of cancer*, *119*(5), 1088-1094.

Corson, T. W., Zhu, C. Q., Lau, S. K., Shepherd, F. A., Tsao, M.-S., & Gallie, B. L. (2007). KIF14 messenger RNA expression is independently prognostic for outcome in lung cancer. *Clinical Cancer Research*, *13*(11), 3229-3234.

Crown, J., O'Leary, M., & Ooi, W. S. (2004). Docetaxel and paclitaxel in the treatment of breast cancer: a review of clinical experience. *The oncologist*, *9*, 24-32.

DePolo, J. (2021, August 18 2021). *Radiation Therapy*. BreastCancer.org. Retrieved December 22 from https://www.breastcancer.org/treatment/radiation

Desai, A., & Mitchison, T. J. (1997). Microtubule polymerization dynamics. *Annual review of cell and developmental biology*, *13*(1), 83-117.

DeSantis, C., Ma, J., Bryan, L., & Jemal, A. (2014). Breast cancer statistics, 2013. *CA: A Cancer Journal for Clinicians*, *64*(1), 52-62.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., . . . Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15-21.

Dufresne, C. J., & Farnworth, E. R. (2001). A review of latest research findings on the health promotion properties of tea. *The Journal of nutritional biochemistry*, *12*(7), 404-421.

Dumontet, C., Duran, G. E., Steger, K. A., Beketic-Oreskovic, L., & Sikic, B. I. (1996). Resistance mechanisms in human sarcoma mutants derived by single-step exposure to paclitaxel (Taxol). *Cancer Research*, *56*(5), 1091-1097.

Ehrlichova, M., Mohelnikova-Duchonova, B., Hrdy, J., Brynychova, V., Mrhalova, M., Kodet, R., . . . Soucek, P. (2013). The association of taxane resistance genes with the clinical course of ovarian carcinoma. *Genomics*, *102*(2), 96-101.

Ehrlichova, M., Vaclavikova, R., Ojima, I., Pepe, A., Kuznetsova, L. V., Chen, J., . . . Gut, I. (2005). Transport and cytotoxicity of paclitaxel, docetaxel, and novel taxanes in human breast cancer cells. *Naunyn-Schmiedeberg's archives of pharmacology*, *372*(1), 95-105.

Emens, L. (2021). *Immunotherapy*. https://www.breastcancer.org/treatment/immunotherapy

Estévez, L. G., & Gradishar, W. J. (2004). Evidence-based use of neoadjuvant taxane in operable and inoperable breast cancer. *Clinical Cancer Research*, *10*(10), 3249-3261.

Etienne-Manneville, S. (2013). Microtubules in cell migration. *Annual review of cell and developmental biology*, *29*, 471-499.

Falchook, G., Coleman, R. L., Roszak, A., Behbakht, K., Matulonis, U., Ray-Coquard, I., . . . Ghamande, S. (2019). Alisertib in combination with weekly paclitaxel in patients with advanced breast cancer or recurrent ovarian cancer: a randomized clinical trial. *JAMA oncology*, *5*(1), e183773-e183773.

Fang, C.-Y., Wu, C.-C., Hsu, H.-Y., Chuang, H.-Y., Huang, S.-Y., Tsai, C.-H., . . . Chen, J.-Y. (2015). EGCG inhibits proliferation, invasiveness and tumor growth by up-regulation of adhesion molecules, suppression of gelatinases activity, and induction of apoptosis in nasopharyngeal carcinoma cells. *International journal of molecular sciences*, *16*(2), 2530-2558.

Formenti, S., Symmans, W., Volm, M., Skinner, K., Cohen, D., Spicer, D., & Danenberg, P. (1999). Concurrent paclitaxel and radiation therapy for breast cancer. Seminars in radiation oncology,

Formenti, S. C., Volm, M., Skinner, K. A., Spicer, D., Cohen, D., Perez, E., . . . Florentine, B. (2003). Preoperative twice-weekly paclitaxel with concurrent radiation therapy followed by surgery and postoperative doxorubicin-based chemotherapy in locally advanced breast cancer: a phase I/II trial. *Journal of clinical oncology*, *21*(5), 864-870.

Fruci, D., CS Cho, W., Romania, P., Nobili, V., Locatelli, F., & Alisi, A. (2016). Drug transporters and multiple drug resistance in the most common pediatric solid tumors. *Current drug metabolism*, *17*(4), 308-316.

Fujiki, H., Watanabe, T., Sueoka, E., Rawangkan, A., & Suganuma, M. (2018). Cancer prevention with green tea and its principal constituent, EGCG: From early investigations to current focus on human cancer stem cells. *Molecules and cells*, *41*(2), 73.

Gan, R.-Y., Li, H.-B., Sui, Z.-Q., & Corke, H. (2018). Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): An updated review. *Critical reviews in food science and nutrition*, *58*(6), 924-941.

Ganguly, A., Yang, H., Pedroza, M., Bhattacharya, R., & Cabral, F. (2011). Mitotic centromere-associated kinesin (MCAK) mediates paclitaxel resistance. *Journal of Biological Chemistry*, *286*(42), 36378-36384.

Gest, C., Joimel, U., Huang, L., Pritchard, L.-L., Petit, A., Dulong, C., . . . Laurent, M. (2013). Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: differences in MDA-MB-231 and MCF-7 breast cancer cell lines. *BMC cancer*, *13*(1), 1-14.

Gianfredi, V., Nucci, D., Abalsamo, A., Acito, M., Villarini, M., Moretti, M., & Realdon, S. (2018). Green tea consumption and risk of breast cancer and recurrence—A systematic review and meta-analysis of observational studies. *Nutrients*, *10*(12), 1886.

Giltnane, J. M., & Balko, J. M. (2014). Rationale for targeting the Ras/MAPK pathway in triple-negative breast cancer. *Discovery medicine*, *17*(95), 275-283.

Glass, G. V. (1966). Testing homogeneity of variances. *American Educational Research Journal*, *3*(3), 187-190.

Goldblatt, E. M., Gentry, E. R., Fox, M. J., Gryaznov, S. M., Shen, C., & Herbert, B.-S. (2009). The telomerase template antagonist GRN163L alters MDA-MB-231 breast cancer cell morphology, inhibits growth, and augments the effects of paclitaxel. *Molecular cancer therapeutics*, *8*(7), 2027-2035.

Goodson, H. V., & Jonasson, E. M. (2018). Microtubules and microtubule-associated proteins. *Cold Spring Harbor perspectives in biology*, *10*(6), a022608.

Gouveia, S. M., Leslie, K., Kapitein, L. C., Buey, R. M., Grigoriev, I., Wagenbach, M., . . . Wordeman, L. (2010). In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. *Current Biology*, *20*(19), 1717-1722.

Gradishar, W. J., Krasnojon, D., Cheporov, S., Makhson, A. N., Manikhas, G. M., Clawson, A., & Bhar, P. (2009). Significantly longer progression-free survival with nab-paclitaxel compared with docetaxel as first-line therapy for metastatic breast cancer. *J Clin Oncol*, *27*(22), 3611-3619.

Groenendijk, F. H., & Bernards, R. (2014). Drug resistance to targeted therapies: deja vu all over again. *Molecular oncology*, *8*(6), 1067-1083.

Gruneberg, U., Neef, R., Li, X., Chan, E. H., Chalamalasetty, R. B., Nigg, E. A., & Barr, F. A. (2006). KIF14 and citron kinase act together to promote efficient cytokinesis. *The Journal of cell biology*, *172*(3), 363-372.

Guan, H., & Yang, K. (2008). RNA isolation and real-time quantitative RT-PCR. In *Adipose Tissue Protocols* (pp. 259-270). Springer.

Gueritte, F. (2001). General and recent aspects of the chemistry and structure activity relationships of taxoids. *Current pharmaceutical design*, *7*(13), 1229-1249.

Guo, B., Villeneuve, D. J., Hembruff, S. L., Kirwan, A. F., Blais, D. E., Bonin, M., & Parissenti, A. M. (2004). Cross-resistance studies of isogenic drug-resistant breast tumor cell lines support recent clinical evidence suggesting that sensitivity to paclitaxel may be strongly compromised by prior doxorubicin exposure. *Breast cancer research and treatment*, *85*(1), 31-51.

Gupta, S., Hussain, T., & Mukhtar, H. (2003). Molecular pathway for (−)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. *Archives of biochemistry and biophysics*, *410*(1), 177-185.

Han, J., Han, B., Wu, X., Hao, J., Dong, X., Shen, Q., & Pang, H. (2018). Knockdown of lncRNA H19 restores chemo-sensitivity in paclitaxel-resistant triple-negative breast cancer through triggering apoptosis and regulating Akt signaling pathway. *Toxicology and applied pharmacology*, *359*, 55-61.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646-674.

Hari, M., Loganzo, F., Annable, T., Tan, X., Musto, S., Morilla, D. B., . . . Greenberger, L. M. (2006). Paclitaxel-resistant cells have a mutation in the paclitaxel-binding region of β-tubulin (Asp26Glu) and less stable microtubules. *Molecular cancer therapeutics*, *5*(2), 270-278.

Hassan, M., Ansari, J., Spooner, D., & Hussain, S. (2010). Chemotherapy for breast cancer. *Oncology reports*, *24*(5), 1121-1131.

Hess, K. R., Anderson, K., Symmans, W. F., Valero, V., Ibrahim, N., Mejia, J. A., . . . Dempsey, P. J. (2006). Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. *Journal of clinical oncology*, *24*(26), 4236-4244.

Horwitz, S. B., Lothstein, L., Manfredi, J. J., Mellado, W., Parness, J., Roy, S., . . . Zeheb, R. (1986). Taxol: Mechanisms of Action and Resistance a. *Annals of the New York Academy of Sciences*, *466*(1), 733-744.

Huang, W., Wang, J., Zhang, D., Chen, W., Hou, L., Wu, X., & Lu, Y. (2015). Inhibition of KIF14 suppresses tumor cell growth and promotes apoptosis in human glioblastoma. *Cellular Physiology and Biochemistry*, *37*(5), 1659-1670.

Hung, P.-F., Hong, T.-M., Hsu, Y.-C., Chen, H.-Y., Chang, Y.-L., Wu, C.-T., . . . Yang, P.-C. (2013). The motor protein KIF14 inhibits tumor growth and cancer metastasis in lung adenocarcinoma. *PLoS One*, *8*(4), e61664.

Janczar, S., Nautiyal, J., Xiao, Y., Curry, E., Sun, M., Zanini, E., . . . Gabra, H. (2017). WWOX sensitises ovarian cancer cells to paclitaxel via modulation of the ER stress response. *Cell death & disease*, *8*(7), e2955-e2955.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer Statistics, 2008. *CA: A Cancer Journal for Clinicians*, *58*(2), 71-96. https://doi.org/https://doi.org/10.3322/CA.2007.0010

Jennifer Armstrong, M. W., and Lisa Weissman. (2020, March 28 2020). *Hormonal Therapy*. BreastCancer.org. Retrieved December 22 from https://www.breastcancer.org/treatment/hormonal

Jeong, Y. J., Kang, J. S., Lee, S. I., So, D. M., Yun, J., Baek, J. Y., . . . Park, S. K. (2016). Breast cancer cells evade paclitaxel-induced cell death by developing resistance to dasatinib. *Oncology letters*, *12*(3), 2153-2158.

Jiang, Y.-Z., Ma, D., Suo, C., Shi, J., Xue, M., Hu, X., . . . Yu, Y. (2019). Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. *Cancer cell*, *35*(3), 428-440. e425.

Jordan, M. A., & Wilson, L. (2004). Microtubules as a target for anticancer drugs. *Nature Reviews Cancer*, *4*(4), 253-265.

Kao, J., Salari, K., Bocanegra, M., Choi, Y.-L., Girard, L., Gandhi, J., . . . Gazdar, A. F. (2009). Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. *PLoS One*, *4*(7), e6146.

Kavallaris, M. (2010). Microtubules and resistance to tubulin-binding agents. *Nature Reviews Cancer*, *10*(3), 194-204.

Kazandjian, D., Blumenthal, G. M., Yuan, W., He, K., Keegan, P., & Pazdur, R. (2016). FDA approval of gefitinib for the treatment of patients with metastatic EGFR mutation–positive non–small cell lung cancer. *Clinical Cancer Research*, *22*(6), 1307-1312.

Kenicer, J., Spears, M., Lyttle, N., Taylor, K. J., Liao, L., Cunningham, C. A., . . . Reis-Filho, J. (2014). Molecular characterisation of isogenic taxane resistant cell lines identify novel drivers of drug resistance. *BMC cancer*, *14*(1), 1-10.

Khan, N., Afaq, F., Saleem, M., Ahmad, N., & Mukhtar, H. (2006). Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. *Cancer Research*, *66*(5), 2500-2505.

Kim, T. K. (2017). Understanding one-way ANOVA using conceptual figures. *Korean J Anesthesiol*, *70*(1), 22-26.

Konecny, G., Pauletti, G., Pegram, M., Untch, M., Dandekar, S., Aguilar, Z., . . . Felber, M. (2003). Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. *Journal of the National Cancer Institute*, *95*(2), 142-153.

Koutsilieris, M., Reyes-Moreno, C., Choki, I., Sourla, A., Doillon, C., & Pavlidis, N. (1999). Chemotherapy cytotoxicity of human MCF-7 and MDA-MB 231 breast cancer cells is altered by osteoblast-derived growth factors. *Molecular Medicine*, *5*(2), 86-97.

Kramer, N., Walzl, A., Unger, C., Rosner, M., Krupitza, G., Hengstschläger, M., & Dolznig, H. (2013). In vitro cell migration and invasion assays. *Mutation Research/Reviews in Mutation Research*, *752*(1), 10-24.

Kumar, P., & Aggarwal, R. (2016). An overview of triple-negative breast cancer. *Archives of gynecology and obstetrics*, *293*(2), 247-269.

Lecumberri, E., Dupertuis, Y. M., Miralbell, R., & Pichard, C. (2013). Green tea polyphenol epigallocatechin-3-gallate (EGCG) as adjuvant in cancer therapy. *Clinical Nutrition*, *32*(6), 894-903.

Lehmann, B. D., Bauer, J. A., Chen, X., Sanders, M. E., Chakravarthy, A. B., Shyr, Y., & Pietenpol, J. A. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *The Journal of clinical investigation*, *121*(7), 2750-2767.

Li, K. K.-W., Qi, Y., Xia, T., Chan, A. K.-Y., Zhang, Z.-Y., Aibaidula, A., . . . Ng, H.-K. (2017). The kinesin KIF14 is overexpressed in medulloblastoma and downregulation of KIF14 suppressed tumor proliferation and induced apoptosis. *Laboratory Investigation*, *97*(8), 946.

Li, Y.-n., Cao, Y.-q., Wu, X., Han, G.-s., Wang, L.-x., Zhang, Y.-h., . . . Liu, J.-m. (2015). The association between Salt-inducible kinase 2 (SIK2) and gamma isoform of the regulatory subunit B55 of PP2A (B55gamma) contributes to the survival of glioma cells under glucose depletion through inhibiting the phosphorylation of S6K. *Cancer cell international*, *15*(1), 1-8.

Lian, B., Hu, X., & Shao, Z.-m. (2019). Unveiling novel targets of paclitaxel resistance by single molecule long-read RNA sequencing in breast cancer. *Scientific reports*, *9*(1), 1-10.

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923-930.

Liu, M., Fu, M., Yang, X., Jia, G., Shi, X., Ji, J., . . . Zhai, G. (2020). Paclitaxel and quercetin co-loaded functional mesoporous silica nanoparticles overcoming multidrug resistance in breast cancer. *Colloids Surf B Biointerfaces*, *196*, 111284. https://doi.org/10.1016/j.colsurfb.2020.111284

Liu, T., Sun, H., Zhu, D., Dong, X., Liu, F., Liang, X., . . . Wang, Y. (2017). TRA2A promoted paclitaxel resistance and tumor progression in triple-negative breast cancers via regulating alternative splicing. *Molecular cancer therapeutics*, *16*(7), 1377-1388.

Liu, X., Tian, X., Wang, F., Ma, Y., Kornmann, M., & Yang, Y. (2014). BRG1 promotes chemoresistance of pancreatic cancer cells through crosstalking with Akt signalling. *European journal of cancer*, *50*(13), 2251-2262.

Liu, Y., Gao, S., Chen, X., Liu, M., Mao, C., & Fang, X. (2016). Overexpression of miR-203 sensitizes paclitaxel (Taxol)-resistant colorectal cancer cells through targeting the salt-inducible kinase 2 (SIK2). *Tumor Biology*, *37*(9), 12231-12239.

Luo, K.-W., Chen, W., Lung, W.-Y., Wei, X.-Y., Cheng, B.-H., Cai, Z.-M., & Huang, W.-R. (2017). EGCG inhibited bladder cancer SW780 cell proliferation and migration both in vitro and in vivo via down-regulation of NF-κB and MMP-9. *The Journal of nutritional biochemistry*, *41*, 56-64.

Luo, T., Wang, J., Yin, Y., Hua, H., Jing, J., Sun, X., . . . Jiang, Y. (2010). (-)-Epigallocatechin gallate sensitizes breast cancer cells to paclitaxel in a murine model of breast carcinoma. *Breast Cancer Research*, *12*(1), R8.

Luo, Y., Li, D., Ran, J., Yan, B., Chen, J., Dong, X., . . . Liu, M. (2014). End-binding protein 1 stimulates paclitaxel sensitivity in breast cancer by promoting its actions toward microtubule assembly and stability. *Protein & cell*, *5*(6), 469-479.

Luqmani, Y. (2005). Mechanisms of drug resistance in cancer chemotherapy. *Medical principles and practice*, *14*(Suppl. 1), 35-48.

Luque-Bolivar, A., Pérez-Mora, E., Villegas, V. E., & Rondón-Lagos, M. (2020). Resistance and Overcoming Resistance in Breast Cancer. *Breast Cancer: Targets and Therapy*, *12*, 211.

Madhavan, J., Mitra, M., Mallikarjuna, K., Pranav, O., Srinivasan, R., Nagpal, A., . . . Kumaramanickavel, G. (2009). KIF14 and E2F3 mRNA expression in human retinoblastoma and its phenotype association. *Molecular vision*, *15*, 235.

Maloney, S. M., Hoover, C. A., Morejon-Lasso, L. V., & Prosperi, J. R. (2020). Mechanisms of Taxane Resistance. *Cancers (Basel)*, *12*(11). https://doi.org/10.3390/cancers12113323

Maloney, S. M., Hoover, C. A., Morejon-Lasso, L. V., & Prosperi, J. R. (2020). Mechanisms of taxane resistance. *Cancers*, *12*(11), 3323.

Manfredi, J. J., & Horwitz, S. B. (1984). Taxol: an antimitotic agent with a new mechanism of action. *Pharmacology & therapeutics*, *25*(1), 83-125.

Manfredi, J. J., Parness, J., & Horwitz, S. B. (1982). Taxol binds to cellular microtubules. *The Journal of cell biology*, *94*(3), 688-696.

Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S., & Baradaran, B. (2017). The different mechanisms of cancer drug resistance: a brief review. *Advanced pharmaceutical bulletin*, *7*(3), 339.

Massey, A. E., Sikander, M., Chauhan, N., Kumari, S., Setua, S., Shetty, A. B., . . . Jaggi, M. (2019). Next-generation paclitaxel-nanoparticle formulation for pancreatic cancer treatment. *Nanomedicine: Nanotechnology, Biology and Medicine*, *20*, 102027.

McDermott, M., Eustace, A., Busschots, S., Breen, L., Clynes, M., O'Donovan, N., & Stordal, B. (2014). In vitro development of chemotherapy and targeted therapy drug-resistant cancer cell lines: a practical guide with case studies. *Frontiers in oncology*, *4*, 40.

McGrogan, B. T., Gilmartin, B., Carney, D. N., & McCann, A. (2008). Taxanes, microtubules and chemoresistant breast cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1785*(2), 96-132.

McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K., & Donehower, R. C. (1989). Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Annals of internal medicine*, *111*(4), 273-279.

Meeran, S. M., Patel, S. N., Chan, T.-H., & Tollefsbol, T. O. (2011). A novel prodrug of epigallocatechin-3-gallate: differential epigenetic hTERT repression in human breast cancer cells. *Cancer prevention research*, *4*(8), 1243-1254.

Mineva, N. D., Paulson, K. E., Naber, S. P., Yee, A. S., & Sonenshein, G. E. (2013). Epigallocatechin-3-gallate inhibits stem-like inflammatory breast cancer cells. *PLoS One*, *8*(9).

Mir, M. A., Qayoom, H., Mehraj, U., Nisar, S., Bhat, B., & Wani, N. A. (2020). Targeting different pathways using novel combination therapy in triple negative breast Cancer. *Current Cancer Drug Targets*, *20*(8), 586-602.

Mohan, N., Karmakar, S., Banik, N. L., & Ray, S. K. (2011). SU5416 and EGCG work synergistically and inhibit angiogenic and survival factors and induce cell cycle arrest to promote apoptosis in human malignant neuroblastoma SH-SY5Y and SK-N-BE2 cells. *Neurochemical research*, *36*(8), 1383-1396.

Mukhtar, H., & Ahmad, N. (2000). Tea polyphenols: prevention of cancer and optimizing health. *The American journal of clinical nutrition*, *71*(6), 1698S-1702S.

Nedeljković, M., & Damjanović, A. (2019). Mechanisms of chemotherapy resistance in triple-negative breast cancer—how we can rise to the challenge. *Cells*, *8*(9), 957.

Němcová-Fürstová, V., Kopperová, D., Balušíková, K., Ehrlichová, M., Brynychová, V., Václavíková, R., . . . Kovář, J. (2016). Characterization of acquired paclitaxel resistance of breast cancer cells and involvement of ABC transporters. *Toxicology and applied pharmacology*, *310*, 215-228.

Nikolaou, M., Pavlopoulou, A., Georgakilas, A. G., & Kyrodimos, E. (2018). The challenge of drug resistance in cancer treatment: a current overview. *Clinical & Experimental Metastasis*, *35*(4), 309-318.

Nogales, E. (2001). Structural insights into microtubule function. *Annual review of biophysics and biomolecular structure*, *30*(1), 397-420.

Nomura, N., Miyajima, N., Sazuka, T., Tanaka, A., Kawarabayasi, Y., Sato, S., . . . Tabata, S. (1994). Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly

sampled cDNA clones from human immature myeloid cell line KG-1. *DNA research*, *1*(1), 27-35.

Oliver, M. H., Harrison, N. K., Bishop, J. E., Cole, P. J., & Laurent, G. J. (1989). A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors. *Journal of cell science*, *92*(3), 513-518.

Önyüksel, H., Jeon, E., & Rubinstein, I. (2009). Nanomicellar paclitaxel increases cytotoxicity of multidrug resistant breast cancer cells. *Cancer letters*, *274*(2), 327-330.

Orr, G. A., Verdier-Pinard, P., McDaid, H., & Horwitz, S. B. (2003). Mechanisms of Taxol resistance related to microtubules. *Oncogene*, *22*(47), 7280-7295.

Oya, Y., Mondal, A., Rawangkan, A., Umsumarng, S., Iida, K., Watanabe, T., . . . Kagechika, H. (2017). Down-regulation of histone deacetylase 4,− 5 and− 6 as a mechanism of synergistic enhancement of apoptosis in human lung cancer cells treated with the combination of a synthetic retinoid, Am80 and green tea catechin. *The Journal of nutritional biochemistry*, *42*, 7-16.

Page, C., Lin, H., Jin, Y., Castle, V., Nunez, G., Huang, M., & Lin, J. (2000). Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. *Anticancer research*, *20*(1A), 407-416.

Panayotopoulou, E. G., Müller, A.-K., Börries, M., Busch, H., Hu, G., & Lev, S. (2017). Targeting of apoptotic pathways by SMAC or BH3 mimetics distinctly sensitizes paclitaxel-resistant triple negative breast cancer cells. *Oncotarget*, *8*(28), 45088.

Paradiso, A., Mangia, A., Chiriatti, A., Tommasi, S., Zito, A., Latorre, A., . . . Lorusso, V. (2005). Biomarkers predictive for clinical efficacy of taxol-based chemotherapy in advanced breast cancer. *Annals of Oncology*, *16*, iv14-iv19.

Paschka, A. G., Butler, R., & Young, C. Y.-F. (1998). Induction of apoptosis in prostate cancer cell lines by the green tea component,(−)-epigallocatechin-3-gallate. *Cancer letters*, *130*(1-2), 1-7.

Pavlopoulou, A., Oktay, Y., Vougas, K., Louka, M., Vorgias, C. E., & Georgakilas, A. G. (2016). Determinants of resistance to chemotherapy and ionizing radiation in breast cancer stem cells. *Cancer letters*, *380*(2), 485-493.

Perou, C. M., Sørlie, T., Eisen, M. B., Van De Rijn, M., Jeffrey, S. S., Rees, C. A., . . . Akslen, L. A. (2000). Molecular portraits of human breast tumours. *nature*, *406*(6797), 747-752.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic acids research*, *29*(9), e45-e45.

Plevritis, S. K., Munoz, D., Kurian, A. W., Stout, N. K., Alagoz, O., Near, A. M., . . . Schechter, C. B. (2018). Association of screening and treatment with breast cancer mortality by molecular subtype in US women, 2000-2012. *Jama*, *319*(2), 154-164.

Qiu, H., Deng, S., Li, C., Tian, Z., Song, X., Yao, G., & Geng, J. (2017). High expression of KIF14 is associated with poor prognosis in patients with epithelial ovarian cancer. *Eur Rev Med Pharmacol Sci*, *21*(2), 239-245.

Ramadass, S. K., Anantharaman, N. V., Subramanian, S., Sivasubramanian, S., & Madhan, B. (2015). Paclitaxel/epigallocatechin gallate coloaded liposome: a synergistic delivery to control the invasiveness of MDA-MB-231 breast cancer cells. *Colloids and surfaces B: Biointerfaces*, *125*, 65-72.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139-140.

Rodrigues-Ferreira, S., Moindjie, H., Haykal, M. M., & Nahmias, C. (2020). Predicting and overcoming taxane chemoresistance. *Trends in Molecular Medicine*.

Rouzier, R., Rajan, R., Wagner, P., Hess, K. R., Gold, D. L., Stec, J., . . . Buchholz, T. A. (2005). Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proceedings of the National Academy of Sciences*, *102*(23), 8315-8320.

Rovini, A., Carré, M., Bordet, T., Pruss, R. M., & Braguer, D. (2010). Olesoxime prevents microtubule-targeting drug neurotoxicity: selective preservation of EB comets in differentiated neuronal cells. *Biochemical pharmacology*, *80*(6), 884-894.

Rowinsky, E. K., & Donehower, R. C. (1995). Paclitaxel (taxol). *New England Journal of Medicine*, *332*(15), 1004-1014.

Roy, A. M., Baliga, M. S., & Katiyar, S. K. (2005). Epigallocatechin-3-gallate induces apoptosis in estrogen receptor–negative human breast carcinoma cells via modulation in protein expression of p53 and Bax and caspase-3 activation. *Molecular cancer therapeutics*, *4*(1), 81-90.

Sakamoto, J., Matsui, T., & Kodera, Y. (2009). Paclitaxel chemotherapy for the treatment of gastric cancer. *Gastric Cancer*, *12*(2), 69-78.

Sanhaji, M., Friel, C. T., Wordeman, L., Louwen, F., & Yuan, J. (2011). Mitotic centromere-associated kinesin (MCAK): a potential cancer drug target. *Oncotarget*, *2*(12), 935.

Sarosy, G., Kohn, E., Stone, D. A., Rothenberg, M., Jacob, J., Adamo, D., . . . Reed, E. (1992). Phase I study of taxol and granulocyte colony-stimulating factor in patients with refractory ovarian cancer. *Journal of clinical oncology*, *10*(7), 1165-1170.

Sauder, D. C., & DeMars, C. E. (2019). An updated recommendation for multiple comparisons. *Advances in Methods and Practices in Psychological Science*, *2*(1), 26-44.

Schiewek, J., Schumacher, U., Lange, T., Joosse, S. A., Wikman, H., Pantel, K., . . . Schmalfeldt, B. (2018). Clinical relevance of cytoskeleton associated proteins for ovarian cancer. *Journal of cancer research and clinical oncology*, *144*(11), 2195-2205.

Schmid, P., Abraham, J., Chan, S., Wheatley, D., Brunt, A. M., Nemsadze, G., . . . Perren, T. (2020). Capivasertib Plus Paclitaxel Versus Placebo Plus Paclitaxel As First-Line Therapy for Metastatic Triple-Negative Breast Cancer: The PAKT Trial.

Schrøder, J. M., Larsen, J., Komarova, Y., Akhmanova, A., Thorsteinsson, R. I., Grigoriev, I., . . . Geimer, S. (2011). EB1 and EB3 promote cilia biogenesis by several centrosome-related mechanisms. *J Cell Sci*, *124*(15), 2539-2551.

Schröder, L., Marahrens, P., Koch, J. G., Heidegger, H., Vilsmeier, T., Phan-Brehm, T., . . . Richter, D. U. (2019). Effects of green tea, matcha tea and their components epigallocatechin gallate and quercetin on MCF-7 and MDA-MB-231 breast carcinoma cells. *Oncology reports*, *41*(1), 387-396.

Sen, T., Moulik, S., Dutta, A., Choudhury, P. R., Banerji, A., Das, S., . . . Chatterjee, A. (2009). Multifunctional effect of epigallocatechin-3-gallate (EGCG) in downregulation of gelatinase-A (MMP-2) in human breast cancer cell line MCF-7. *Life sciences*, *84*(7-8), 194-204.

Sha, L., Zhang, Y., Wang, W., Sui, X., Liu, S., Wang, T., & Zhang, H. (2016). MiR-18a upregulation decreases Dicer expression and confers paclitaxel resistance in triple negative breast cancer. *Eur Rev Med Pharmacol Sci*, *20*(11), 2201-2208.

Shemesh, O. A., & Spira, M. E. (2010). Paclitaxel induces axonal microtubules polar reconfiguration and impaired organelle transport: implications for the pathogenesis of paclitaxel-induced polyneuropathy. *Acta neuropathologica*, *119*(2), 235-248.

Shi, X., & Sun, X. (2017). Regulation of paclitaxel activity by microtubule-associated proteins in cancer chemotherapy. *Cancer Chemotherapy and Pharmacology*, *80*(5), 909-917.

Shirazi, F. H., Zarghi, A., Ashtarinezhad, A., Kobarfard, F., Nakhjavani, M., Anjidani, N., . . . Mohebi, S. (2011). *Remarks in successful cellular investigations for fighting breast cancer using novel synthetic compounds*. INTECH Open Access Publisher Croatia.

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer Statistics, 2021. *CA: A Cancer Journal for Clinicians*, *71*(1), 7-33. https://doi.org/https://doi.org/10.3322/caac.21654

Siegel, R. L., Miller, K. D., & Jemal, A. (2015). Cancer statistics, 2015. *CA: A Cancer Journal for Clinicians*, *65*(1), 5-29. https://doi.org/https://doi.org/10.3322/caac.21254

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians*, *67*(1), 7-30. https://doi.org/https://doi.org/10.3322/caac.21387

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(1), 7-34. https://doi.org/10.3322/caac.21551

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J Clin*, *70*(1), 7-30. https://doi.org/10.3322/caac.21590

Simonian, M., Ghaffari, M. H., & Negahdari, B. (2021). Immunotherapy for Breast Cancer Treatment. *Iranian Biomedical Journal*, *25*(3), 140.

Singel, S. M., Cornelius, C., Batten, K., Fasciani, G., Wright, W. E., Lum, L., & Shay, J. W. (2013). A targeted RNAi screen of the breast cancer genome identifies KIF14 and TLN1 as genes that modulate docetaxel chemosensitivity in triple-negative breast cancer. *Clinical Cancer Research*, *19*(8), 2061-2070.

Singel, S. M., Cornelius, C., Zaganjor, E., Batten, K., Sarode, V. R., Buckley, D. L., . . . Sadeghi, N. (2014). KIF14 promotes AKT phosphorylation and contributes to chemoresistance in triple-negative breast cancer. *Neoplasia*, *16*(3), 247-256. e242.

Sledge, G. W., Neuberg, D., Bernardo, P., Ingle, J. N., Martino, S., Rowinsky, E. K., & Wood, W. C. (2003). Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). *Journal of clinical oncology*, *21*(4), 588-592.

Smoter, M., Bodnar, L., Duchnowska, R., Stec, R., Grala, B., & Szczylik, C. (2011). The role of Tau protein in resistance to paclitaxel. *Cancer Chemotherapy and Pharmacology*, *68*(3), 553-557.

Snyder, J. P., Nettles, J. H., Cornett, B., Downing, K. H., & Nogales, E. (2001). The binding conformation of Taxol in β-tubulin: a model based on electron crystallographic density. *Proceedings of the National Academy of Sciences*, *98*(9), 5312-5316.

Society, A. C. (2019). Breast cancer facts & figures 2019-2020. *Am. Cancer Soc*, 1-44.

Sørlie, T., Perou, C. M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., . . . Jeffrey, S. S. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proceedings of the National Academy of Sciences*, *98*(19), 10869-10874.

Sparano, J. A., Wang, M., Martino, S., Jones, V., Perez, E. A., Saphner, T., . . . Davidson, N. E. (2008). Weekly paclitaxel in the adjuvant treatment of breast cancer. *New England Journal of Medicine*, *358*(16), 1663-1671.

Sprouse, A. A., & Herbert, B.-S. (2014). Resveratrol augments paclitaxel treatment in MDA-MB-231 and paclitaxel-resistant MDA-MB-231 breast cancer cells. *Anticancer research*, *34*(10), 5363-5374.

Stage, T. B., Bergmann, T. K., & Kroetz, D. L. (2018). Clinical pharmacokinetics of paclitaxel monotherapy: an updated literature review. *Clinical pharmacokinetics*, *57*(1), 7-19.

Stearns, M. E., & Wang, M. (2011). Synergistic effects of the green tea extract epigallocatechin-3-gallate and taxane in eradication of malignant human prostate tumors. *Translational oncology*, *4*(3), 147-156.

Steding, C. E. (2016). Creating chemotherapeutic-resistant breast cancer cell lines: advances and future perspectives. *Future Oncology*, *12*(12), 1517-1527.

Steding, C. E. (2016). Creating chemotherapeutic-resistant breast cancer cell lines: advances and future perspectives. *Future Oncol*, *12*(12), 1517-1527. https://doi.org/10.2217/fon-2016-0059

Steding, C. E., Wu, S. t., Zhang, Y., Jeng, M. H., Elzey, B. D., & Kao, C. (2011). The role of interleukin-12 on modulating myeloid-derived suppressor cells, increasing overall survival and reducing metastasis. *Immunology*, *133*(2), 221-238.

Stengel, C., Newman, S., Leese, M., Potter, B., Reed, M., & Purohit, A. (2010). Class III β-tubulin expression and in vitro resistance to microtubule targeting agents. *British journal of cancer*, *102*(2), 316-324.

Suganuma, M., Saha, A., & Fujiki, H. (2011). New cancer treatment strategy using combination of green tea catechins and anticancer drugs. *Cancer science*, *102*(2), 317-323.

Sun, R., Liu, Z., Wang, L., Lv, W., Liu, J., Ding, C., . . . Xu, C. (2015). Overexpression of stathmin is resistant to paclitaxel treatment in patients with non-small cell lung cancer. *Tumor Biology*, *36*(9), 7195-7204.

Sun, X., Song, J., Li, E., Geng, H., Li, Y., Yu, D., & Zhong, C. (2019). (-)-Epigallocatechin-3-gallate inhibits bladder cancer stem cells via suppression of sonic hedgehog pathway. *Oncology reports*, *42*(1), 425-435.

Sur, S., & Panda, C. K. (2017). Molecular aspects of cancer chemopreventive and therapeutic efficacies of tea and tea polyphenols. *Nutrition*, *43*, 8-15.

Surveillance, E., and End Results (SEER) Program. (November 2020). *Female Breast Cancer Subtypes*. National Cancer Institute. https://seer.cancer.gov/statfacts/html/breast-subtypes.html#:~:text=There%20are%20four%20main%20female%20breast%20cancer%20subtypes%2C,Negative%22%29%203%20HR%2B%2FHER2%2B%20%28%22Luminal%20B%22%29%204%20HR-%2FHER2%2B%20%28%22HER2-enriched%22%29

Tabuchi, Y., Matsuoka, J., Gunduz, M., Imada, T., Ono, R., Ito, M., . . . Takaoka, M. (2009). Resistance to paclitaxel therapy is related with Bcl-2 expression through an estrogen receptor mediated pathway in breast cancer. *International journal of oncology*, *34*(2), 313-319.

Tanaka, S., Nohara, T., Iwamoto, M., Sumiyoshi, K., Kimura, K., Takahashi, Y., & Tanigawa, N. (2009). Tau expression and efficacy of paclitaxel treatment in metastatic breast cancer. *Cancer Chemotherapy and Pharmacology*, *64*(2), 341-346.

Tang, S.-N., Fu, J., Shankar, S., & Srivastava, R. K. (2012). EGCG enhances the therapeutic potential of gemcitabine and CP690550 by inhibiting STAT3 signaling pathway in human pancreatic cancer. *PLoS One*, *7*(2), e31067.

Thériault, B. L., Basavarajappa, H. D., Lim, H., Pajovic, S., Gallie, B. L., & Corson, T. W. (2014). Transcriptional and epigenetic regulation of KIF14 overexpression in ovarian cancer. *PLoS One*, *9*(3), e91540.

Thériault, B. L., Cybulska, P., Shaw, P. A., Gallie, B. L., & Bernardini, M. Q. (2014). The role of KIF14 in patient-derived primary cultures of high-grade serous ovarian cancer cells. *Journal of ovarian research*, *7*(1), 123.

Thomas, G. E., Sreeja, J. S., Gireesh, K., Gupta, H., & Manna, T. K. (2015). + TIP EB1 downregulates paclitaxel-induced proliferation inhibition and apoptosis in breast cancer cells through inhibition of paclitaxel binding on microtubules. *International journal of oncology*, *46*(1), 133-146.

Thuerigen, O., Schneeweiss, A., Toedt, G., Warnat, P., Hahn, M., Kramer, H., . . . Schuetz, F. (2006). Gene expression signature predicting pathologic complete response with gemcitabine, epirubicin, and docetaxel in primary breast cancer. *J Clin Oncol*, *24*(12), 1839-1845.

Tommasi, S., Mangia, A., Lacalamita, R., Bellizzi, A., Fedele, V., Chiriatti, A., . . . Lorusso, V. (2007). Cytoskeleton and paclitaxel sensitivity in breast cancer: the role of β-tubulins. *International journal of cancer*, *120*(10), 2078-2085.

Tsai, S.-J., & Wiltbank, M. C. (1996). Quantification of mRNA using competitive RTPCR with standard-curve methodology. *Biotechniques*, *21*(5), 862-866.

Tuma, R. S. (2003). Taxol's journey from discovery to use: lessons & updates. *Oncology Times*, *25*(18), 52-57.

Tyagi, T., Treas, J. N., Mahalingaiah, P. K. S., & Singh, K. P. (2015). Potentiation of growth inhibition and epigenetic modulation by combination of green tea polyphenol and 5-aza-2′-deoxycytidine in human breast cancer cells. *Breast cancer research and treatment*, *149*(3), 655-668.

van der Vaart, B., Akhmanova, A., & Straube, A. (2009). Regulation of microtubule dynamic instability. In: Portland Press Limited.

Verweij, J., Clavel, M., & Chevalier, B. (1994). Paclitaxel (TaxolTM) and docetaxel (TaxotereTM): Not simply two of a kind. *Annals of Oncology*, *5*(6), 495-505.

Wang, J., Yang, M., Li, Y., & Han, B. (2015). The role of microRNAs in the chemoresistance of breast cancer. *Drug development research*, *76*(7), 368-374.

Wang, W., Shi, Y., Li, J., Cui, W., & Yang, B. (2016). Up-regulation of KIF14 is a predictor of poor survival and a novel prognostic biomarker of chemoresistance to paclitaxel treatment in cervical cancer. *Bioscience reports*, *36*(2).

Wang, Z.-Z., Yang, J., Jiang, B.-H., Di, J.-B., Gao, P., Peng, L., & Su, X.-Q. (2018). KIF14 promotes cell proliferation via activation of Akt and is directly targeted by miR-200c in colorectal cancer. *International journal of oncology*, *53*(5), 1939-1952.

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., & McPhail, A. T. (1971). Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *Journal of the American Chemical Society*, *93*(9), 2325-2327.

Welsh, J. (2013). Animal models for studying prevention and treatment of breast cancer. In *Animal models for the study of human disease* (pp. 997-1018). Elsevier.

Werner, H. M., Trovik, J., Halle, M. K., Wik, E., Akslen, L. A., Birkeland, E., . . . Salvesen, H. B. (2014). Stathmin protein level, a potential predictive marker for taxane treatment response in endometrial cancer. *PLoS One*, *9*(2), e90141.

Wolff, A. C., Tung, N. M., & Carey, L. A. (2019). Implications of neoadjuvant therapy in human epidermal growth factor receptor 2–positive breast cancer. In (Vol. 37, pp. 2189-2192): American Society of Clinical Oncology.

Xiao, L., Zhang, S., Zheng, Q., & Zhang, S. (2021). Dysregulation of KIF14 regulates the cell cycle and predicts poor prognosis in cervical cancer: a study based on integrated approaches. *Brazilian Journal of Medical and Biological Research, 54*.

Xie, S., Ogden, A., Aneja, R., & Zhou, J. (2016). Microtubule-binding proteins as promising biomarkers of paclitaxel sensitivity in cancer chemotherapy. *Medicinal research reviews*, *36*(2), 300-312.

Xu, H., Choe, C., Shin, S.-H., Park, S.-W., Kim, H.-S., Jung, S.-H., . . . Chung, Y.-J. (2014). Silencing of KIF14 interferes with cell cycle progression and cytokinesis by blocking the p27 Kip1 ubiquitination pathway in hepatocellular carcinoma. *Experimental & molecular medicine*, *46*(5), e97-e97.

Xu, X.-Y., Zhao, C.-N., Cao, S.-Y., Tang, G.-Y., Gan, R.-Y., & Li, H.-B. (2019). Effects and mechanisms of tea for the prevention and management of cancers: An updated review. *Critical reviews in food science and nutrition*, 1-13.

Yang, C., Wu, J., de Heus, C., Grigoriev, I., Liv, N., Yao, Y., . . . Qi, R. Z. (2017). EB1 and EB3 regulate microtubule minus end organization and Golgi morphology. *J Cell Biol*, *216*(10), 3179-3198.

Yang, T., Zhang, X. B., & Zheng, Z. M. (2013). Suppression of KIF 14 expression inhibits hepatocellular carcinoma progression and predicts favorable outcome. *Cancer science*, *104*(5), 552-557.

Yang, Z., Li, C., Yan, C., Li, J., Yan, M., Liu, B., . . . Gu, Q. (2019). KIF14 promotes tumor progression and metastasis and is an independent predictor of poor prognosis in human gastric cancer. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1865*(1), 181-192.

Yuan, J.-M., Sun, C., & Butler, L. M. (2011). Tea and cancer prevention: epidemiological studies. *Pharmacological research*, *64*(2), 123-135.

Zasadil, L. M., Andersen, K. A., Yeum, D., Rocque, G. B., Wilke, L. G., Tevaarwerk, A. J., . . . Weaver, B. A. (2014). Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Science translational medicine*, *6*(229), 229ra243-229ra243.

Zhang, Y., Yuan, Y., Liang, P., Zhang, Z., Guo, X., Xia, L., . . . Ying, Y. (2017). Overexpression of a novel candidate oncogene KIF14 correlates with tumor progression and poor prognosis in prostate cancer. *Oncotarget*, *8*(28), 45459.

Zohrap, N., Saatci, Ö., Ozes, B., Coban, I., Atay, H. M., Battaloglu, E., . . . Bugra, K. (2018). SIK2 attenuates proliferation and survival of breast cancer cells with simultaneous perturbation of MAPK and PI3K/Akt pathways. *Oncotarget*, *9*(31), 21876.

APPENDIX A: SUPPLEMENTARY MATERIALS FROM CHAPTER 2

Table 2.S1

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 0.0005 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	0.07	.38	0.06
MDA-MB-231 Paclitaxel Treated	3	0.44	0.14			

Table 2.S2

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 0.005 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	0.17	.49	.14
MDA-MB-231 Paclitaxel Treated	3	0.43	0.13			

Table 2.S3

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 0.05 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	0.36	.37	0.30
MDA-MB-231 Paclitaxel Treated	3	0.41	0.13			

Table 2.S4

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 0.5 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	0.88	.41	0.72
MDA-MB-231 Paclitaxel Treated	3	0.37	0.12			

Table 2.S5

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 5 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	2.2	.82	1.80
MDA-MB-231 Paclitaxel Treated	3	0.31	0.07			

Table 2.S6

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 50 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	2.69	.33	2.20
MDA-MB-231 Paclitaxel Treated	3	0.15	0.018			

Table 2.S7

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 500 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	2.63	.31	2.14
MDA-MB-231 Paclitaxel Treated	3	0.14	0.02			

Table 2.S8

Results of Independent t-Test for Short-Term Dose-Response to Paclitaxel Concentration 5,000 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.44	0.08	5.64	.13	4.61
MDA-MB-231 Paclitaxel Treated	3	0.17	0.03			

Table 2.S9

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 0.0005 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	2.88	.52	2.35
MDA-MB-231 Paclitaxel Treated	3	0.44	0.18			

Table 2.S10

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 0.005 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	4.81	.87	3.93
MDA-MB-231 Paclitaxel Treated	3	0.35	.11			

Table 2.S11

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 0.05 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	8.26	0.92	6.74
MDA-MB-231 Paclitaxel Treated	3	0.25	0.039			

Table 2.S12

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 0.5 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	9.66	.041*	7.88
MDA-MB-231 Paclitaxel Treated	3	0.18	0.02			

$*p < .05$

Table 2.S13

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 5 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	10.08	.033*	8.23
MDA-MB-231 Paclitaxel Treated	3	0.16	0.01			

**p < .05*

Table 2.S14

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 50 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	10.21	.038*	8.33
MDA-MB-231 Paclitaxel Treated	3	0.15	0.018			

**p < .05*

Table 2.S15

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 500 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	10.42	.03*	8.51
MDA-MB-231 Paclitaxel Treated	3	0.14	0.02			

**p < .05*

Table 2.S16

Results of Independent t-Test for Long-Term Dose-Response to Paclitaxel Concentration 5,000 ng/mL

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.79	0.11	10.62	0.03*	8.67
MDA-MB-231 Paclitaxel Treated	3	0.13	0.01			

**p < .05*

Table 2.S17

Results of Independent t-Test for Cell Invasion Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Sensitive}	3	0.14	0.01	2.19	0.14	1.79
MDA-MB-231^{Resistant}	3	0.44	0.14			

Table 2.S18

Results of Independent t-Test for Colony Formation Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Sensitive}	3	113.61	56	1.35	0.12	1.10
MDA-MB-231^{Resistant}	3	66.22	23.91			

Table 2.S19

Descriptive Statistics for Cytotoxicity Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	8.85	6.88	-			
2. MDA-MB-231^{Resistant} Px Treated	3	7.65	2.32	0.99	-		
3. MDA-MB-231^{Sensitive}	3	13.31	3.73	0.71	0.54	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	21.17	5.84	0.06	0.04*	0.29	-

*$p < .05$

Table 2.S20

Descriptive Statistics of Apoptosis Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	5.78	1.06	-			
2. MDA-MB-231^{Resistant} Px Treated	3	6.32	2.35	0.99	-		
3. MDA-MB-231^{Sensitive}	3	6.19	1.83	1	1	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	8.28	3.98	0.64	0.78	0.75	-

Table 2.S21

Descriptive Statistics of Short-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	1.11	0.09	-			
2. MDA-MB-231^{Resistant} Px Treated	3	1.06	0.09	0.96	-		
3. MDA-MB-231^{Sensitive}	3	1.03	0.10	0.81	0.97	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	0.93	0.16	0.28	0.50	0.73	-

Table 2.S22

Descriptive Statistics of Long-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	0.91	0.09	-			
2. MDA-MB-231^{Resistant} Px Treated	3	0.39	0.08	0.001***	-		
3. MDA-MB-231^{Sensitive}	3	0.80	0.13	0.58	0.003**	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	0.32	0.07	0.001***	0.84	0.001***	-

***p < .01*, *** p* < .001

Table 2.S23

Descriptive Statistics of Short-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	4.53	2.79	-			
2. MDA-MB-231^{Resistant} Px Treated	3	4.44	1.71	1	-		
3. MDA-MB-231^{Sensitive}	3	6.14	2.16	0.89	0.87	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	4.78	3.79	1	1	0.93	-

Table 2.S24

Descriptive Statistics of Long-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	46.15	5.76	-			
2. MDA-MB-231^{Resistant} Px Treated	3	9.52	2.54	0.001***	-		
3. MDA-MB-231^{Sensitive}	3	32.49	4.43	0.014*	0.001***	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	5.10	2.53	0.001***	0.57	0.001***	-

*$p < .05$, *** $p < .001$

Table 2.S25

Descriptive Statistics of Short-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	85.83	18.97	-			
2. MDA-MB-231^{Resistant} Px Treated	3	67.19	33.97	0.84	-		
3. MDA-MB-231^{Sensitive}	3	97.75	1.64	0.73	0.54	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	82.44	13.99	0.99	0.88	0.44	-

Table 2.S26

Descriptive Statistics of Long-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	98.5	0	-			
2. MDA-MB-231^{Resistant} Px Treated	3	67.11	1.92	0.003**	-		
3. MDA-MB-231^{Sensitive}	3	99.33	0.14	0.03*	0.003**	-	
4. MDA-MB-231^{Sensitive} Px Treated	3	52.75	8.27	0.03*	0.22	0.02*	-

*$p < .05$, *** $p < .001$

Table 2.S27

Results of Independent t-Test for RT-PCR of MAP2

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Sensitive}	3	27.74	0.21	13.99	0.71	11.43
MDA-MB-231^{Resistant}	3	30.45	0.26			

APPENDIX B: SUPPLEMENTARY MATERIALS FROM CHAPTER 3

Table 3.S1

Descriptive Statistics for RT-PCR of KIF14 Knockdown

Treatment	*n*	*M*	*SD*	1	2	3	4	5	6
1. MDA-MB-231	3	26.82	0.24	-					
2. MDA-MB-231 48 hours KIF14 KD	3	29.55	0.14	.002**	-				
3. MDA-MB-231 72 hours KIF14 KD	3	30.58	0.18	.000***	.01**	-			
4. MDA-MB-231 96 hours KIF14 KD	3	27.56	0.23	.098	.003**	.000***	-		
5. MDA-MB-231 5 days KIF14 KD	3	27.83	1.04	.65	.33	.14	.99	-	
6. MDA-MB-231 6 days KIF14 KD	3	26.21	0.06	.16	.000***	.001***	.024*	.36	-

* $p < .05$ ** $p < .01$, *** $p < .001$

Table 3.S2

Results of Independent t-Test for Cell Invasion Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	0.14	0.019	0.30	.83	0.24
MDA-MB-231 KIF14 KD	3	0.13	0.016			

Table 3.S3

Results of Independent t-Test for Colony Formation Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231	3	129.44	59.32	2.24	.19	1.83
MDA-MB-231 KIF14 KD	3	46.33	24.97			

Table 3.S4

Descriptive Statistics for Cytotoxicity Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	28.03	27.09	-			
2. MDA-MB-231 Px Treated	3	18.77	9.46	.93	-		
3. MDA-MB-231 KIF14 KD	3	23.94	9.67	.99	.99	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	26.24	21.99	1	.96	.99	-

Table 3.S5

Descriptive Statistics for Apoptosis Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	8.11	4.36	-			
2. MDA-MB-231 Px Treated	3	10.80	6.28	.99	-		
3. MDA-MB-231 KIF14 KD	3	11.72	8.13	.97	1	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	22.71	17.68	.38	.54	.59	-

Table 3.S6

Descriptive Statistics for Short-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	1.09	0.10	-			
2. MDA-MB-231 Px Treated	3	1.04	0.15	.94	-		
3. MDA-MB-231 KIF14 KD	3	0.96	0.045	.41	.71	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	0.90	0.022	.14	.30	.84	-

Table 3.S7

Descriptive Statistics for Long-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	0.87	0.13	-			
2. MDA-MB-231 Px Treated	3	0.58	0.05	.14	-		
3. MDA-MB-231 KIF14 KD	3	0.84	0.12	.99	.19	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	0.36	0.21	.01**	.29	.013*	-

* $p < .05$, ** $p < .01$

Table 3.S8

Descriptive Statistics for Short-Term Relative Population Doubling

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	7.71	3.23	-			
2. MDA-MB-231 Px Treated	3	15.29	20.87	.92	-		
3. MDA-MB-231 KIF14 KD	3	6.33	1.41	.90	.87	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	4.71	4.89	.81	.83	.94	-

Table 3.S9

Descriptive Statistics for Long-Term Relative Population Doubling

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	45.40	29.40	-			
2. MDA-MB-231 Px Treated	3	11.13	10.41	.40	-		
3. MDA-MB-231 KIF14 KD	3	28.00	15.69	.81	.50	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	2.82	0.54	.30	.61	.25	-

Table 3.S10

Descriptive Statistics for Short-Term Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	99.08	0.80	-			
2. MDA-MB-231 Px Treated	3	85.25	19.06	.66	-		
3. MDA-MB-231 KIF14 KD	3	94.00	5.57	.54	.87	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	72.61	19.39	.32	.85	.43	-

Table 3.S11

Descriptive Statistics for Long-Term Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231	3	98.33	0.52	-			
2. MDA-MB-231 Px Treated	3	80.5	3.53	.029*	-		
3. MDA-MB-231 KIF14 KD	3	98.75	0.25	.65	.03*	-	
4. MDA-MB-231 KIF14 KD Px Treated	3	58.58	2.96	.004**	.005**	.004**	-

* $p < .05$, ** $p < .01$

Table 3.S12

Results of Independent t-Test for MDA-MB-231^{Resistant} Cell Invasion Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Resistant}	3	0.14	0.02	1.69	.58	0.01
MDA-MB-231^{Resistant} KIF14 KD	3	0.12	0.01			

Table 3.S13

Results of Independent t-Test for MDA-MB-231^{Sensitive} Cell Invasion Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Sensitive}	3	0.13	0.00	-0.79	.11	0.01
MDA-MB-231^{Sensitive} KIF14KD	3	0.14	0.01			

Table 3.S14

Results of Independent t-Test for MDA-MB-231^{Resistant} Colony Formation Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Resistant}	3	113.78	61.62	1.31	.068	45.18
MDA-MB-231^{Resistant} KIF14 KD	3	65.33	16.92			

Table 3.S15

Results of Independent t-Test for MDA-MB-231^{Sensitive} Colony Formation Assay

Treatment	*n*	*M*	*SD*	*t*(4)	*p*	*Cohen's d*
MDA-MB-231^{Sensitive}	3	69.89	30.11	-0.30	.59	27.02
MDA-MB-231^{Sensitive} KIF14KD	3	76.44	23.52			

Table 3.S16

Descriptive Statistics for MDA-MB-231^{Resistant} Cytotoxicity Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	16.46	16.75	-			
2. MDA-MB-231^{Resistant} Px Treated	3	14.37	11.79	1	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	34.63	47.31	.87	.83	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	39.68	27.78	.77	.72	.83	-

Table 3.S17

Descriptive Statistics for MDA-MB-231^{Sensitive} Cytotoxicity Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	18.99	10.39	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	23.43	5.93	.97	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	10.92	2.87	.96	.61	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	14.14	6.72	.85	.79	.99	-

Table 3.S18

Descriptive Statistics for MDA-MB-231^{Resistant} Apoptosis Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	7.72	4.38	-			
2. MDA-MB-231^{Resistant} Px Treated	3	6.32	2.35	.97	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	9.08	4.09	.97	.81	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	13.63	3.89	.29	.16	.49	-

Table 3.S19

Descriptive Statistics for MDA-MB-231^{Sensitive} Apoptosis Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	6.19	1.83	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	6.34	0.98	1	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	10.04	4.01	.53	.53	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	12.18	0.54	.06	.008**	.80	-

** $p < .01$

Table 3.S20

Descriptive Statistics for MDA-MB-231^{Resistant} Short-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	1.11	0.09	-			
2. MDA-MB-231^{Resistant} Px Treated	3	1.06	0.09	.90	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	0.99	0.02	.31	.66	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	0.84	0.10	.015*	.039*	.20	-

** $p < .05$*

Table 3.S21

Descriptive Statistics for MDA-MB-231^{Sensitive} Short-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	1.03	0.10	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	0.93	0.16	.70	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	1.00	0.07	.99	.87	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	0.89	0.07	.45	.97	.64	-

Table 3.S22

Descriptive Statistics for MDA-MB-231^{Resistant} Long-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	0.91	0.09	-			
2. MDA-MB-231^{Resistant} Px Treated	3	0.39	0.08	.002**	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	0.91	0.15	1	.002**	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	0.22	0.11	.001***	.35	.001***	-

** $p < .01$, *** $p < .001$

Table 3.S23

Descriptive Statistics for MDA-MB-231^{Sensitive} Long-Term Proliferation Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	0.80	0.13	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	0.32	0.07	.005**	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	0.83	0.15	.99	.003**	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	0.37	0.11	.008**	.97	.005**	-

** $p < .01$

Table 3.S24

Descriptive Statistics for MDA-MB-231^{Resistant} Short-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	5.62	2.74	-			
2. MDA-MB-231^{Resistant} Px Treated	3	7.35	3.31	.87	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	7.24	3.46	.89	1	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	2.44	0.90	.54	.22	.23	-

Table 3.S25

Descriptive Statistics for MDA-MB-231^{Sensitive} Short-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	6.22	2.06	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	4.98	3.53	.90	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	7.09	1.04	.96	.66	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	2.90	1.39	.33	.67	.17	-

Table 3.S26

Descriptive Statistics for MDA-MB-231^{Resistant} Long-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	43.77	13.78	-			
2. MDA-MB-231^{Resistant} Px Treated	3	7.80	4.78	.003**	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	28.49	8.28	.20	.065	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	3.44	1.15	.002**	.92	.027*	-

* $p < .05$, ** $p < .01$

Table 3.S27

Descriptive Statistics for MDA-MB-231^{Sensitive} Long-Term Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	32.49	4.43	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	7.87	4.47	.002**	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	31.85	7.12	1	.002**	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	6.11	4.38	.001***	.98	.001***	-

** $p < .01$, *** $p < .001$

Table 3.S28

Descriptive Statistics for MDA-MB-231^{Resistant} Short-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	97.50	1.98	-			
2. MDA-MB-231^{Resistant} Px Treated	3	88.11	5.72	.36	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	93.08	5.71	.84	.79	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	67.89	10.22	.003**	.023*	.007**	-

* $p < .05$, ** $p < .01$

Table 3.S29

Descriptive Statistics for MDA-MB-231^{Sensitive} Short-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	97.75	1.64	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	85.08	13.23	.51	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	95.00	4.34	.75	.66	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	84.42	8.67	.27	1	.39	-

Table 3.S30

Descriptive Statistics for MDA-MB-231^{Resistant} Long-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant}	3	98.83	0.38	-			
2. MDA-MB-231^{Resistant} Px Treated	3	60.58	7.13	.028*	-		
3. MDA-MB-231^{Resistant} KIF14 KD	3	98.00	1.52	.80	.024*	-	
4. MDA-MB-231^{Resistant} KIF14 KD Px Treated	3	53.58	4.63	.008**	.56	.005**	-

* $p < .05$, ** $p < .01$

Table 3.S31

Descriptive Statistics for MDA-MB-231^{Sensitive} Long-Term Viability Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive}	3	99.33	0.14	-			
2. MDA-MB-231^{Sensitive} Px Treated	3	53.58	9.52	.035*	-		
3. MDA-MB-231^{Sensitive} KIF14 KD	3	98.67	0.38	.21	.036*	-	
4. MDA-MB-231^{Sensitive} KIF14 KD Px Treated	3	57.58	8.06	.030*	.94	.031*	-

* $p < .05$

APPENDIX C: SUPPLEMENTARY MATERIALS FROM CHAPTER 4

Table 4.S1

Descriptive Statistics for MDA-MB-231 Proliferation Assay 0ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.73	0.24	-		
2. MDA-MB-231^{Sensitive}	3	0.59	0.24	0.91	-	
3.MDA-MB-231 Ctrl	3	0.66	0.24	0.98	0.99	-

Table 4.S2

Descriptive Statistics for MDA-MB-231 Proliferation Assay 12.5ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.72	0.20	-		
2. MDA-MB-231^{Sensitive}	3	0.13	0.08	0.73	-	
3.MDA-MB-231 Ctrl	3	0.69	0.31	0.99	0.83	-

Table 4.S3

Descriptive Statistics for MDA-MB-231 Proliferation Assay 25ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.73	0.26	-		
2. MDA-MB-231^{Sensitive}	3	0.49	0.31	0.71	-	
3.MDA-MB-231 Ctrl	3	0.67	0.29	0.99	0.84	-

Table 4.S4

Descriptive Statistics for MDA-MB-231 Proliferation Assay 50ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.58	0.21	-		
2. MDA-MB-231^{Sensitive}	3	0.20	0.08	0.13	-	
3.MDA-MB-231 Ctrl	3	0.64	0.25	0.98	0.08	-

Table 4.S5

Descriptive Statistics for MDA-MB-231 Proliferation Assay 100ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.29	0.079	-		
2. MDA-MB-231^{Sensitive}	3	0.20	0.12	0.91	-	
3.MDA-MB-231 Ctrl	3	0.66	0.19	0.11	0.043*	-

* $p < .05$

Table 4.S6

Descriptive Statistics for MDA-MB-231 Proliferation Assay 200ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MDA-MB-231^{Resistant}	3	0.25	0.06	-		
2. MDA-MB-231^{Sensitive}	3	0.29	0.17	0.98	-	
3.MDA-MB-231 Ctrl	3	0.59	0.22	0.12	0.20	-

Table 4.S7

Descriptive Statistics for MCF7 Proliferation Assay 0ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. $MCF7^{Resistant}$	3	0.86	0.68	-		
2. $MCF7^{Sensitive}$	3	1.05	0.10	0.96	-	
3.MCF7 Ctrl	3	1.36	0.18	0.67	0.20	-

Table 4.S8

Descriptive Statistics for MCF7 Proliferation Assay 12.5ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. $MCF7^{Resistant}$	3	0.75	0.62	-		
2. $MCF7^{Sensitive}$	3	1.30	0.28	0.59	-	
3.MCF7 Ctrl	3	1.22	0.18	0.66	0.97	-

Table 4.S9

Descriptive Statistics for MCF7 Proliferation Assay 25ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MCF7^{Resistant}	3	0.72	0.59	-		
2. MCF7^{Sensitive}	3	1.26	0.22	0.30	-	
3.MCF7 Ctrl	3	1.21	0.21	0.37	0.99	-

Table 4.S10

Descriptive Statistics for MCF7 Proliferation Assay 50ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. MCF7^{Resistant}	3	0.50	0.29	-		
2. MCF7^{Sensitive}	3	1.1.	0.15	0.019*	-	
3.MCF7 Ctrl	3	1.15	0.16	0.017*	1.0	-

* $p < .05$

Table 4.S11

Descriptive Statistics for MCF7 Proliferation Assay 100ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. $MCF7^{Resistant}$	3	0.36	0.26	-		
2. $MCF7^{Sensitive}$	3	0.83	0.16	0.062	-	
3.MCF7 Ctrl	3	1.12	0.17	0.005**	0.33	-

** $p < .01$

Table 4.S12

Descriptive Statistics for MCF7 Proliferation Assay 200ug/mL EGCG

Treatment	*n*	*M*	*SD*	1	2	3
1. $MCF7^{Resistant}$	3	0.39	0.26	-		
2. $MCF7^{Sensitive}$	3	0.62	0.23	0.58	-	
3.MCF7 Ctrl	3	1.11	0.19	0.013*	0.082	-

* $p < .05$

Table 4.S13

Descriptive Statistics for MDA-MB-231^{Sensitive} Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive} EGCG + Px	3	1.32	0.11	-			
2. MDA-MB-231^{Sensitive} Px	3	2.13	0.56	0.87	-		
3. MDA-MB-231^{Sensitive} EGCG	3	1.92	1.58	0.94	0.99	-	
4. MDA-MB-231^{Sensitive} Ctrl	3	5.91	1.99	0.011*	0.031*	0.023*	-

* $p < .05$

Table 4.S14

Descriptive Statistics for MDA-MB-231^{Resistant} Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant} EGCG + Px	3	1.31	0.48	-			
2. MDA-MB-231^{Resistant} Px	3	2.20	0.21	0.18	-		
3. MDA-MB-231^{Resistant} EGCG	3	1.22	0.45	1.0	0.13	-	
4. MDA-MB-231^{Resistant} Ctrl	3	4.26	3.28	0.54	0.73	0.53	-

Table 4.S15

Descriptive Statistics for MCF7^{Sensitive} Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MCF7^{Sensitive} EGCG + Px	3	1.51	0.48	-			
2. MCF7^{Sensitive} Px	3	2.28	1.01	0.64	-		
3. MCF7^{Sensitive} EGCG	3	1.24	0.19	0.97	0.42	-	
4. MCF7^{Sensitive} Ctrl	3	2.22	1.08	0.69	1.0	0.46	-

Table 4.S16

Descriptive Statistics for MCF7^{Resistant} Relative Population Doubling Assay

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MCF7^{Resistant} EGCG + Px	3	1.20	0.68	-			
2. MCF7^{Resistant} Px	3	1.84	1.13	0.71	-		
3. MCF7^{Resistant} EGCG	3	1.13	0.17	1.0	0.65	-	
4. MCF7^{Resistant} Ctrl	3	1.86	0.61	0.70	1.0	0.64	-

Table 4.S17

Descriptive Statistics for MDA-MB-231^{Sensitive} Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Sensitive} EGCG + Px	3	52.50	8.95	-			
2. MDA-MB-231^{Sensitive} Px	3	81.50	6.91	0.004**	-		
3. MDA-MB-231^{Sensitive} EGCG	3	50.03	7.32	0.97	0.002**	-	
4. MDA-MB-231^{Sensitive} Ctrl	3	93.33	3.41	0.000***	0.24	0.000***	-

** $p < .01$, *** $p < .001$

Table 4.S18

Descriptive Statistics for MDA-MB-231^{Resistant} Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MDA-MB-231^{Resistant} EGCG + Px	3	50.53	10.86	-			
2. MDA-MB-231^{Resistant} Px	3	76.58	6.11	0.024**	-		
3. MDA-MB-231^{Resistant} EGCG	3	58.11	10.77	0.71	0.11	-	
4. MDA-MB-231^{Resistant} Ctrl	3	93.53	4.67	0.001***	0.15	0.004**	-

** $p < .01$, *** $p < .001$

Table 4.S19

Descriptive Statistics for MCF7^{Sensitive} Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MCF7^{Sensitive} EGCG + Px	3	57.92	12.41	-			
2. MCF7^{Sensitive} Px	3	87.75	13.00	0.046*	-		
3. MCF7^{Sensitive} EGCG	3	58.67	8.03	1	0.051	-	
4. MCF7^{Sensitive} Ctrl	3	88.42	10.61	0.041*	1	0.046*	-

* $p < .05$

Table 4.S20

Descriptive Statistics for MCF7^{Resistant} Viability

Treatment	*n*	*M*	*SD*	1	2	3	4
1. MCF7^{Resistant} EGCG + Px	3	55.33	19.50	-			
2. MCF7^{Resistant} Px	3	76.00	9.50	0.43	-		
3. MCF7^{Resistant} EGCG	3	58.75	17.54	0.99	0.57	-	
4. MCF7^{Resistant} Ctrl	3	89.50	14.94	0.11	0.73	0.16	-