DECREASED EXPRESSION OF MICROTUBULES IN CONFINED MICROENVIRONMENTS LEADS TO A REDUCED EFFECT OF PACLITAXEL ON BREAST CANCER CELLS

by

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Abstract

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Despite the current medical options available to patients, cancer cells frequently remain after treatment and metastasize. This is particularly significant because secondary cancers account for 90% of cancer-related fatalities. Using standard lithography techniques to make PDMS-based microchannel devices, different microenvironments for studying metastasizing (MDA-MB-231) and non-metastasizing (MCF-7) breast cancer cells were created. While the cells were in the narrow and wide microchannels, effects of the anti-cancer drug Paclitaxel at different concentrations were examined and compared with the traditional Transwell assay. Paclitaxel stabilizes microtubules inside the cell and restricts replication, making it an ideal drug to target for quickly dividing cancer cells. Based on tests quantifying migration and viability of the cancer cells, it was found that Paclitaxel's effect significantly decreases when cells are in narrow microchannels, which is a similar environment to when they are migrating inside the body. A powerful finding included the decreased expression of microtubules in the confined microchannels, indicating a potential reason why migrating cells are not as susceptible to common chemotherapy drugs.

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Chapter 1

Introduction

Breast Cancer Diagnosis

One of the most important factors in the successful long term survival of breast cancer patients is early detection[1]. The most common method of detection for breast cancer is X-ray mammography, followed by magnetic resonance imaging (MRI) and ultrasound[2, 3]. There are, however, limitations with each of these techniques including a limited ability to detect cancerous masses in women with dense breast tissue as well as the inability to distinguish between benign and malignant masses[4]. The result of the latter limitation is that women must undergo an emotionally difficult and physically painful biopsy that could potentially be unnecessary. Still, breast cancer screenings have lead to a reduction in fatal cases and are considered an important part of womens' health due to the fact that they can contribute to early detection and therefore a decreased risk of metastasis[5].

Breast Cancer Stages and Histology Grades

Cancers can be categorized in several different ways depending on the information that is being gathered. Clinically, physicians designate cancer into stages based on how advanced it is. The stage is determined prior to treatment and is based on physical exams, images such as those from a mammogram, or a biopsy of the tumor tissue[6]. There are five stages of breast cancer designated as stage 0, I, II, III, and IV; stages I and II have two sub-stages while stage III has three[7]. Breast cancer is placed into a stage or sub-stage based on the presence and size of a tumor, if cancer cells are found in local lymph nodes, and whether it has metastasized to other parts of the body[6, 7]. For example, stage 0 is defined as carcinoma *in situ* with no lymph node involvement or metastasis to other parts of the body, and stage IV breast cancer is a tumor of any size

that has metastasized. The goal of the cancer staging system is to help physicians with a prognosis as well as optimal treatment strategies[6].

Another way to classify cancers is through histological grading. This is a way to measure the aggressiveness of the tumor using the Nottingham Grading System[8]. The grades are determined by three features of the cancer cells: the difference between cancer cells and normal tissue, nuclear pleomorphism, and how quickly the cells divide[9]. Based on the characteristics of the cancer cells, each of these three features is assigned a score of one through three. Those scores are summed to get an overall score. The sums of 3-5, 6-7, and 8-9 give histology grades of 1, 2, and 3, respectively; grade 1 represents a slow growing cancer while grade 3 is fast growing[8]. Although histological grades have been shown to correlate with cancer prognosis, it is not as widely used as the staging[10].

Cancer Treatment Options

It is important to begin treatment as quickly as possible once a cancer diagnosis has been made. All of the treatments options should be discussed so that the wishes of the patient are met. Surgery, radiation, and chemotherapy are three of the most commonly used therapies, and combinations of these with others are frequently implemented.

Surgery

The goal of surgery is to remove as many of the cancerous cells as possible and is generally considered necessary for a positive prognosis[11]. One way to do this is by a removal of the tumor while the surrounding, healthy tissue is left in place. This is referred to as breast conserving and is usually preferred by patients due to aesthetic as well as emotional reasons[12]. Alternatively, a more aggressive method is to perform a mastectomy, which is the removal of all breast tissue[13]. In order to determine which is the

optimal surgical method, the location and aggressiveness of the cells should be taken into account.

Radiation

Radiation is performed on about half of cancers that are diagnosed[14]. Radiation affects all cells, but healthy cells are more stable and therefore repair themselves more easily than cancer cells[15]. While the use of radiation combined with other treatments has been shown to reduce the cancer recurrence rate after five years[16], about a third of all women who have surgery do not have radiation because of its cost and side effects[17].

Chemotherapy

Chemotherapy involves the use of chemical agents to treat cancer. Post-operative chemotherapy is used to inhibit cancer cells that have separated from the primary tumor and would not be targeted by surgery or radiation occurring in the breast tissue[18]. It can also be performed pre-operatively to reduce the size of the primary tumor so that breast conserving surgery could be performed instead of a full mastectomy[19, 20]. Additionally, the use of two anti-cancer agents, poly-chemotherapy, has proven to be beneficial in the treatment of cancer[21].

Paclitaxel

Paclitaxel (Taxol) is a chemotherapy agent that is used to treat several types of cancer including breast cancer. Its mechanism involves stabilizing microtubules, which are made of α-tubulin and β-tublin[22]. By hindering the microtubules, Taxol inhibits the proliferation as well as migratory processes of cells[23]. Recently, a study has reported that Taxol creates chromosome missegregation, which does not allow the cell to divide and eventually causes cell death[24].

Cancer Treatments by Stage

In order to determine the best treatment for a patient, physicians examine several prognostic markers. The goal is to balance the risk of treatment against the potential positive response of the body. The location, size, and histological grade of the tumor are taken into account[25]. One of the biggest considerations, however, is the stage of the cancer at the time of diagnosis. It is important to remember that all patients and their cancers are different, and even people with similar cancers can be treated using different methods.

Stage 0

The classification of stage 0, *in situ*, breast cancer depends on its location; it can either be found in a lobule or duct. Lobular carcinomas generally do not progress to become invasive cancer, so regular monitoring and appointments with a physician are condsidered acceptable treatments to make sure the cell growth remains within an acceptable range[11]. Alternatively, ductal carcinoma can invade surrounding tissue. Thus, the treatment is generally breast conserving surgery followed by radiation[11].

Stage I and II

Stages I and II are early stage cancer but are deemed invasive[26]. Usually, surgery, radiation, and sometimes chemotherapy are ustilized in treating cancers at these stages[11]. Mastectomies were the standard surgical method for early stage cancers, but this trend is deacreasing as breast conserving surgery has shown to be sufficient in treating these cancers and improving survival rates[11].

Stage III

When a cancer is locally advanced, it is categorized as stage III. Locally advanced can mean several different things including a tumor that has extended into the chest wall or one in which local lymph nodes contain cancerous cells[7]. Due to the

advanced nature of this cancer, treatment generally begins with pre-operative chemotherapy[20]. Depending on how the body responds to the chemotherapy, the physician and patient will decide if a mastectomy is necessary or if breast conserving surgery will suffice in improving the prognisis. After surgery, radiation and chemotherapy are often the next steps in treatment[27, 28].

Stage IV

In stage IV cancer, the cells have metastasized, or migrated to different parts of the body. At this point in the disease, treatments are used as a way to make the patient as confortable as possible and prolong life with the disease as opposed to cure the cancer[29]. Surgery to remove the primary tumor is rare because survival time is usually dependent on the secondary cancer, so removing the original cancer mass is considered an unnecessary procedure[30]. Recently, however, the potential benefit in removing the primary tumor has been shown in terms of increased survival times[31, 32]. Furthermore, physicians or patients may make the decision to undergo surgery if they are experiencing pain or other complications at the primary tumor site[30].

Breast Cancer Progression

Breast Cancer Development

Despite the previously stated medical options, cancer continues to be the leading causes of death in the developed world with breast cancer being the second leading cause of death for women[33]. Breast cancer develops through gene mutations that lead to the uncontrolled proliferation of cells[34]. It has been suggested that tumor initiation involves cancer stem cells, which are in relatively low concentration within a tumor; they are capable of differentiating to form a progenitor cell and another cancer stem cell[35]. The progenitor cell can then divide to create clones of itself, thereby forming a tumor while the other cancer stem cell that was formed maintains the cancer's property of immortality.

Breast cancer cells reside in the extracellular matrix (ECM) of the breast tissue, where collagen is the most common protein[25]. During tumor formation, the ECM is first broken down and then remodeled, allowing for an easier pathway for metastasizing cells[25, 34].

Breast Cancer Metastasis

Metastasizing cancer is that which spreads from a primary part of the body to a secondary part of the body and is responsible for 90% of cancer related fatalities[36]. The majority of breast cancer patients diagnosed with the metastatic form of the disease die within two years[37]. Primary breast cancer develops in the breast tissue and can then metastasize and become a secondary cancer in parts of the body such as the lungs, liver, bones, and brain[38]. Metastasis involves the primary cancer cells migrating through the tissue surrounding the tumor and infiltrating the circulatory or lymphatic system[34, 39].

Current Assays

Developing drugs aimed at treating cancer takes many years, and even after development they must be tested in order to demonstrate safety and effectiveness. One testing requirement for a drug such as Taxol is that it reduce the migration of cancer cells[40]. In order to test this, various concentrations of the drug can be introduced to cancer cells. A successful drug should give a dose-dependent response meaning that larger concentrations of the drug should have more of an effect, for example reducing cell migration tendency, than smaller concentrations[40]. A number of different assays exist that are able to compare the migration of cells in the presence of anti-cancer drugs, but some have limitations[40].

Boyden Chamber/ Transwell Assay

A common test used to quantify the efficacy of anti-cancer agents is the Boyden chamber/ Transwell assay (described throughout the rest of the paper as the Transwell assay). Here, cells are seeded on top of a membrane containing pores through which

they can migrate[41]. Anti-cancer agents are then introduced into the assay in different concentrations. The number of cells that migrate through the pores can be isolated and quantified to determine if the specific drug is successful at slowing cell migration. This assay is limited in the fact that individual cells cannot be tracked or seen as they migrate[42]. Addititionally, this assay is considered 2D because the cells are in an open air environment, which is not similar to *in vivo* conditions.

Microfluidics

Microfluidic-based devices have been increasing in popularity and complexity over the last two decades and have demonstrated great promise in testing anti-cancer agents[43]. A common method of fabrication is through the use of polydimethylsiloxane (PDMS) and soft-lithography techniques to create microchannels within the device[44]. The microchannels can be modified in height, width, and length to meet a specific need; the dimensions are usually on the micron scale. The microchannels are a controlled 3D environment that are similar to what migrating cancer cells would encounter *in vivo,* causing them to exhibit the same morphology and biochemical properties as cells migrating through the body[45]. In comparison with the Transwell assay, this device does allow single cells to be monitored over long time periods.

Objectives of This Research

Cancer remains one of the most fatal diseases in the world even with the substantial amount of therapies available for treatment. It is the goal of this work to explore the potential reasons behind this. Here, the efficacy of Taxol is investigated on cells in various microenvironments including narrow and wide microchannels and 2D surfaces. The effect on cell migration and viability are determined, and the reasons behind and implications of these results are discussed.

Chapter 2

Materials and Methods

Device Design

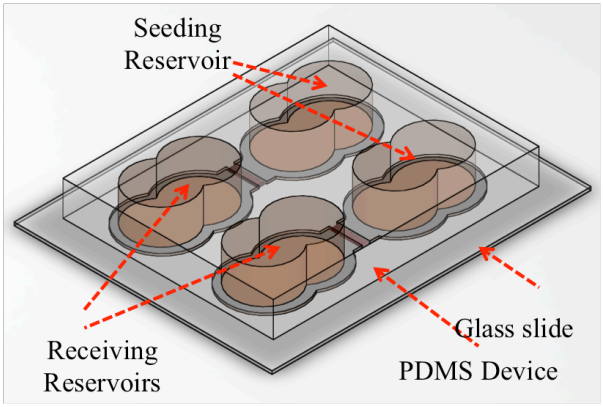
Two different PDMS-based microchannel devices were used in these experiments. The Cell Migration Assay (CMA) device and the Flower device both contained microchannels that were either 5 by 5μm (denoted 5_5) or 15 by 15μm (denoted 15_15) in width and height. The rationale behind using these dimensions was to create a 3D microenvironment through which the cells could migrate. While both dimensions are considered 3D, the 5_5 microchannels are herein considered narrow microchannels, and the 15_15 are considered wide microchannels.

Cell Migration Assay Device

The first PDMS device used was the Cell Migration Assay (CMA) device. It had twenty separate units, each with one cell seeding reservoir and one cell receiving reservoir (Figure 2-1). Connecting the two reservoirs was one microchannel array containing microchannels that were 400μm in length and either 5_5 or 15_15 in width and height.

This device had the benefit of reducing the time spent for each experiment. Because the device contained twenty separate units, more criteria could be tested at one time. This helped to reduce the total number of trials for each experiment.

(A)



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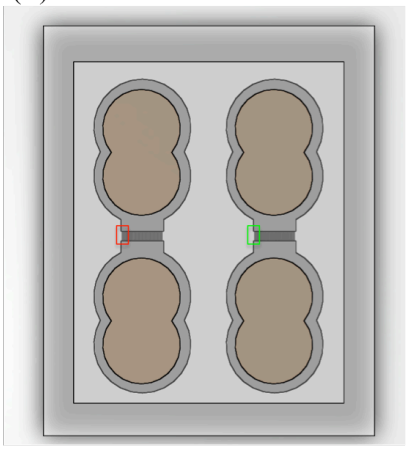


(C)

Microchannel Type	Cross section Width x Height	Length
15_15	15µm x 15µm	400µm
5_5	5µm x 5µm	400µm

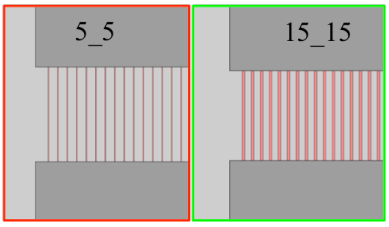


Figure 2-1 The Microfluidic Device for Migration Study. (A) Three dimensional schematic of the PDMS device used. (B) Top view of the device with different types of microchannels. (C) Details of 5_5 and 15_15 microchannels.

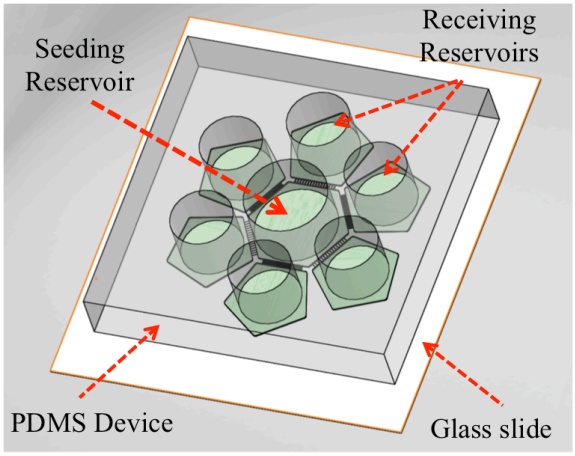
Flower Device

The second PDMS device used to culture cells for these studies was the Flower device. It consisted of one central reservoir, where cells were seeded, with six surrounding satellite reservoirs. Connecting the central to the satellite reservoirs were arrays of microchannels that were 530µm long. The width and height of each microchannel array alternated between either 5_5 or 15_15 for each satellite reservoir so that the central reservoir had three satellite reservoirs connected by 5_5 microchannels and the other three satellite reservoirs connected by 15_15 microchannels (Figure 2-2).

The benefit of this device was that three different conditions (5_5 microchannels, 15_15 microchannels, and a 2D area (the central reservoir)) could monitored at the same

time and under the exact same conditions. This helped to reduce batch-to-batch inconsistencies between different experimental trials.

(A)



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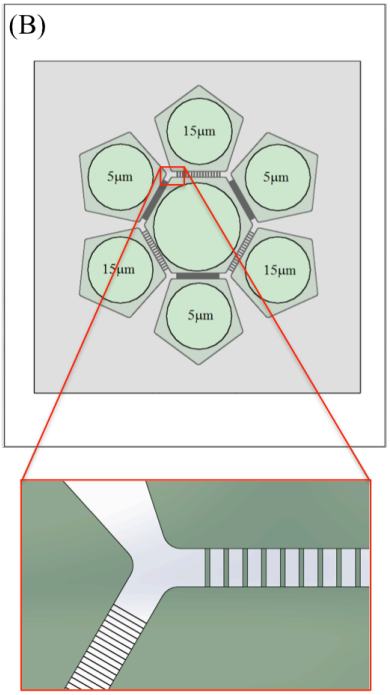

(C)

Microchannel Type	Cross section Width x Height	Length
15µm	15µm x 15µm	530µm
5µm	5µm x 5µm	530µm

Figure 2-2 The Microfluidic Flower Device for Quantifying Microtubule Expression and Cell Viability. (A) Three dimensional schematic of the PDMS device used. (B) Top view of the device with alternating arrays of 5_5 and 15_15 microchannels. (C) Details of 5_5 and 15_15 microchannels.

Fabrication of Devices

Silicon wafers with microchannel patterns were fabricated by using SU8 photoresists (Microchem) and conducting standard photolithography procedures. All of the devices were prepared by mixing PDMS (Dow Corning) with a curing agent in a 10:1 ratio (v/v) and pouring the mixture onto the silicon wafer. After seven minutes on a 150° C hotplate, the devices were cut and peeled off of the wafer. After a biopsy punch was used to create the reservoir inlets, the devices were cleaned and sterilized in 70% ethanol for

thirty minutes. They were then air plasma treated for 10 minutes (PSD Pro Series, Novascan) and assembled on precut coverglass.

Cell Lines and Culture

The breast cancer cell lines MDA-MB-231 and MCF-7 were obtained from University of Texas Southwestern Medical Center at Dallas. These cells were maintained in Dulbecco's Modified Eagle's Medium/F-12 medium with 10% fetal bovine serum (FBS), which was renewed as necessary, indicated by a color change of the medium, during cell culture. The devices were incubated at a temperature of 37˚C and 5% CO_2; if fixing was necessary, 4% paraformaldehyde was used.

Substrate Coating

After assembly, the devices were coated with Collagen Type 1. To produce a collagen coating of 50µg/ml, 140µl of 3.67 mg/ml Collagen Type 1 (BD Biosciences) was mixed with 9.86 ml sterilized 0.02 N acetic acid. Immediately, the mixture was introduced into the devices and incubated at 37˚ C overnight. The devices were washed three times with sterilized phosphate buffer solution (PBS) prior to cell seeding.

Quantitative Comparison of Breast Cancer Cell Migration in Microchannels vs Boyden Chamber/ Transwell Assay

Cell Migration Assay Device

In order to test the response of migrating cancer cells to anti-cancer drugs such as Paclitaxel (Taxol) in different microenvironments, both MDA-MB-231 and MCF-7 cells were cultured in both the 5_5 and 15_15 CMA devices. These results were compared with the more conventional methods for studying migration, the Boyden chamber/Transwell assay.

When using the 5_5 devices, $20x10^3$ cells in a seeding volume of 10µl were seeded into each of the twenty units of the devices. $10x10^3$ cells in a seeding volume of

10µl were seeded when using the 15_15 devices. The cells were followed until a sufficient number migrated into the microchannels. Five different concentrations of Taxol (0, 1nM, 10nM, 100nM, 1µM) in fresh medium were then introduced to the various units in the device. Three and eight hours after the introduction of the drug, pictures of the cells' movement in the microchannels were taken. Using ImageJ, the displacement of the cells ($n \geq 20$ cells/concentration) for each condition was measured and the cell migration speed in µm/hr was calculated.

Boyden Chamber/ Transwell Assay

Transwell assays were run by seeding $10x10^3$ MDA-MB-231 and MCF-7 cells separately on top of the membrane (n=150/cell line). After 24 hours, five different concentrations of Taxol (0, 1nM, 10nM, 100nM, 1µM) in fresh medium were introduced. The cells were allowed to migrate for 48 hours before they were isolated, stained with DAPI, and quantified (n=30 trials/concentration).

Quantification of Microtubule Expression in Three Different Physical Confinements

Taxol is a microtubule targeting drug, so the next experiment's purpose was to quantify the difference in expression of microtubules in various microenvironment conditions. For this study, $60x10^3$ of either MDA-MB-231 or MCF-7 cells in a volume of 60µl were seeded in the central punch of the Flower device. The cells were seeded as to avoid creating a bias in cell distribution. When a sufficient number of cells had migrated into the microchannels, they were stained with DAPI (Invitrogen) as well as for F-actin (phalloidin, Cytoskeleton Inc.) and either alpha tubulin (Thermo Scientific) or beta 3 tubulin. Pictures of the cells in the three different conditions (i.e. 5_5, 15_15, and 2D) were taken using a fluorescent microscope (n=10 cells/condition). For consistent analysis of the data, the exposure time of the microscope was kept constant at 10ms and 200ms

when imaging DAPI and alpha or beta tubulin, respectively. Image J was used to quantify the intensity of the tubulin; this data was then graphed using Microsoft Excel.

Quantitative Comparison of Breast Cancer Cell Viability in Three Different Physical Confinements

The survival rate of breast cancer cells to Taxol was examined with the Flower device. In this case, $60x10^3$ cells with a volume of 60µl were seeded into the central punch. When a sufficient number of cells had migrated into the microchannels, the devices were treated with 100nM Taxol in fresh medium or maintained in fresh, Taxol-free medium as a control. After, 48 hours, the viability of the cells was determined using Propidium Iodide/Fluorescein Diacetate (PI/FDA) staining. The number of alive and dead cells under the three different conditions (i.e. 5_5, 15_15, and 2D) was determined, and the percentage was calculated and graphed using Microsoft Excel.

Chapter 3

Results and Discussion

The Efficacy of Taxol is Reduced in Narrow Microchannels

In this study, the migratory tendencies of metastasizing and non-metastasizing breast cancer cells in different conditions were compared. The tendencies of all of the cells were normalized so that the cells with no drug, which acted as the control, were migrating at 100%. The migration tendency of the cells in the presence of different concentrations of Taxol was then reported as a percentage of the control migration tendency.

In the 15_15 microchannels, the MDA-MB-231 cells migrated slower than when they were located in the 5_5 microchannels for all concentrations of Taxol. Also, the cells used in the Transwell assay migrated slower than both the 5_5 and 15_15 microchannels for concentrations above 10nM (Figure 3-1). A one-way analysis of variance (ANOVA) was run between the 5_5, 15_15, and Transwell results for each of the five concentrations. The p-values were less that 0.05 for all concentrations except the control, so Tukey-Kramer tests were run to determine individual significance. Above 10nM, all three values were significantly different.

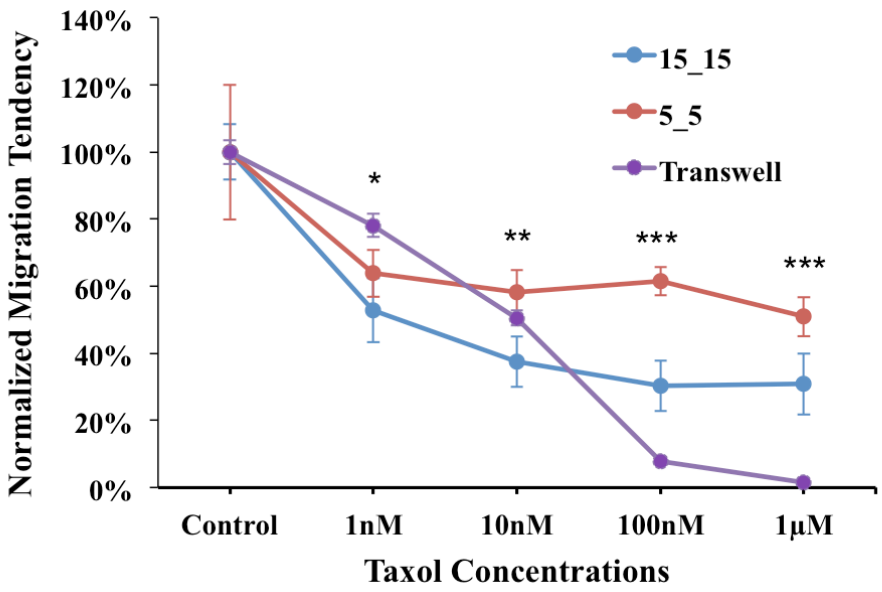

Figure 3-1 Migration Response of MDA-MB-231 to Taxol in Various Microenvironments. Results are presented as the averages ± SEM. *P<0.05 compares the migration tendency between the Transwell assay and 15_15 microchannels. **P<0.05 compares the migration tendency between the 5_5 and 15_15 microchannels. ***P<0.05 compares the migration tendency between 15_15 and Transwell assay, 5_5 and Transwell assay, and 15_15 and 5_5 microchannels. For the Transwell devices, n = 30. For the microchannels, n ≥ 20 cells/cell line.

The MCF-7 cells are non-metastasizing cells and therefore migrate very slowly. Due to this, only a fraction of the cells needed for analysis were able to migrate into the narrow microchannels. Thus, the migration tendency of MCF-7 cells in response to Taxol was quantified for 15_15 and the Transwell assay only. The migration tendencies were similar for both of these microenvironments throughout the range of concentrations (Figure 3-2). Unpaired student T-Tests were run to compare the migration tendency of

the cells in the 15_15 and Transwell assay for each concentration, and none of the values showed significant difference.

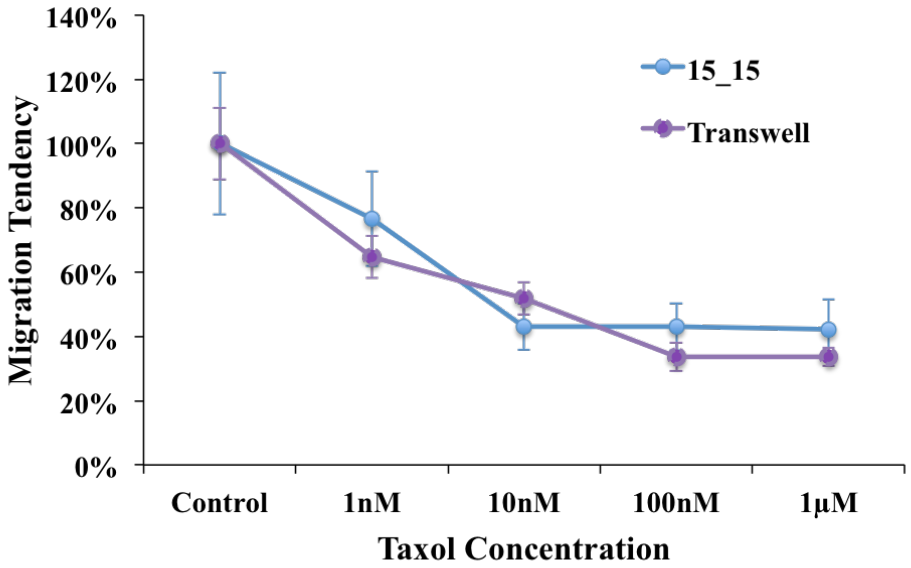

Figure 3-2 Migration Response of MCF-7 to Taxol in Various Microenvironments. Results are presented as the averages ± SEM. For the Transwell devices, n = 30; for the microchannels, n ≥ 20 cells/cell line.

There are many implications to these findings. 2D assays such as the Transwell assay are traditionally used to test the efficacy of anti-cancer drugs. In order to be considered effective, a drug that targets for microtubules as Taxol does, would need to show a reduction the migration tendency of the cells. From the results, the Transwell assay shows the significant reduction of migration tendency of MDA-MB-231 at increasing concentrations of Taxol; at 100nM the cells migrate at only 1% of their speed in the cases with no drug. However in the 3D environment of the narrow microchannels, the overall decrease in migration tendency is reduced. The cells continue to move more quickly in the 15_15 and especially in the 5_5 microchannels with the migration speed

still 30% and 62%, respectively, of the migration tendency with no drug when measured at 100nM.

While both the 15_15 and 5_5 are considered 3D microchannels, the 5_5 microchannels have the cells surrounded on all sides, which makes this microenvironment more similar to the one where the cancer cells migrate when inside the body. Thus, it is important to take these results into consideration when testing anti-cancer agents. The 2D assays currently used show a more dramatic response to the drugs by the cells, but the results from the 5_5 microchannels show this may not be the case when the drugs interact with cells *in vivo*. It can be assumed that there is an adequate amount of Taxol within each microchannel to have an effect on the cell even in the narrow microchannels. This is because the drug is added to fresh medium and then introduced to the devices; it is known that medium fills the microchannels as soon as it is introduced to the devices, so the drug in solution should enter the microchannels as well. Thus, the results presented here are not a case of lack of Taxol to affect the cells in the 5_5 microchannels, but instead a reduced potency of Taxol on those cells.

Microtubule Expression is Reduced in Narrow Microchannels

In the previous migration experiment, the cells located in 5_5 microchannels had less of a response to Taxol at various concentrations. This could be explained if the cells in narrow microchannels had a lower expression of microtubules than those in the wide microchannels or in the 2D environment. To test this, both MDA-MB-231 and MCF-7 cells within 15_15 and 5_5 microchannels as well as those in the 2D environment were stained for alpha and beta III tubulin. The results showed that the expression of both types of tubulin decreased for both cell lines when they were located within the narrow microchannels (Figures 3-3-3-6).

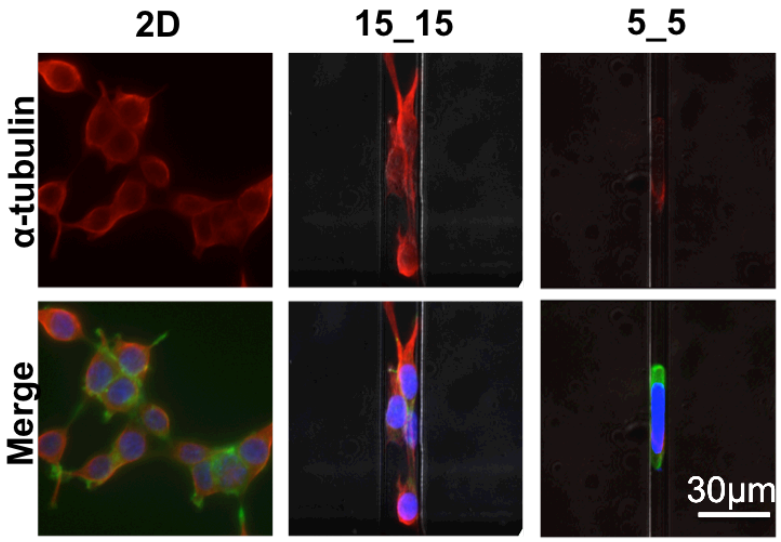

Figure 3-3 Reduction of α-tubulin Expression of MB-231 in Narrow Microchannels (5_5). Fluorescent images of α-tubulin expression (red, top panel) and merged images (F-actin expression: green, DAPI: blue, bottom panel).

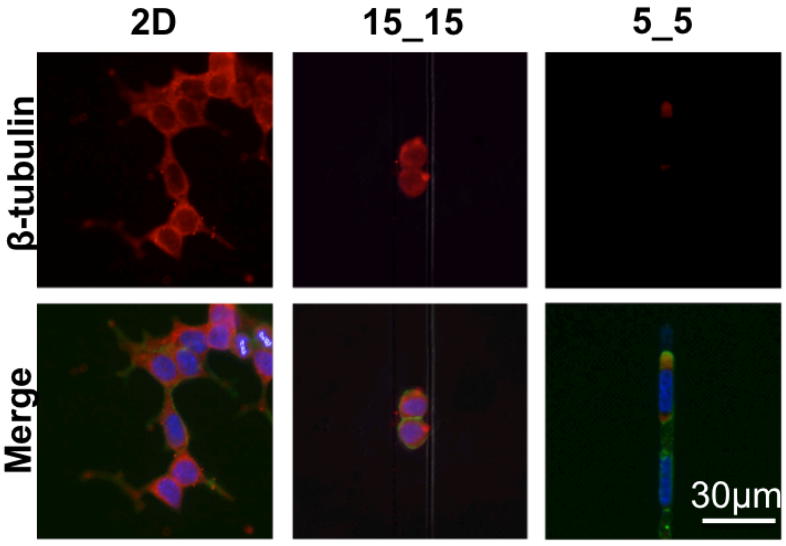

Figure 3-4 Reduction of β-tubulin Expression of MB-231 in Narrow Microchannels (5_5). Fluorescent images of β-tubulin expression (red, top panel) and merged images (F-actin expression: green, DAPI: blue, bottom panel).

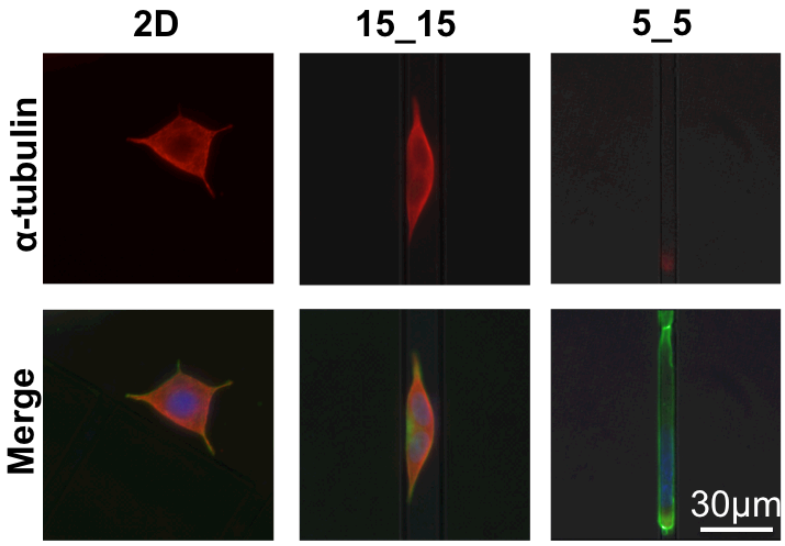

Figure 3-5 Reduction of α-tubulin Expression of MCF-7 in Narrow Microchannels (5_5). Fluorescent images of α-tubulin expression (red, top panel) and merged images (F-actin expression: green, DAPI: blue, bottom panel).

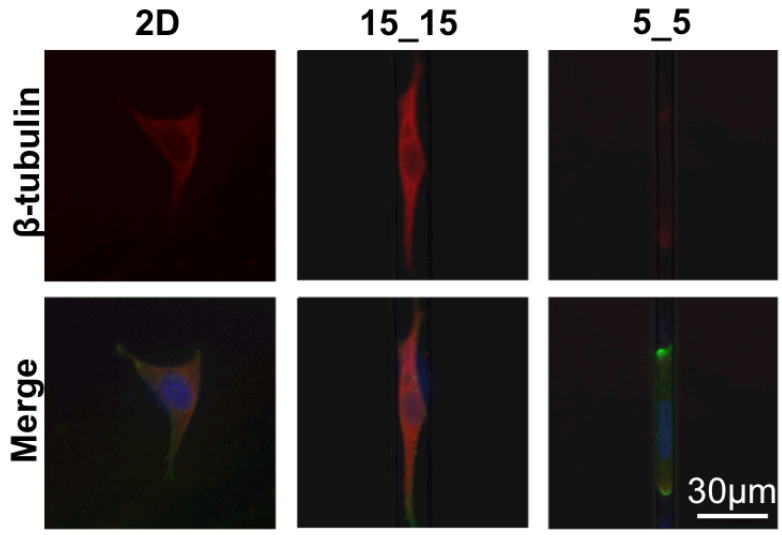

Figure 3-6 Reduction of β-tubulin Expression of MCF-7 in Narrow Microchannels (5_5). Fluorescent images of β-tubulin expression (red, top panel) and merged images (F-actin expression: green, DAPI: blue, bottom panel).

The intensity of the microtubules' fluorescent coloring was quantified and was found to be the highest in the 2D environment for both cell types and tubulin types. Furthermore, the expression of microtubules in the 2D environment was significantly higher than the expression of the cells in the 5_5 microchannels (Figure 3-7). These results show that when cells are migrating inside the body, they reduce the expression of microtubules.

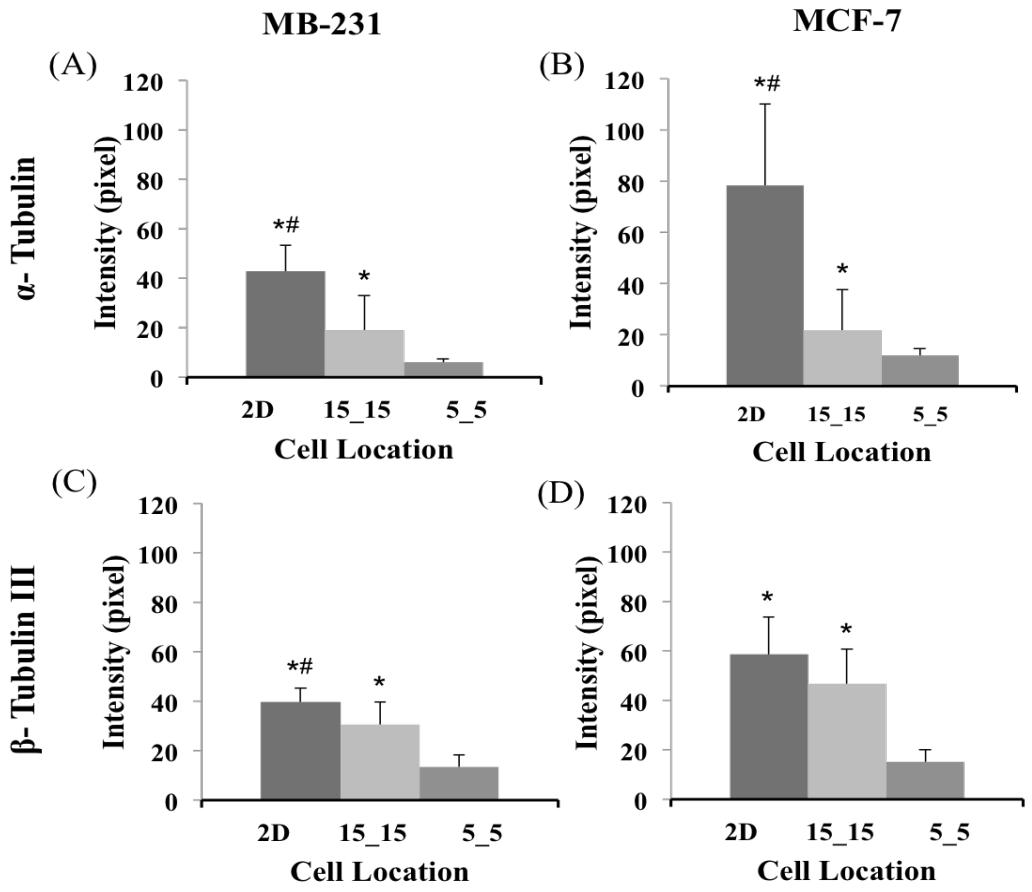

Figure 3-7 Quantification of Intensity of α-tubulin and β-tubulin Expression in 2D and Various Microchannel Environments. Results were presented as the averages ± STDs. *P<0.05 Compare 5_5 microchannels and others. #P<0.05 Compare 15_15 microchannels and others. n ≥ 9 cells/cell line.

Viability of Breast Cancer Cells in the presesnce of Taxol Increases in Narrow Microchannels

100nM was chosen as the concentration used in this experiment because 48 hours at this concentration reduced the viability of the cells to 3% as compared with other concentrations or time points (Figure 3-8 A). This significant decrease in cell viability allowed for the comparison of the viability of the cells in the 15_15 and 5_5 microenvironments.

The metastasizing and non-metastasizing cancer cells located in 5_5 microchannels showed an increased survival rate compared to those in the 15_15 microchannels and 2D environment when exposed to 100nM Taxol (Figure 3-8 B, C). Unpaired student T-tests showed a significant difference between the Taxol treated cells and control cells for the MDA-MB-231 cells in all three microenvironments and in the 2D and 15_15 microchannels for the MCF-7 cells. This result further enhances the hypothesis that cancer cells are less affected by Taxol when they are in narrow microchannels.

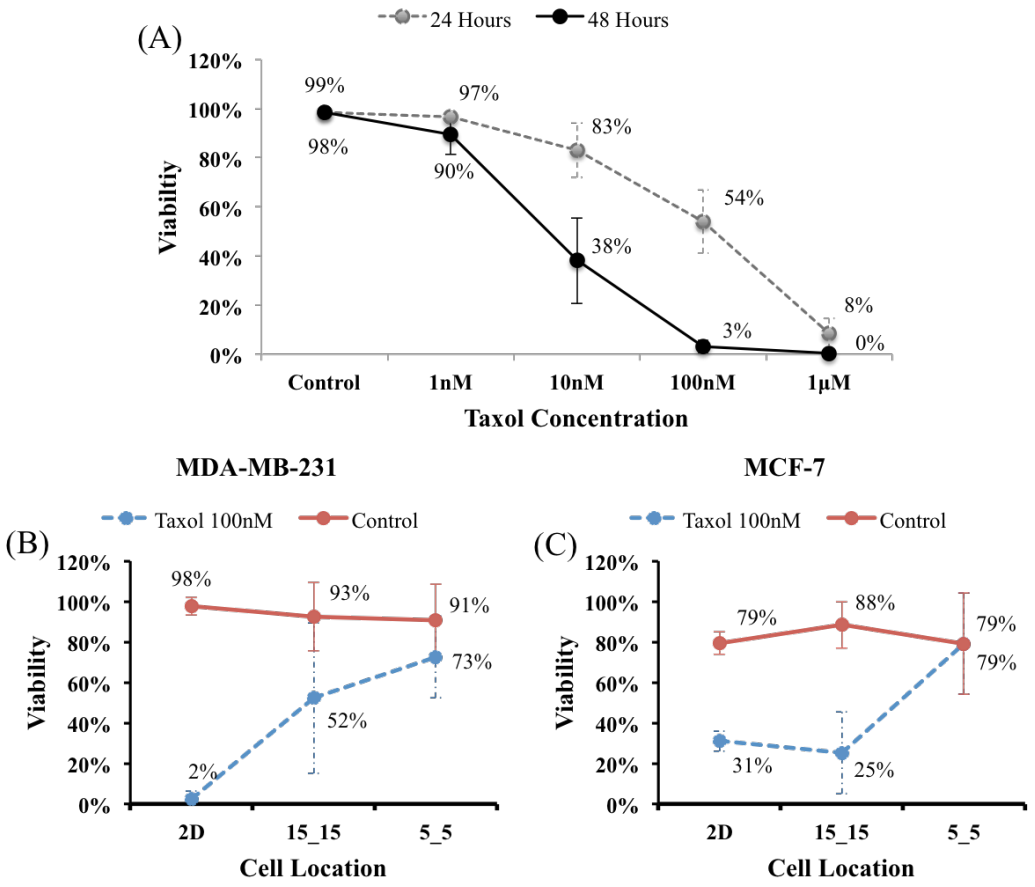

Figure 3-8 Increasing Cell Viability of MDA-MB-231 and MCF-7 in Narrow Microchannels. (A) Effect of Paclitaxel (Taxol) on cell viability of MDA-MB-231 for 24 hours and 48 hours in a 2D environment. (B) Comparison of survived MDA-MB-231 within 2D and 15_15 and 5_5 microchannels. (C) Comparison of survived MCF-7 within 2D and 15_15 and 5_5 microchannels. Results were presented as the averages ± STDs. *P<0.01 Compares the viability of Taxol treated cells vs. the control. n ≥ 10 cells/cell line.

Chapter 4

Closing Remarks

Conclusion

The ability of Taxol to effectively target for cancer cells depends on the microtubule expression of cells. It was shown that traditional 2D assays have the cells in a microenvironment where they highly express microtubules. Therefore the anti-cancer agents can effectively target the cells and reduce their migration and viability. However, when the same agents were tested using 3D microchannel devices with narrow microchannels, the effect was not as pronounced because of a decrease in microtubule expression.

These results highlight the need for testing of anti-cancer agents on cells that are in microenvironments similar to *in vivo* dimensions. The lack of these types of tests could contribute to the reasons that cancer remains one of the most deadly diseases. If the anti-cancer agents target for microtubules, which are not highly expressed when the cells are in confined spaces such as when they are migrating, the drug will not have an effect on those cells.

Future Work

There are many ways to expand these experiments in order to further confirm the results. First, more cell lines could be included to see if the trend of decreased efficacy of anti-cancer agents in narrow microchannels extends to them. There are also more anti-cancer agents that work by the same mechanism, microtubule targeting, which could be used in further tests.

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