Artistic Interpretation of the Significance of Human Breast Cancer Cell Vinculin Distribution in Different Extracellular Matrix Conditions

by
Yu-Tin Hsiao

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biochemistry & Biophysics
(Honors Associate)

Presented June 5, 2018
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AN ABSTRACT OF THE THESIS OF

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Abstract approved:_____

Bo Sun

There are clues that suggest human breast cancer cells use collagen fibers to migrate, and vinculin is a transmembrane protein associated with how cells attach to their environment. Therefore, immunostaining for the vinculin will shed some light in how human breast cancer cells are attaching to their environment. Immunostaining for vinculin has been very difficult in MDA-MB-231 cells, and the procedure that educed the most noise was to fixate and permeabilize the cells simultaneously before treating the cells with the blocking solutions and the antibodies. The more rigid the extracellular matrix (ECM) the more vinculin there are in a human breast cancer cell. The focal adhesion area decreases with decreasing ECM rigidity. The aspect ratio of the focal adhesions does not change with the varying ECM rigidity. The inter-focal adhesions distance does not seem to have much correlation with ECM rigidity. Moreover, an artistic interpretation of the scientific research may shed some light into how to encourage the general public to be interested and engaged in scientific conversations. There is no need for accurate depictions of vinculin staining, but an artistic expression of vinculin staining can be used to create more interest in the topic of human breast cancer cell attachment to different ECM rigidity.

Key Words: Human breast cancer, collagen fibers, vinculin, actin, focal adhesion, artistic interpretation of science, scientific conversation, artistic expression

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Honors Baccalaureate of Science in Biochemistry & Biophysics project of Yu-Tin Hsiao presented on June 5, 2018.

APPROVED:

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Bo Sun, Mentor, representing Physics

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Stephen Hayes, Committee Member, representing Art

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Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Yu-Tin Hsiao, Author

Introduction

Art and Science

Art and science have often influenced each other, and driven each other to a different levels of accomplishment (1). Science and art are very similar in that both artists and scientists have to have three qualities: (1) passionate, (2) creative, and (3) obsessively curious (2). Passion can be defined as the ability to see past the current reality, and create and bring ideas and objects that do not exist yet into the new reality (3). Passionate people have a vision of finding their true purpose, and it often requires "introspection, self-analysis, and written expression." Creativity has several components that often has the following features: bringing new things into existence, possible in any discipline in human activity, and achievable by anyone (4). Curiosity in science is often thought of as a compulsion to understand (5). However, curiosity can also be understood more generally as a radical force that breaks down old ideas and constructs. From this level, it is conceivable how art and science are similar due to the nature of both disciplines are striving to push the known boundaries to create a new reality with new knowledge and perspective. Science is inseparable from art no matter how hard a scientist tries to deny the presence of artistic elements in their work (6). Scientific ideas and results are better understood when visualized, and visualization is done with artistic skills and knowledge. Even if some scientists were able to completely exclude art from their research work, there still exists the fact that scientific knowledge needs to be communicated to the general public in order to grain public support (7). To gain public interest and support, the scientific results needs to be culturally impactful, and scientific training and mindset is not aimed at achieving high cultural impact the way the arts is. Art is able to obtain public interest because of its unpredictable perception and flexibility interpretation, and this nature of art creates a lasting conversation about a particular topic that the art piece touches on.

Human Breast Cancer Cells: The Science

Breast cancer is the most common cancer in women in the world (8). There are more reported cases in the developed countries compared to the developing countries. However, the mortality rates from breast cancer are much higher in developing countries. In the United States, breast cancer is the primary cause of cancer-related deaths in women, with a 5-year survival rate of 23% for patients diagnosed with distant metastases. Currently, the diagnosis of breast cancer is by the use of a mammogram, which is not a very good tool because a mammogram looks at the density of breast cancer cells in a particular sample(8). However, the use of a mammogram to detect the cancers is not very accurate because the progressiveness of breast cancer depends on factors other than the density, such as the type of environment the breast cancer cells are in (9).

To determine the effects of the environment on breast cancer cell migration we can look at the vinculin distribution within human breast cancer (MDA-MB-231) cells (10). Vinculin is a protein that regulates focal adhesion dynamics, which is associated with how cells attach to the extracellular environment (ECM) (11). Analyzing how the cells attach to ECM will give more clues as to how breast cancer cells invade and migrate. The objective of this research project is to discover the optimal conditions for staining for

vinculin, a trans membrane protein that is associated with how the breast cancer cells attach to the ECM, and analyze how focal adhesions vary with different ECM rigidity.

Human Breast Cancer Cells: The Art

People's perception of fine art is often dependent on cultural experiences, which results in art being very subjective and allow multiple interpretations (12). There are many ways to express art such as fine art and photography. The birth of photography has lifted the obligations of portraying an accurate depiction of reality off from fine arts (12). Photography can now be used as both a tool for artistic expression and as medical intervention due to its nature of being able to accurately capture reality (13). Therefore, fine art artists can now have the freedom to explore new ways of expression. Artistic expression has the potential to move, engage, and inspire its viewers (13). Fine art can be focused on how certain reality makes the artist feel in order to depict the significance of the original inspiration (14). The significance of the inspiration manifests in many ways including an artwork's size, color choice, composition, and title selection.

In order to explore this notion of art being an expression of reality and not a depiction of reality, this project aims to draw on scientific images collected for inspiration to create a series of paintings that showcase the significance of the scientific work through size, color, composition, and title selection. The scientific images taken with a confocal microscope are of human breast cancer cells, which are accurate a depiction of the cells, will serve as inspiration for the artworks.

Methods

The science

This study used glass bottom dishes (μ-$^{\text{Dish35 mm, high}}$ Glass Bottom) purchased from ibidi®. Putting in different microliters of collagen gel created the different thicknesses of collagen gels, and the gels had a concentration of 2 microgram per microliter. The MDA-MB-231 cells were grown in 37˚C on different thickness of collagen that were created at room temperature. The cells were fixed and permeabilized simultaneously by mixing the fixative solution (4% formaldehyde in Phosphate-Buffered Saline, PBS, from ThermoFisher Scientific) and the permeabilization solution (0.5% Triton X-100 bought from Thermo Fisher Scientific) in a 1 to 1 ratio, and adding the mixed solution to the cell sample. The cells were incubated at room temperature for 15 minutes with the mixed solution. Then, the cells were washed in PBS for three times two minutes each before adding the blocking solution (3% BSA, fraction V, de-lipidated, New Zealand source, in DPBS bought from ThermoFisher Scientific) to the sample, which was incubated at room temperature for 1.5 hours. After adding the blocking solution, the cells were washed with PBS three times with two minutes each. The primary antibody (Vinculin Antibody (42H89L44), ABfinity™ Rabbit Monoclonal bought from ThermoFisher Scientific) was diluted in blocking solution in a 1:1000 ratio. The primary antibody was incubated with the sample in 4˚C for 24 hours before washing the sample with PBS for 6 times with 5 minutes each wash. The secondary antibody (Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 647 conjugate bought from ThermoFisher Scientific) was diluted in PBS in a 1:1000 ratio, and was incubated with the cells in 4˚C in the dark for 5 hours. The sample was then washed with PBS 6 times with 5 minutes each wash. The cells were then stained for actin, a protein that is attached to the vinculin. The actin stain

serves as a biomarker for vinculin. The actin antibody (ActinGreen™ 488 ReadyProbes® Reagent bought from ThermoFisher Scientific) was diluted in PBS (2 drops for every mL of PBS). The sample was then washed for 3 times with two minutes each wash. The sample was preserved in PBS for imaging. The images were taken with the confocal microscope (Leica DMI4000B). The vinculin and actin were excited with 635mm and 488mm wavelengths respectively.

The Art

The scale of the pieces were chosen to try to comprehend how significant the scientific results have impacted me. All of the piece are on canvas (Artist's Loft) to have a more prominent platform for my piece to be displayed on. The two smaller pieces were chosen because they are just trying to capture the way I feel about the scientific research project before I started to work in the laboratory and during the work in the laboratory. The larger piece was on a larger scale because it was to capture the impact of the scientific results as opposed to the emotional experiences while working. Moreover, the material chosen for these piece was acrylic paint (Pro Art Student Acrylic), to create a thick and opaque texture to signify how the cancer have a strong hold in people's lives. Each of these paintings were created in two parts: background painting and subject painting, to signify the multistep nature of my scientific research. The background was painted with a thick brush to generate the fibrous texture for all the paintings, and the small details are created with thin brushes that can make precise markings throughout all three pieces.

Results and Discussion

The Science

Figures 1A-D shows the images of human breast cancer cells in different ECM rigidity stained for vinculin (shown in red), actin (shown in blue), and computer recognized shapes of vinculin (shown in green). There should be more vinculin attached to more rigid ECM. Figures 1A-D visualizes the amount of focal adhesions to ECM with increasing ECM rigidity, and it seems that the focal adhesions decrease with increasing ECM rigidity.

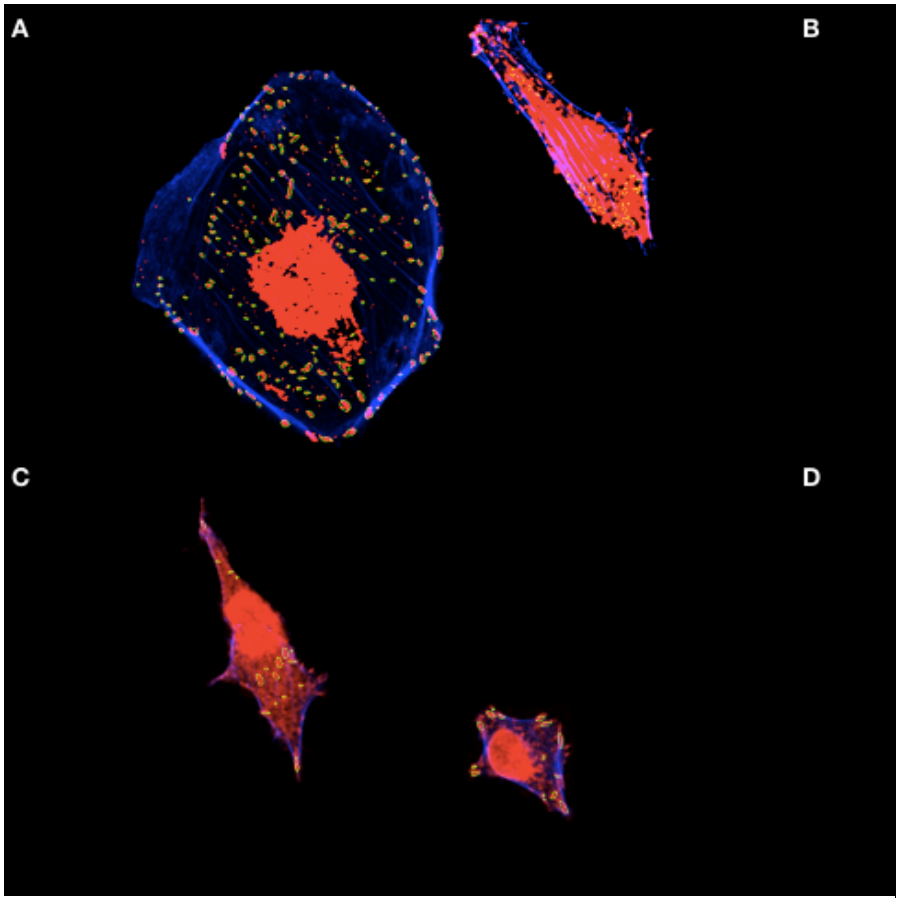

Figure 1: (A) Combined images of stained vinculin (red), actin (blue), and computer recognized vinculin (green) of MDA-MB-231 cell on glass. (B) Combined images of stained vinculin (red), actin (blue), and computer recognized vinculin (green) of MDA-MB-231 cell on 10μl of 2mg/ml concentration of collagen gel. (C) Combined images of stained vinculin (red), actin (blue), and computer recognized vinculin (green) of MDA-MB-231 cell on 20μl of 2mg/ml concentration of collagen gel. (D) Combined images of stained vinculin (red), actin (blue), and computer

A decrease in focal adhesion area is expected with an increase in volume of collagen that the cells were placed one. The focal adhesion area on glass, $10\mu l$, $20\mu l$, and $30ul$, have averages of $2.5\mu m^2$, $1.8\mu m^2$, $2.0\mu m^2$, and $1.9\mu m^2$ respectively, shown in Figure 2a. The results show that focal adhesion area changes slightly with varying rigidity. The aspect ratio should increase with ECM rigidity (15). The aspect rations of the focal adhesions on glass, $10\mu l$, $20\mu l$, and $30ul$ had averages of 1.8, 1.9, 1.9, and 2.0 respectively, shown in Figure 2b. The shape of focal adhesion does seem to correlate with ECM rigidity. The more rigid the ECM the less round and circular the focal adhesions seem. The distance between focal adhesions should not change with ECM rigidity (15). The inter-focal adhesion distance of the focal adhesion on glass, $10\mu l$, $20\mu l$, and $30ul$ are $5.2\mu m$, $5.0\mu m$, $5.1\mu m$, and $4.9\mu m$ respectively, shown in Figure 2c. From the results, the distance between focal adhesions does not seem to correlate with ECM rigidity.

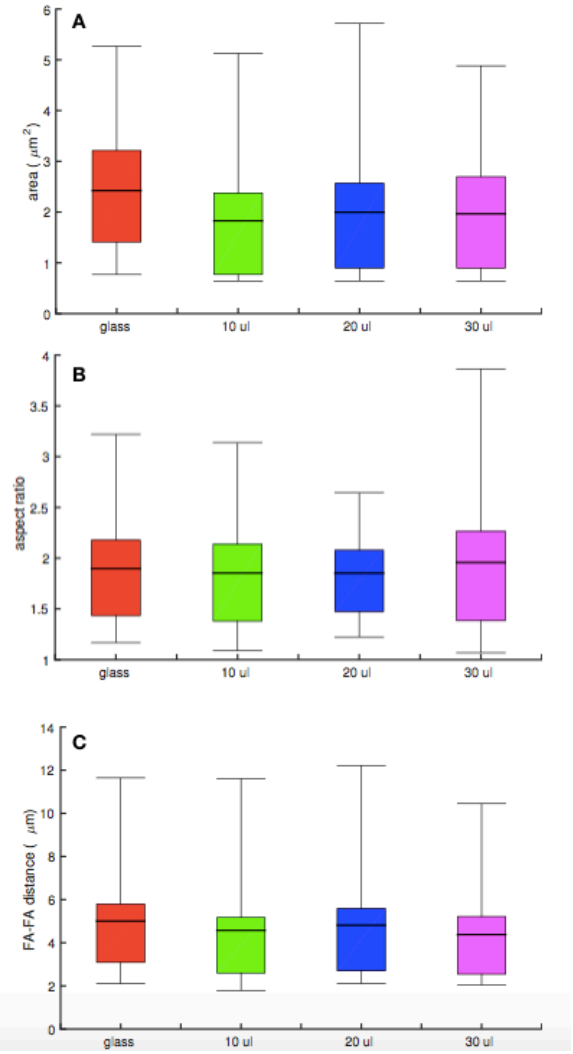

Figure 2: (A) The focal adhesion area of vinculin in various thickness of collagen gel. (B) The focal adhesion aspect ratio of vinculin in various thickness of collagen gel. (C) The inter focal adhesion distance of vinculin in various thickness of collagen gel.

The Art

Figures 3, 4, and 5 show the finished art piece that were inspired by figure 1.

Figure 3: Expression of how human breast cancer cells feel to people.

Figure 3 has a white and baby blue gradient in the bottom right and top left corners for the background. It has four deep, dark red circles of different sizes with long stems coming out of the circles. The choice to make this piece with such as high contrast between the light and dark is to show how awkward cancer cells can be when the general public think about them. Real cancer cells do not look like a round circle with "arms" extending from it in all directions, however, the goal of this piece was not reproduce reality. The goal of this piece was to capture what my feelings towards cancer cells were before working in the research laboratory. The deep, dark, desaturated red hues in the cancer cells reflect how I viewed them as scary and mysterious living organisms. The many "arms" extending outwards away from the cell center was made to display how cancer cells often have a strong hold in people's lives as they tend to be the center of attention for anyone who either has cancer or know someone who has cancer. The light value, tint of blue in the corners are meant to create a very calm feeling for the background in contrast to the uncomfortable feeling produced by the cancer cells. The brush strokes for the background in this piece is made to be in a bottom left to top right direction to create a visual cue for the viewers to move between those two corners when looking at this painting. I used only the three primary hues: red, yellow, and blue in the making of this piece. The blue was only used in the background to add a sense of tranquility, and the shades of red made the dark cells with a few tints of yellow in there to create visual depth within each cell, thus show casing the cells to be three dimensional.

Figure 4: Expression of how human breast cancer cells feel after working with them through immunofluorescent staining for extended periods of time.

Figure 4 has a completely black background with a lot of different metallic and neon colored shapes scattered throughout the piece radiating from the bottom left corner towards the top right corner. This piece was created to reflect my feelings towards the research when I first started my project in the research laboratory. I made the cancer cell samples to be imaged, and when doing so I had to use immunofluorescent stain in order to visualize the different proteins. Inspired by the fluorescent dyes, in the piece I only used bright, neon, metallic colors to reflect on the nature of the fluorescent dyes used in my research work. The single cancer cell extending from the bottom left to top right of the canvas piece signifies how when imaging cells, only one cell is imaged at a time. The cells are often in an extended fashion with protrusions away from the cell nucleus. Moreover, the flatness in the painting reflects on how the cells were imaged two dimensionally. The shapes that make up the cell are fun and unrepresentative of the actual proteins in the cancer cell. They display my emotions towards cancer cells after staining many cells. The cells no longer looked mysterious and scary to me, instead they felt rather harmless and bright with the fluorescent dyes. In some ways, the cells felt beautiful to me due to the increased understanding of cancer cells.

Figure 5: Inspired by the focal adhesion of human breast cancer cells on different ECM rigidity.

In figure 5 shows a dark green paint for the bottom half of the piece with light green in the middle and a fluorescent green towards the top of the painting. There are also four pairs of saturated red circles surrounded by streaks of pure blue and pure red paint around each of the circles. There are also some yellow dots at the ends of the blue streaks that surround the circles. Scattered throughout the piece also has five thick red paint swirls surrounded by straight saturated blue streaks with yellow dots at both ends of each blue line. In making this piece, the goal was not to create an accurate depiction of human breast cancer cell attachment to ECM, but to create an abstract representation of the research work. The piece has many blue lines extending from bottom left to top right manipulates the viewers to see the cells moving along in that direction. The contrast in complementary colors: red and green allows the viewers to easily pick out the reds because they pop out against the green background in various values of the green hue.

Conclusion

Human breast cancer cell focal adhesions can be studied by staining and visualizing vinculin, and the focal adhesions of vinculin on various ECM rigidity sheds clues on how well a cancer cell is able to attach to its environment. This study shows (1) focal adhesion area increases with increasing ECM rigidity, (2) focal adhesion shape seems to be more rounded as ECM rigidity increases, and (3) the distance between each focal adhesion is not dependent on ECM rigidity. With the images collected to obtain these results, an artistic interpretation of these scientific data was created to spike interest in the general public in both the art and the science aspect of this project. The science inspired the art, which sparks more interest in the science. This projects shows just how interdependent art and science can be. Scientific research often uses computational methods to model and quantify real world observations, and art uses different qualities of

color to amplify the real world observations to evoke a feelings in the viewers. Therefore, if more projects can have both a scientific and an artistic component, many people would likely be more interested in the sciences.

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