THE ROLE OF CADHERINS AND RYK ON METASTASIS AND INVASION IN TRIPLE NEGATIVE BREAST CANCER CELL MODEL: Hs578T/Hs578Ts(i)$_8$

BY

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A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Biochemistry

South Dakota State University

2023

DISSERTATION ACCEPTANCE PAGE
Ibis Iser

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This is book is dedicated to these special individuals in my life:

Madison

Mila

Heriberto

Ignacio

&

Olga: TNBC Survivor

I love you all.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the support of many individuals, and it is my pleasure to thank those that have facilitated my dream into a reality. I would like to express my deepest gratitude to my advisors, Severine Van Slambrouck and Matthew Miller for their patience, supervision and expertise during my PhD studies. It's your endless support and advice that carried me through all the stages of my dissertation.

I would also like to thank my committee member for their knowledge shared with me during my defense. Additionally, many thanks to my lab mates for all their advice, suggestions, and moral support. I would like to extend my sincere thanks to South Dakota State University Biochemistry and Chemistry staff, who helped me in my journey in more ways than I can count.

Lastly, I would be remiss in not mentioning my family for their support and belief in me through this journey. Special thanks to *mami y papi* for your constant support and always showing me the way. Warm thanks to my grandparents*, mima y pipo* for your endless warmth and encouragement in my dreams. To my loving husband- *Heriberto,* words cannot express how thankful and grateful I am for your daily support, your faith and confidence in me. Finally, I would like to thank God, for getting me through all of my difficulties and giving me the opportunity and guidance in achieving my goals.

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ABSTRACT

THE ROLE OF CADHERINS AND RYK ON METASTASIS AND INVASION IN TRIPLE NEGATIVE BREAST CANCER CELL MODEL: Hs578T/Hs578Ts(i)$_8$

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Breast cancer is a major cause of death among women in European and North American countries, even with improved methods for diagnosis and therapy. The mortality of breast cancer is mainly due to the migration of the primary tumor to distinct sites in the body and is very common in triple negative breast cancer (TNBC). This type of breast cancer affects younger woman and has a high recurrence rate. Unfortunately, TNBC is extremely difficult to control because of the absence of specific targets for treatment. Therefore, our research aim is to discover new therapeutic targets and identify novel approaches for treatment of TNBC. In this study the isogenic Hs578T/Hs578Ts(i)$_8$ cell model is used. This model consists of two cell lines: the Hs578T cells represent a primary tumor whereas the Hs578Ts(i)$_8$ cells have undergone biochemical changes that makes it more migratory and invasive. This cell model allows studying breast cancer progression since it resembles breast cancer patients that show metastatic disease. The focus of this project is on cell-cell adhesion molecules (*cadherins*), and the novel receptor tyrosine kinase, Ryk, their interactions and spatiotemporal rearrangements. These molecules are studied using various techniques and methods such as behavioral assays, western blots, co-immunoprecipitation, and immunofluorescence microscopy. The results of our experiments

have allowed for determining a role for N-cadherin and cadherin-11, their location, and potential connection with Ryk in invasive TNBC. In summary, this study provides novel valuable insights on the role of cadherins and Ryk in the progression of TNBC.

Chapter 1: Introduction

1.1 Triple Negative Breast Cancer

Breast cancer is the most common type of cancer and the leading cause of death due to its metastatic behavior (Redig and Mcallister 2014) . Among the various breast cancer subtypes, Triple Negative Breast Cancer (TNBC) is known for having the poorest prognosis and limited treatment options. TNBC is an aggressive and invasive cancer that lacks the expression of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2, (HER2) receptors. The spreading pattern in TNBC is different from other invasive breast cancer subtypes in that it grows and spreads faster to the brain, lungs and other organs and tissue (Martin, et al. 2013). Treatment options for TNBC patients are limited to mastectomy, chemotherapy, and radiation. The lack of receptors in TNBC make it difficult to treat with hormone therapy at these specific receptors (ER, PR and HER2). Researchers have found that small-molecule inhibitors have demonstrated to work on TNBC but over a short period of time develops a rapid resistance through multiple mechanisms (Li, et al. 2022). Determining the internal and external changes that occur in TNBC environment can provide a clearer understanding of it' metastatic and invasive behavior. Therefore, focusing on the cell-cell and cell-matrix biochemical changes and behavior that occur during invasion can lead to more effective therapeutic approaches that can improve the low response rate in triple negative breast cancer patients.

1.1.2 Metastasis and Invasion

Metastasis continues to be the leading cause for mortality in cancer patients despite of all the remarkable progress that has been made in investigating the molecular and cellular basis of this fatal disease (Martin, et al. 2013).These medical advancements have led to early detection and successful treatments that target cancer metastasis, while very detrimental, aggressive and invasive cancers such as TNBC late diagnosis is considered a death sentence for most patients. While all cancers grow and proliferate, not all behave or function in the same manner. Making the investigation of key mechanisms involved in the invasion and migration of these cancerous cell is crucial in treating cancer progression and reducing the mortality rate.

Metastasis is the spread of malignant cells to distant organs and tissues in the body (Martin, et al. 2013). This is the result of cancer cells undergoing molecular changes through intracellular and extracellular matrix (ECM) interactions, causing malignant cells to migrate. In order for these tumor cells to effectively metastasize, they must complete a number of sequential events called the *metastatic cascade*. This progression is broadly separated into three main processes: *invasion*, *intravasation* and *extravasation*. The loss of cell-cell adhesion allows malignant tumor cells to disassociate from the primary tumor site, promoting changes within the extra cellular matrix, causing the cells to *invade* into the surroundings stroma. Subsequently, finding a nearby blood/lymph vessel that provides a route to enter the circulatory or lymphatic system is essential for progression from local invasion to distant metastasis, this process is called *intravasation*. When these malignant

cells arrive to other areas in the body like the lungs, liver stomach through the blood/lymph vessels, they will adhere to its new surroundings by forming strong bonds that allows new tumor colonies to form. Altogether, this cascade is primarily driven by the loss of cell-cell adhesion molecules between neighboring cells leading to a dissociation from the primary tumor site, and subsequently initiating changes in the cell- matrix interaction that endorses the cell to attain a motile phenotype (Martin, et al. 2013). Moreover, recent advances have suggested that RTK phosphorylation are generally thought to weaken cell-cell adhesion by disrupting the association of cadherins and actin cytoskeleton (Ciasson-MacKenzie and McClatchey 2018).

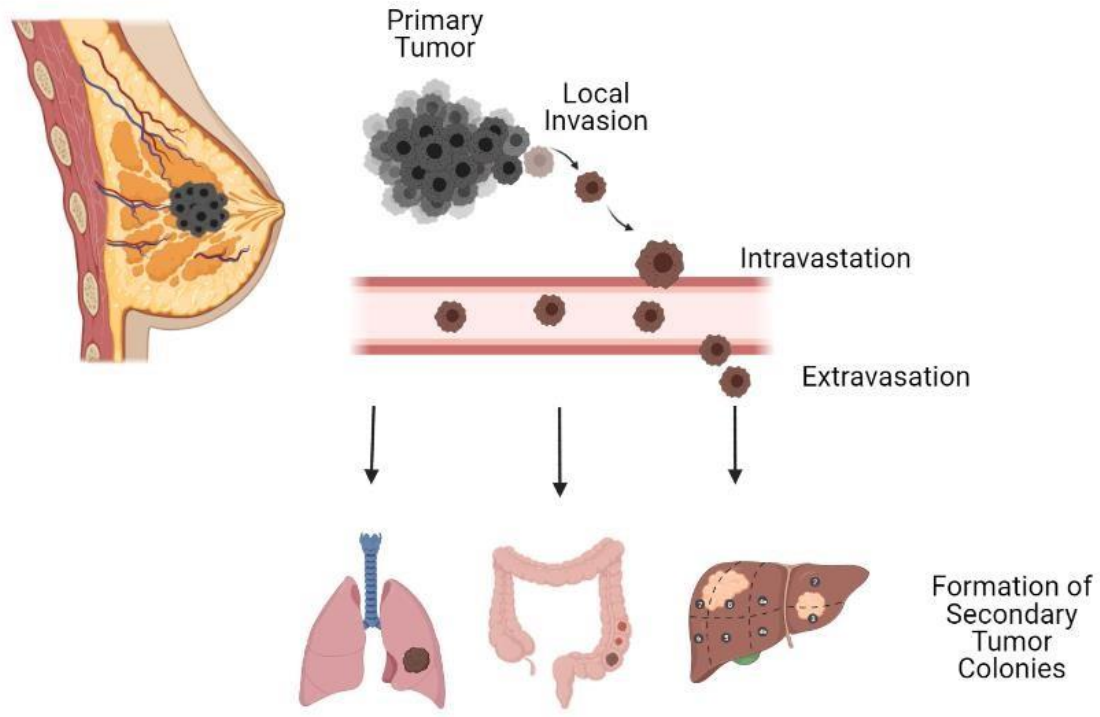

Figure 1 Breast Cancer migration: This is an illustration showing how a primary tumor can invade ECM and find the nearest lymph node or blood vessel and be able to travel to other organs of the body and form secondary tumors.

1.1.3 Cell Model

Many cell line subtypes are investigated to understand these tumorigenic and aggressive behaviors in cancer for decades given that tumor invasion and metastasis are the most fatal aspects of TNBC (Rana, et al. 2021). While many of these studies are vital for diagnosis and treatments, Dr. Susan McDonnell, created an isogenic subclone from the TNBC Hs578T cell line, forming a cell model that provides an opportunity to explain changes occurring at the cell-cell and cell matrix changes that occur in the process of tumor invasion. As shown in Figure 2 isogenic subclones were isolated from the Hs578T cell lines using sequential passages; through a BD Matrigel$_{TM}$ Invasion Chamber assay system to isolated cells that undergone phenotype changes that can mimic the invasion and migration processes from a primary tumor site. Dr. Donell succeeded with Hs578T cell line and for a new invasive variant Hs578Ts(i)8 and it showed to be 3 times more invasive and 2.5 times more migratory that the parental cell line (Hs578T) (Hughes, et al. 2008).

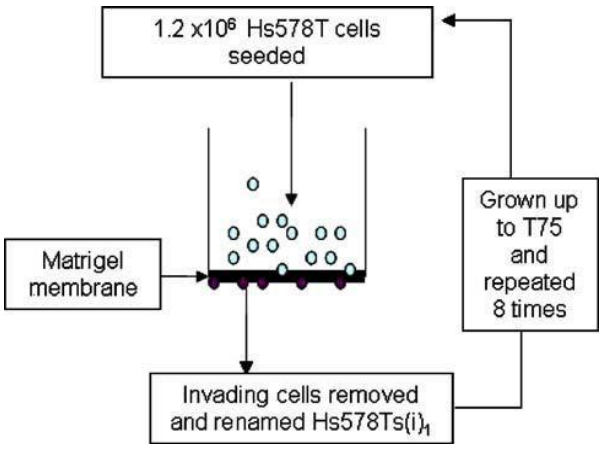

Figure 2 BD Matrigel Invasion Chamber assay system demonstrates how isogenic subclones were isolated from Hs578T cell lines using sequential passages (Hughes, et al. 2008).

To identify the differences between the Hs578T (parental cell line) and the Hs578Ts(i)$_8$ (invasive cell line) a series of functional assays were performed—*cell aggregation, fibronectin cell adhesion*, and *wound heal assays*. The cell aggregation assay examines the function of the epithelial cancer (Mareel 1997) (Bracke 1996). The parental cell line forms a compact cell aggregate (Figure 3 A), while the variant forms loose cell aggregates (Figure 3 A). Subsequently, a cell adhesion assay coated with fibronectin demonstrated that the invasive cells interact approximately 40% more with fibronectin cell-matrix than the parental cell line. To confirm migratory behavior a wound-heal assay later demonstrated that invasive subclone behaved 2.5 times more migratory. These experiments demonstrate behavioral differences between the Hs578TT TNBC cancer cell line and its more invasive subclone Hs578ts(i)$_8$ making this isogenic cell model embodies an elegant experimental model for studying cancer invasion and migration.

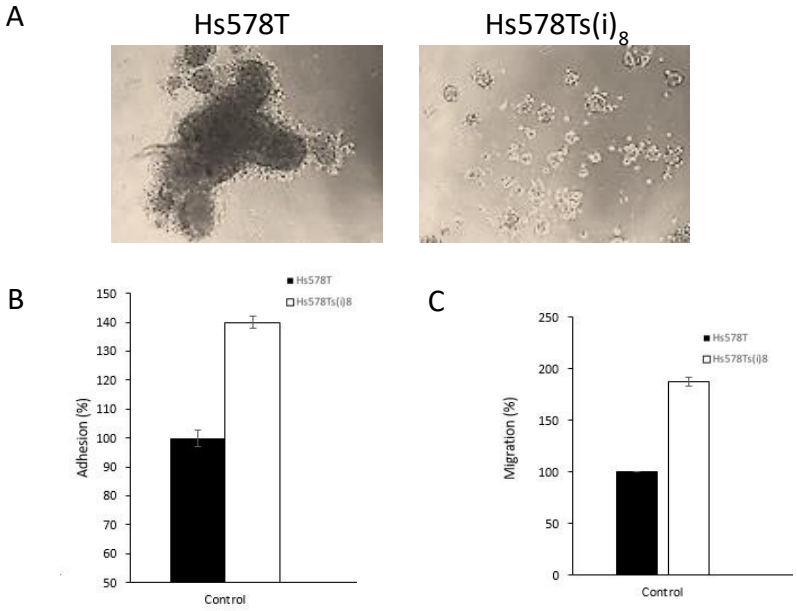

Figure 3 Phenotypic and Behavioral differences between Hs578T and Hs578Ts(i)$_8$ confirmed. A) Cell aggregation of both cell lines Hs578T create a large aggregate and

Hs578Ts(i)8 create disperse aggregates. B) Migration assay demosntrates that the invasive cell line is 2.5 times more migratory than the parental cell line.

1.1.4 Receptor Tyrosine Kinases

Receptor Tyrosine Kinases (RTKs) are cell surface receptors that heavily contribute significantly to cancer progression. RTKs have specialized biological structures that respond to environmental cues by initiating signaling cascades in tumor cells, which are initially activated through growth factors and chemokines (Butti, et al. 2018) . Dysregulated binding and activation of numerous signaling pathways in mammary epithelial cell leads to breast cancer development. RTKs act as single-pass transmembrane proteins that are expressed on various cell types, including tumor microenvironments. Elevated levels of RTKs are linked to increased breast cancer aggressiveness. Overexpression of RTK epidermal growth factor receptors (EGFRs), vascular endothelial growth factor receptor (VEGFRs), and or fibroblast growth factors receptors (FGFRs) are found in different types of cancer including breast (Butti, et al. 2018). Studies demonstrates the prominent role of receptor tyrosine kinases in the formation and progression of tumors and the development of targeted therapies against RTK has been evaluated in clinical trials and later approved in many cancer types including breast cancer. However, these target therapies is not a comprehensive solution due to the variances among subtypes such as TNBC shows more aggressive and invasive tendencies and RTKs signaling within TNBC is dissimilar to other triple negative breast cancer subtypes (Butti, et al. 2018). Therefore, investigating RTK's function in TNBC is likely to shed new light on the influence and performance of RTKs in metastatic behavior.

RTKs bind and respond to growth factors, hormones and/ other ligands and the binding that occurs between these molecules and RTKs by providing stimuli to neighboring RTK. This done by ligand binding to a tyrosine kinase site activating by phosphorylating multiple tyrosine sites as shown in Figure 4. This process is also known as *cross-phosphorylation,* which activates other signaling pathways. Cell surface RTKs are sent to lysosomal degradation, recycled to the cell surface, or translocated into subcellular membranes like the nucleus (Scitable by nature education 2010) . According to recent publications in the last decades, there is mounting evidence that indicates an interrelationship between cell-cell adhesion and RTKs, since RTKs localization and function influences cell contacts and spatiotemporal control (Ciasson-MacKenzie and McClatchey 2018). Therefore, it is one intention of this paper to study the involvement of these cell surface receptors in cell adhesion molecules associated with invasion.

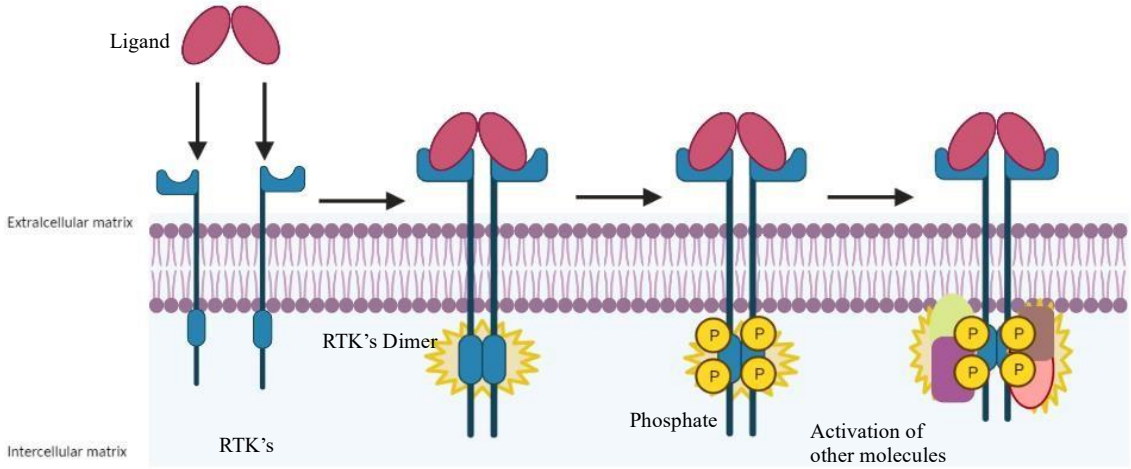

Figure 4 RTK Activation: This Figure illustrates how a ligand binds to an inactivated RTK. Ligand binding causes RTK dimmers to phosphorylate causing an activation of other signaling pathway.

1.1.5 Cell Adhesion Molecules (CAMs)

Cell adhesion molecules promote intercellular and extracellular connections while serving as a form of communication between cells and their environment. One of the effects of this interaction is the continuous regulation of cell functions by proliferation, gene expression, differentiation, apoptosis, and migration (Murray, Frampton and Nelson 1999). These molecules are critical to many physiological processes therefore any alterations to the structure or function of these cell adhesion molecules results in diseases including cancer (Moh and Shen 2009). CAMs are classified into five main groups: *selectins, cadherins, integrins, immunoglobulins, and CD44 family.* Of these five molecules cadherin and integrin continue to be a primary focus in understanding cell invasion and migration.

Cadherins are transmembrane proteins that function through calcium-dependent homophilic and heterophilic interactions responsible for the cell adhesion and communication between cells (Andrews, Kim and Hens 2012) (Maitre and Heisenberg 2013) . These interactions justify the key participation of cadherins in cell recognition and adhesion. Any dysregulation in cadherin signaling has shown to influences tumor formation and progression and are considered as promising targets for cancer therapies.

Integrins are a family of transmembrane glycoprotein receptors that mediate cell matrix and cell-cell interactions (Johnson and Lewis 2002). The role of these receptors is fundamental to cellular process that are not only responsible for cell growth, differentiation, and death, while also regulating malignant cell growth metastasis and cancer induced angiogenesis (Taherian, et al. 2011). Integrins are fundamentally valuable in cellular processes because they offer a dynamic linkage between the extra cellular matrix and the

actin cytoskeleton. The involvement of integrin with ECM ligands trigger integrin clustering and the formation disassembly reorganization of actin filaments stress fiber and focal adhesion complexes. This dynamic reorganization allows integrin's to function as a regulator of cell adhesion, cell migration and cell division (Taherian, et al. 2011).

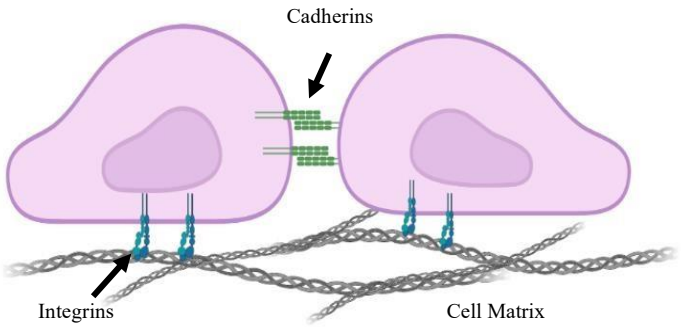

Figure 5 Cadherin and Integrins: illustration of cadherins location at cell-cell junctions and integrins are located between cell and extracellular matrix.

Because local invasion is understood to be a necessary first step in metastatic dissemination, Epithelial-mesenchymal transition (EMT) and epithelial plasticity are hypothesized to contribute to tumor progression (Micalizzi, Farabaugh and Ford 2010). The EMT theory refers to epithelial cells that lose their cell polarity and cell-cell adhesion, gaining migratory and invasive properties that can lead to mesenchymal cells. As shown in Figure 6 specialized epithelized cells that are found in cavities, organs and vessels undergo biochemical changes within their cell-cell adhesion molecules and cell-matrix interactions. This process begins with the disassembly of cell surface protein such as E-cadherin and integrin, which mediate epithelial connections to neighboring cell and basement membrane. These molecules are then respectively replaced by N-cadherin and other integrins that provide adhesive and priming for the cell to become more mesenchymal. This

causes a change in cell polarity from cuboidal shape to a spindle like shape (mesenchymal). This changes in cell polarity is caused by the reorganization of the cells cytoskeleton causing peripheral actin to be replaced by stress fibers all while the cell matrix is being degraded ((Micalizzi, Farabaugh and Ford 2010). The changes provide these cells with ability to invade and reduce cell-cell contact (Douglas Micalizzi 2010).

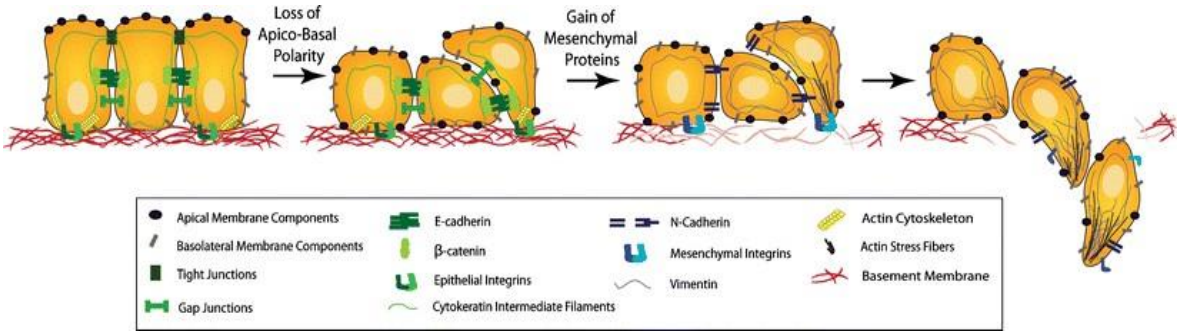

Figure 6. EMT and Epithelial Plasticity. This cartoon demonstrates epithelial cells lose their apico-basal polarity. (Douglas Micalizzi 2010).

The loss of E-cadherin expression is common in high grade tumors and is directly correlated to increased invasiveness (Micheal J Pishvainian Carolyn M. Feltes 1999). However, studies have shown an inverse correlation in invasive breast cancer cell lines, namely increased levels of expression of N-cadherin minimal to no detection of E-cadherin as shown in the Figure 7 (Micheal J Pishvainian Carolyn M. Feltes 1999). N-cadherin, also known as neural-cadherin, has been found in intercellular contacts and has shown to promote cell-cell adhesion between invasive breast cancer cells. Most cells that lack E-cadherin still retain cell-adhesion, indicating the presence of another cadherin family

member is taking over that role. Studies have shown that N-cadherin and cadherin-11 expression in TNBC cell lines, strongly suggesting that there's a gap to the mainstream literature on the EMT on the behavior of cell-cell adhesion molecules in migratory and invasive tumors. For instance, in the isogenic cell lines the parental line expressed higher levels of N-cadherin than the invasive variant as Figure 7 while novel cadherin-11 protein expression was presented in both cell lines. Cadherin-11 has shown to appear in cell membrane, cell-cell junction and at lamellipodia projection that does not interact with other cells (Hazan 1997). These studies suggest that cadherin-11 may have more than one function in the cell and may be involved with facilitating tumor cell invasion and metastasis.

The expression of cadherins previously discussed varies within the actual cell line in question. N-cadherin is evidently expressed higher in the parental than in the invasive cell line while the overall expression of cadherin-11 has been observed equal in both cell lines. Through immunofluorescence N-cadherin is observed through immunofluorescence imaging in cell-cell adhesion as well at the leading edge of the cell membrane in both cell lines. However, N-cadherin is more predominant in the invasive variant.

Conclusively, the overall protein expression of these cell lines are expressed higher in the parental Hs578T and observed differently in the immunofluorescence images of the invasive Hs578Ts(i)$_8$ cell line. These findings bring into question that protein expression is directly correlated to the activity of the cell" (ML, et al. 2010). Considering the spatial localization of these molecules in the cell provides meaningful insight on its function than the overall protein expression within the cell, which suggests that quantity of this protein expression does not solely portrait a cause for these invasive and migratory properties. In

a fluorescent image of Cadherin-11 it can be appreciated the presence of cell-cell adhesion site but also in cell membrane protrusions, in both the parental Hs578T cell line and invasive cells. However, in comparison to the parental cell line, the invasive cell lines demonstrate less cell contact and is observed present in more cell membrane extensions. These observations, along with the results protein expression, further promotes the interest in understanding the correlation of these molecules and their localization. Cadherin-11 protein expression is higher expressed in the parental Hs578T cell line in comparison to

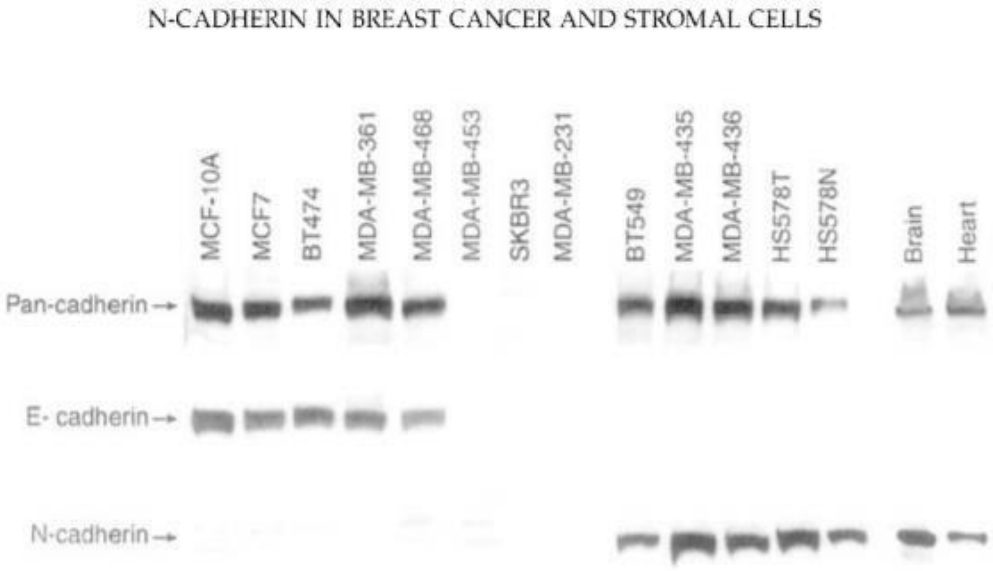

Figure 7 Immunoblot analysis of breast cancer cell lines. (Hazan 1997)

the invasive cell line Hs578Ts(i)$_8$ but protein localization is visualized differently, presenting the opportunity to question its real function.

1.1.5 Cell Migration and Motor Proteins

Cell migration is a complex mechanism that is mainly driven by actin and myosin cytoskeleton inside the cell membrane (Ananthakrishnan and Ehrlicher 2007). This migration process of the result of sequential steps: 1) protrusion of the leading edge of the

cell, 2) simultaneously adhesion molecules at the cell body and rear end and anchors at the leading edge, 3) followed by cytoskeletal contraction to propel cell forward as shown in Figure 8. The cytoskeleton is an essential component in creating the motility-driving forced that drives this complex phenomenon. It's composed of three distinct biopolymers— actin, microtubules and intermediate filaments that propel motility. The actin cytoskeleton is

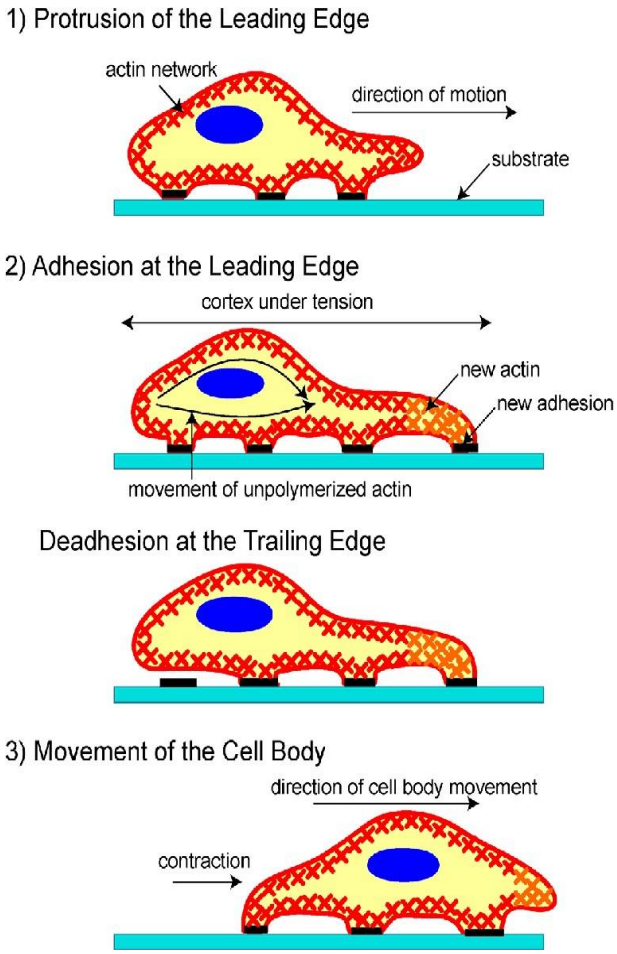

Figure 8 Illustration of the forces behind cell movement1) protrusion of the leading edge of the cell, 2) simultaneously adhesion molecules at the cell body and rear end and anchors at the leading edge, 3) followed by cytoskeletal contraction to propel cell forward (Ananthakrishnan and Ehrlicher 2007).

considered as the engine that drives cell protrusion of the leading edge. Being also vital for the adhesion at leading edge and detachment at the rear end and cell movement. Actin filaments are semi flexible polymers built from pairs of globular actin monomers. Actin is recognized as a highly dynamic protein due to its constant restructuring and transition from one actin structure to another that transforms shape and movement of the cell based on internal or external influences (Ananthakrishnan and Ehrlicher 2007).

1.1.6 Actin-myosin contraction

The function of actin filament is associated with myosin motors. The structure of myosin motors consists generally of a head, neck and tail region, action on structural variations in myosin may be shown as one head and neck or even two heads. These myosin molecules participate in the cellular process by requiring force and translocation in cell movement through tension on actin filaments. The head and necks amino-terminal is responsible for the carboxyl-terminal is responsible for caring cargo, or connecting to myosin, vesicles, or filaments. The effects of myosin motor works on actin filaments take part in sequential processes— binding, power stroke, and unbinding. This biophysical mechanism is continuous and leads to acto-myosin contractile forces that propel the cell forward (GM 2000).

This study led to investigate class II myosin's since these cadherins are responsible for producing actomyosin contraction in non-muscle cells (Liu and Cheney 2012). Non-muscle myosin II (NM II) molecules like muscle myosin's are composed of three pair of peptides— two heavy chains of 230 kDa, two 20 kDa regulatory light chains (RLCs) that

regulate NM II activity and two 17 kDa essential light chains that stabilizes the heavy chain structure as shown in Figure 9 (Vicente-Manzanares, et al. 2009). NMII are fundamentally involved in cell reshaping and movement specifically through cell adhesion, cell migration and cell division. Studies have demonstrated that NMII as a critical regulator of cells adhesion and polarity in cell migration and this mechanism requires dynamic remodeling of the actin cytoskeleton and interaction of the cell with its environment.

The structure of NMIIA contain two globular head domains with ATP binding or actin, followed by neck region that binds the two different light chains as demonstrated in Figure 9. This neck domain acts as a lever arm that allows for the head rotation while the ATP mechanism convert to mechanical movement lead by the myosin head. There are three different genes that encode for myosin heavy chains, namely, NMIIA, NMIIB, NMIIC of the three NMIIA has the highest rate of ATP hydrolysis while allowing actin filaments to propel more rapidly than NMIIB & C. While on the other hand, NMIIB has a higher duty ratio than NMIIA, and a high affinity for ADP. This observation suggests that NMIIB is well suited for exert tension on actin filaments for a longer period and be energetically favorable (Vicente-Manzanares, et al. 2009). Moreover, researchers have found that NMII is necessary in contributing to cancer cell migration. Cancer cells ability to react to its microenvironment is dependent on NMII mediated adhesion due to ability to generate mechanical force (Aguilar-Cuenca, Juanes-Garcia and Vicente-Manzanares 2014) (Halder, et al. 2021). A number of cancer cells have shown to reveal expressions that are altered and localization leading to diversity of spatiotemporal differences in the formation of NMII (Shutova and TM 2018) (Halder, et al. 2021). This behavior allows to cancer cells to adapt to various modes of migration. NMII behaviors previously observed influence act through

actin and promotes adhesion-related proteins, like integrins and other signal molecules such as cadherins and RTKs, in proximity. Therefore, this acto-myosin contracting force is the power engine of migrating cells, making the relationship of this mechanism in mutation and disease of increasingly important focus in research its influence in cell-cell adhesion and migration. Understanding these behaviors and relationships in TNBC can provide insight the migratory mechanism in an invading cell.

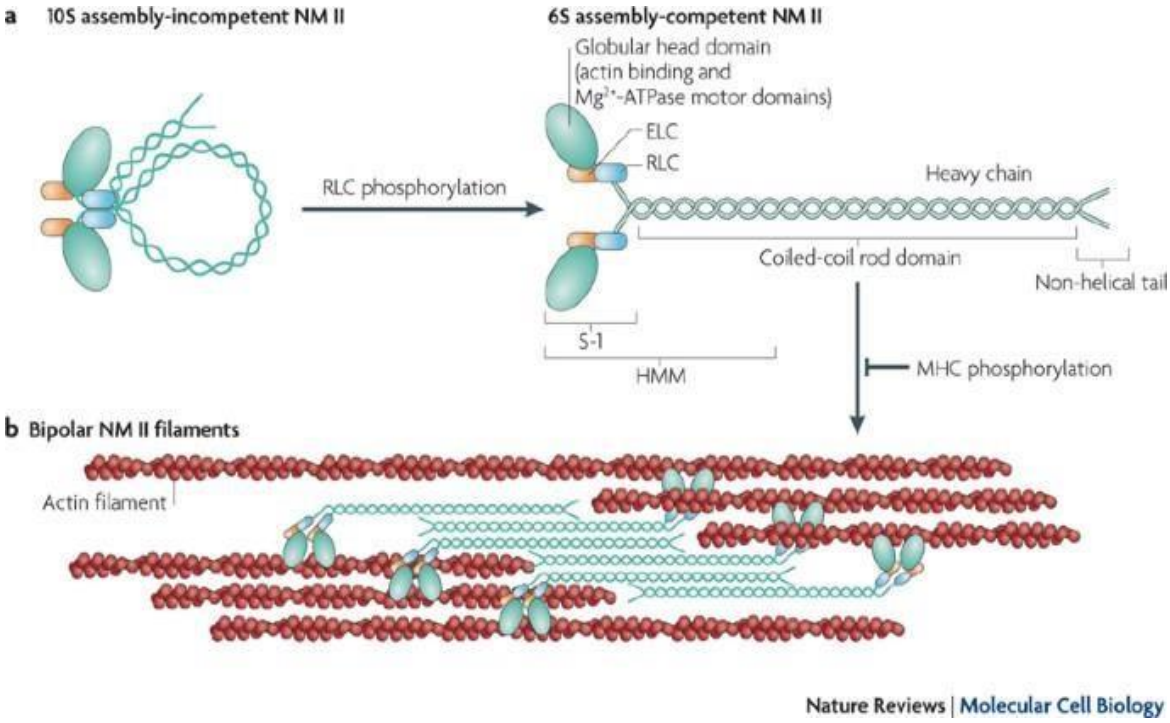

Figure 9. Illustration of Non-muscle myosin II (NM II) molecules like muscle myosin's are composed of three pair of peptides— two heavy chains of 230 kDa, two 20 kDa regulatory light chains (RLCs) that regulate NM II activity and two 17 kDa essential light chains that stabilizes the heavy chain structure (Vicente-Manzanares, et al. 2009)

Chapter 2. Materials and methods

2.1. Antibodies and other reagents

Antibodies directed against integrin α5 (#4705), N-cadherin (clone D4R1H), nonmuscle myosin II-A (#3403), non-muscle myosin II-B (clone D8H8), Ser19 p-NMIIRLC

(#3671), Ser1943 p-NMII-A (clone D7Z7T), Rho antibody from the Active Rho Detection Kit (#8789), talin-1 (clone C45F1), paxillin (clone D9G12), β-actin (clone D6A8), blebbistatin, mouse and rabbit monoclonal IgG isotypes, Alexa Fluor® 488 and

Dylight™ Phalloidin, and Alexa Fluor® 488 and 594 secondary antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-mouse and anti-rabbit alkaline phosphatase-labeled secondary antibodies, Alexa Fluor® 594 Deoxyribonuclease-I and the BCA protein assay reagent kit were from Thermo Fisher

Scientific (Waltham, MA, USA). The function-blocking antibody against integrin

α5β1was (clone JBS5) obtained from EMD Millipore (Billerica, MA, USA) while the anti-MyoGEF (IF: #ab22096, WB: #ab19412) and anti-non-muscle myosin II-B (clone 3H2) antibodies were purchased from Abcam (Cambridge, MA, USA). Anti-N-cadherin antibody (clone 8C11) for immunofluorescence was obtained from Novus Biologicals

(CO, USA) while function-blocking anti-N-cadherin antibody (clone GC-4) was obtainedfrom Sigma-Aldrich (St. Louis, MO, USA). The anti-integrin β1antibody (clone

18/CD29) was purchased from BD Biosciences (San Jose, CA, USA). The directly labeled (Alexa Fluor® 488) polyclonal antibody against NMII-A was kindly offered by Dr. Anne Bresnick (Albert Einstein College of Medicine, NY, USA)34. Pharmacological inhibitor toxicity was evaluated through measurement of mitochondrial dehydrogenase 18 activities with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma-Aldrich).

2.2. Cell culture

The human mesenchymal breast cancer Hs578T cells and the derivative cell line Hs578Ts(i)8 were a kind gift from Dr. S. McDonnell (UCD School of Chemical and Bioprocess Engineering, University College Dublin, Ireland) and were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.01 mg/ml bovine insulin (Thermo Fisher Scientific) at 37°C equilibrated with 5% (v/v) CO2 in humidified air. The TNBC cells used in the present study were frozen in liquid nitrogen when not in use and were not passaged in our laboratory for >15 weeks.

2.3. Western blotting

Cell lysates were made from 80 to 90% confluent cultures using 0.5 ml lysis buffer containing 1% Triton X-100 and 1% Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific). Aliquots of lysates, containing 30 μg of protein, were boiled for 5 min in SDS-PAGE sample buffer containing 5% (v/v) β-mercaptoethanol, electrophoresed on

7.5 or 10% TGXTM precast gels and transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA, USA). After transfer, membranes were blocked and incubated with relevant primary antibodies followed by incubation using alkaline phosphatase-labeled secondary antibodies and developed using NBT/BCIP substrate (Roche, 1:50 in 0.1 M Tris-HCl, 0.05 M MgCl2 and 0.1 M NaCl at pH 9.5). All experiments were carried out with β-tubulin loading controls.

2.4. Co-immunoprecipitation of cell surface molecules

Cells at 80-90% confluency, were lysed, as described under 'Western blotting'. Lysates, containing 1000-1500 μg protein. Antibodies to relevant cell surface molecules (1:250) were added to the collected supernatant and rotated at 4°C overnight. Subsequently, protein G-Sepharose beads (Amersham Biosciences, NJ or GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) were used to recover the immunocomplexes. Immunoprecipitates were resolved in 150 μl SDS-PAGE sample buffer and heated to 95°C for 5 min. The supernatants were then subjected to SDS-PAGE, transferred electrophoretically to PVDFmembranes. After transfer, the membranes were analyzed and developed as described in'Western blotting'.

2.5. Fluorescence immunostaining

To examine the distribution and co-localization of proteins under standard conditions, cells were grown on glass cover slips (diameter, 12 mm) placed in 24-well plates, then removed, washed with PBS and fixed in 4% paraformaldehyde for 1 hour (EMS, Hatfield, PA, USA) or in methanol for 10 minutes. To examine the reorganization

of proteins upon pharmacological inhibitor treatment, 50 μM of blebbistatin was added to cells grown on glass coverslips in a 24-well plate for 10 mins and fixed with 4% paraformaldehyde. Next, fixed cells were washed, permeabilized with 0.1% Triton X-100 for 10 mins, blocked, and incubated with directly labeled antibodies or primary antibodies, Alexa Fluor® 488 or 594 Phalloidin, or Deoxyribonuclease I Alexa Fluor®594 conjugate followed by incubation with Alexa Fluor®-labeled secondary antibodies.Stained cells were mounted with Vectashield mounting medium (Vector Labs, Burlington, CA) and images were acquired using an Olympus IX71 fluorescence 20 microscope (Tokyo, Japan) with a digital Zyla sCMOS camera and a 60X PlanApo/1.20 water objective, and in the BioTek Cytation 3 Cell Imaging Multimode reader (Winooski,VT, USA) with a 40X or 20X objective. Control stainings were performed without primary antibodies and with IgG isotype control antibodies to measure possible cross reaction or non-specific binding.

2.6. Wound healing assay

Cells were grown in 24-well plates until confluency and washed twice with phosphate buffered saline (PBS). A scratch was made using a P200 pipette tip and 1 ml of mediumin the presence of DMSO, 10 uM blebbistatin or integrin α5β1 function-blocking antibody (10 ug/ml) was added. Cell migration was monitored, and images were collected after 17 h, with an EVOS® XL Core Cell Imaging (Thermo Fisher Scientific). ImageJ software was used to estimate the cell free area of the wounds39. The distances over which the cells migrated were measured in three independent experiments and expressed as percentage compared to DMSO-treated Hs578T and Hs578Ts(i)8 cells.

2.7. Cell adhesion assay

Cells were detached with 0.2% (w/v) EDTA, to avoid proteolytic degradation of cell surface proteins, and washed with serum-free medium. Next, 5×105 cells were resuspended in 1 ml DMEM supplemented with 2% (v/v) FBS. Cell suspensions (100 µl), in the presence or absence of function-blocking antibodies (5–10 µg/ml) were added to fibronectin pre-coated 96-well plates (BD Biosciences, San Jose, CA, USA), and centrifuged for 1 min at 115 g. After 90 min incubation at 37°C, wells were washed four times with PBS to remove non-adherent cells. The adherent cells were then detected and quantified by measuring the acid phosphatase activity, through solublization of the 21 remaining cells with 0.2% Triton X-100 and by the addition of the substrate, PNPP (pnitrophenyl phosphate; Sigma, St. Louis, MO, USA). Absorbance values of the lysates were determined on a microplate reader at 405 nm (BioTek, Cytation 3, Winooski, VT, USA) and expressed as relative absorbance (%). Mouse and rabbit IgG isotype control antibodies were included to estimate the non-specific binding.

2.8. Cell aggregation assay

Semisolid agar pre-coated 96-well plates were prepared by adding 50 µl/well (ratio of agar to Ringer's salt solution in 7:1, w/v%). Single cell suspension of 10 000-100 000 cell/100 µl medium were mixed with function-blocking antibodies (5-10 µg/ml) or blebbistatin (7.5, 10 or 20 µM) and seeded onto the plate and incubated for 24 h in a humidified atmosphere of 5% CO2 at 37°C. Aggregate formation was evaluated by phase contrast microscopy using an EVOS® XL Core Cell Imaging System.

2.9. Flow cytometry

Cells were detached with 0.2% (w/v) EDTA, to avoid proteolytic degradation of cell surface proteins, neutralized with cold PBS and resuspended in PBS containing 0.1% (w/v) BSA (Sigma). Next, 2.5x105 cells were incubated with the relevant primary antibodies for 90 min at 4°C, followed by secondary FITC or Alexa Fluor®-labeled antibodies for 45 min at 4°C. After washing, 1x104 stained cells were analyzed for fluorescence using the CytoFlex (Beckman CoulterÔ, Miami, FL, USA). Stainings without primary antibodies and IgG isotype antibodies were used as controls.

2.10. Statistics

All treatments were matched and carried out at least 3 times. Data were analyzed using Excel, for determination of mean, standard deviation (SD) and Student's t-test (95%). The intensity of the immunoblotted bands was quantified by densitometry, using statistical software Scion Image (Scion Corp., Frederick, MD, USA).

Chapter 3: Results

3.1.1 Cadherin's cell-adhesion behavior in Hs578T/Hs578Ts(i)$_8$

Cadherin's are cell adhesion molecules that are responsible for cell communication and cell-cell attachment; any loss in the adherent function is a direct influence on migratory and invasive behavior in cancer cells. Identifying the cadherins responsible for cell adhesion among cells is necessary to study the metastatic cascade in greater detail. Therefore, through western blot assays it was determined that the isogenic sub clone cell model Hs578T/Hs578Ts(i)$_8$ and other aggressive TNBC cell lines do not express the typical E-and P-cadherin as shown in table (1). Nonetheless, these cells still retain weak calcium dependent adhesion, indicating the presence of another cadherin member. N-cadherin is an indicator of ongoing EMT, and its protein expression has shown to be correlated with progression of various types of carcinomas (Loh, et al. 2019). Detection of N-cadherin protein expression was examined in aggressive TNBC cells Hs578T/Hs578Ts(i)$_8$, MDA-MB231, and MDA-MB436 (table 1). In addition, the detection of cadherin-11 also became of interest since studies have demonstrated its expression in the most invasive cell line but not in any noninvasive cell line. Indicating that cadherin-11 serves as molecular marker for he more aggressive and invasive subset tumors. It can be appreciated in table 1 that Cadherin-11 was positively detected in the isogenic subclone model Hs578T/Hs578Ts(i)$_{8,}$ and MDA-MB-231. N-cadherin and Cadherin-11 were detected in three out of the four aggressive TNBC cell lines of which both cadherins showed higher protein expression in the parental Hs578T in comparison to its isogenic sub clone Hs578Ts(i)$_8$.

	E-cadherin	N-cadherin	P-cadherin	Cadherin-11
Hs578T	-	++	-	++
Hs578Ts(i)$_8$	-	+	-	+
MDA-MB-231	-	-	-	+
MDA-MB-436	-	+	-	-

Table 1. Protein Detection of E-cadherin, N-cadherin, P-Cadherin and Cadherin-11 on Hs578T/Hs578Ts(i), MDA-MB-231 and MDA-MB-436.

3.1.2 Cadherin cell adhesion function on TNBC cells

Cancer invasion is promoted by the loss of cell-cell adhesion. A simple, elegant and inexpensive *in vitro* assay to study the differences between invasive and non-invasive cell phenotypes can be achieved with a slow aggregation assay. This aggregation assay is used to detect molecules that can maintain/restore functional integrity of the cadherin complex in epithelial cells (Mareel 1997). Therefore, to determine the function of N-cadherin and Cadherin-11 in TNBC cells, function-blocking antibodies were used in a cell aggregation assay. N-cadherin and Cadherin-11 functioning role was determined by using the function-blocking antibodies: clone GC-4 and 16G5, respectively Figure (10). These experiments demonstrated that the N-cadherin and cadherin-11 antibody interrupted these cadherin homotypic ligations and aggregation of the parental Hs578T cells, forming loose disperse aggregates while no significant changes were observed after this function-blocking antibody treatment of the invasive sub clone Figure 10. These experiments were also performed in the MDA-MB-231 but only using function-blocking antibody cadherin-11 16G5, since MDA-MB-231 does not express N-cadherin. The same concentration and

antibody was used on the MDA-MB-231 and it does not demonstrate any significant difference from the control (Figure 10).

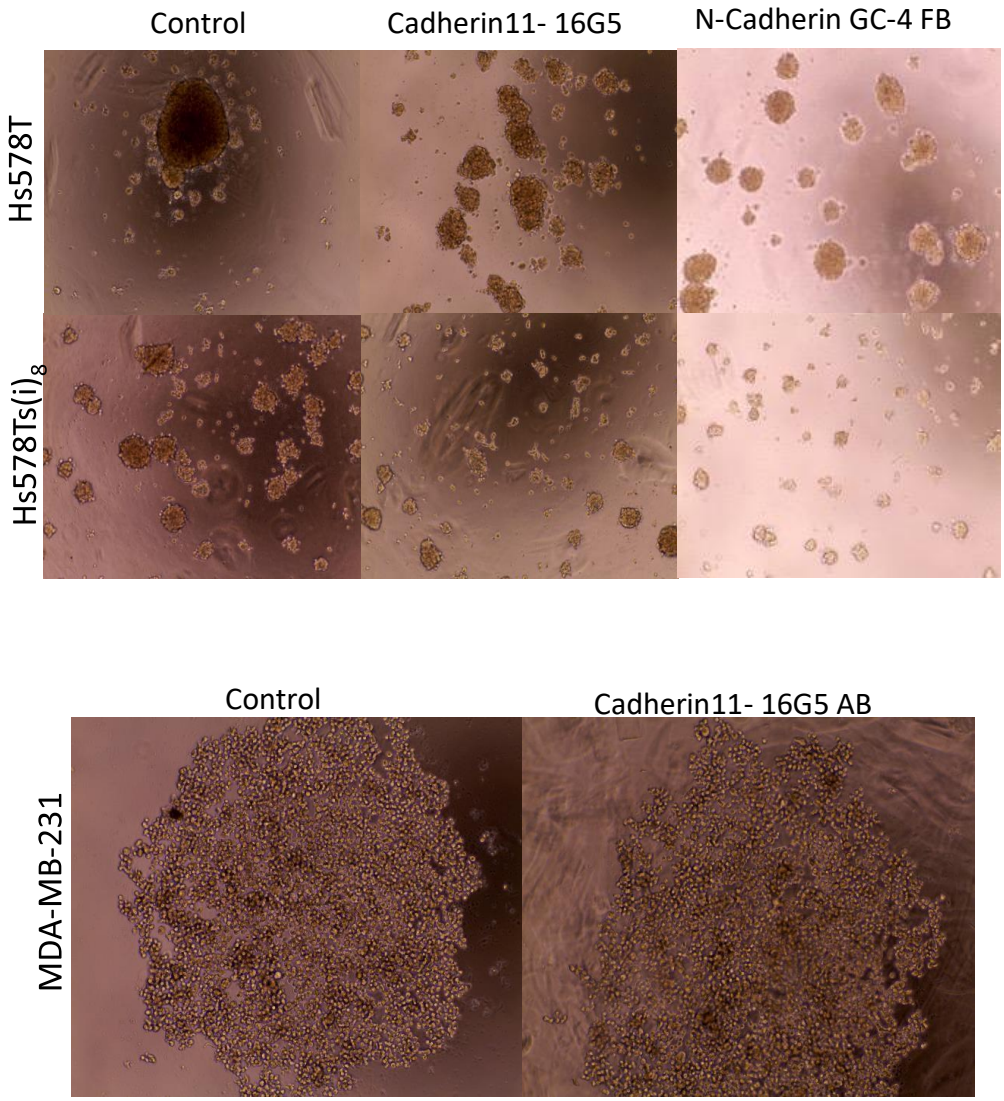

Figure 10: Cell aggregation assay on Hs578T/Hs78Ts(i)8 and MDA-MB-231. A function-blocking antibody were used for N-cadherin and Cadherin-11.

3.1.3 Cadherin-11 and N-cadherins localization Hs578T/Hs578Ts(i)$_8$

While these findings do not follow mainstream literature on expression levels of these cell adhesion molecules (cadherin-11 and N-cadherin) in advanced breast cancer, spatiotemporal localization of these molecules have gained a notable interest, as it may support the novel idea that cadherin's have more than one function and interact with other cellular adhesion and signaling processes. To determine the localization of both cadherin11 and N-cadherin, immunocytochemistry experiments show how these cadherin localization within the subclone cell lines are different by localizing in other area that are not cell-cell contacts, they are observed at lamellipodia like projections. In the parental Hs578T cell line as shown in the solid white arrow, cadherin-11 is observed at cell-cell junction sites, while in the invasive cell line not only is cell-cell junctions observed but also in membrane projections, and leading edge of a cell as shown in the white solid arrows. As for N-cadherin, it is visualized at cell-cell junction and in polarized cell N-cadherin can be observed in the cell-membrane not neat a cell-cell junction site as indicated with the white solid arrows, while in the invasive Hs578Ts(i)8 N-cadherin is found in cell-cell junction, in lamellipodia like projection as well. These finding confirm with studies that cadherins can be found at other site in the cell membranes that are not at cell-cell junctions' sites.

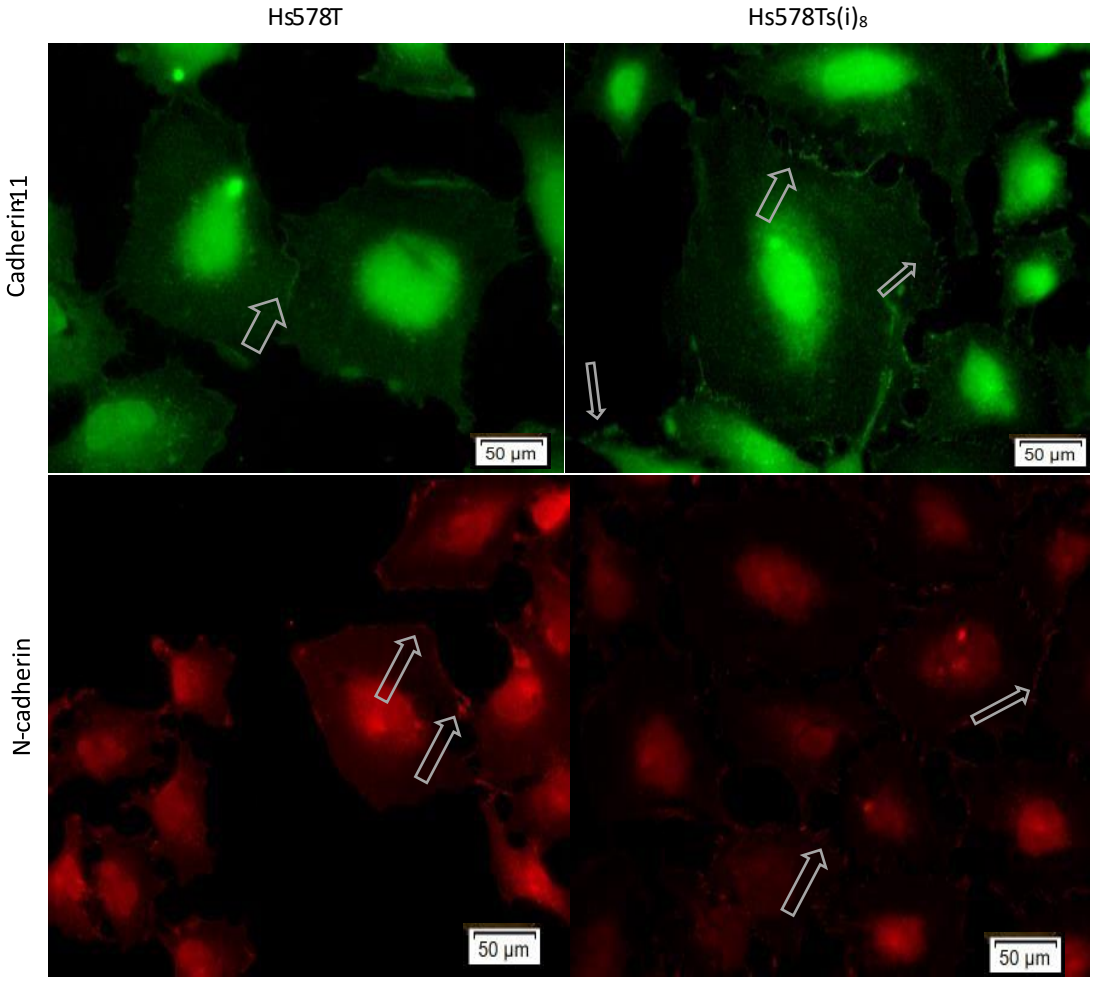

Figure 11 Cadherin-11 and N-cadherin localization on Hs578T and Hs578Ts(i)$_8$. Cadherin-11 is found on cell junction cites on both Hs578T and Hs578Ts(i)$_8$. N-cadherin is found on cell junction cites and non-cell junction sites on both Hs578T and Hs578Ts(i)$_8$.

3.1.4 Cadherins spatiotemporal localization in Hs578T/Hs578Ts(i)$_8$

To further validate their localization a dual staining of N-cadherin and cadherin11 was performed as shown Figure 12 Cadherin-11 was fluorescently tagged with a mouse 488 secondary antibody and shown in the green channel, while N-cadherin can be seen in the red channel and was fluorescently tagged with a secondary rabbit 594 antibody. These images were merged and shown in closer detailed (10um as) Figure (11). As observed N-cadherin and cadherin-11 in the merge image demonstrate co-localization at cell-cell adhesion sites in the Hs578T cells as compared to the Hs578Ts(i)$_8$ cell line. Moreover, two distinct observations were observed in the spatiotemporal rearrangement of these molecules: 1) N- and Cadherin 11 were closing together not only at cell-cell adhesion sites but also lamellipodia like projections and 2) significant differences of these molecules attachment intensity to cells between the parental Hs578T and invasive Hs578Ts(i)$_8$ cells.

The arrangement of these molecules in these cell line reveal a different attachment pattern. As shown in the merged Figure (12) the parental cell-cell adhesion attachment force appears more concentrated at these sites, while in the invasive subclone these cadherin molecules appear more loosely connected to other cells. The observations made in this isogenic model can be aligned with studies that indicate that cadherin adhesion possess some sort of mechanosensory to adapt to their strength to rigidity of the intra and extra cellular environments (Strength dependence of cadherin mediated adhesion).

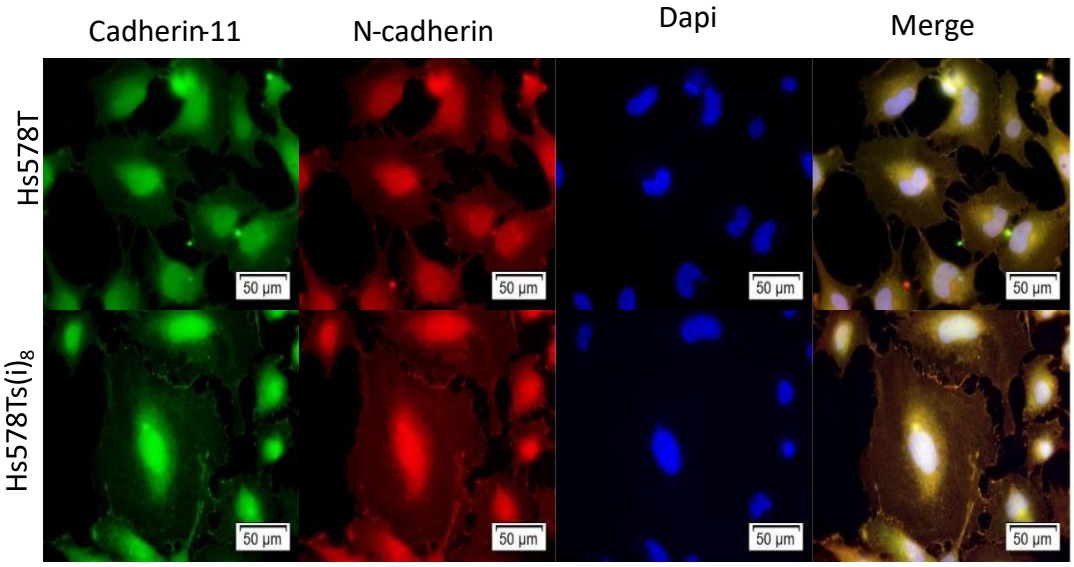

Figure 12 Dual staining f Cadherin-11 and N-cadherin on HS578T/Hs578Ts(i)8. Cadherin-11 was fluorescently tagged with a mouse 488 secondary antibody and shown in the green channel, while N-cadherin can be seen in the red channel and was fluorescently tagged with a secondary rabbit 594 antibody.

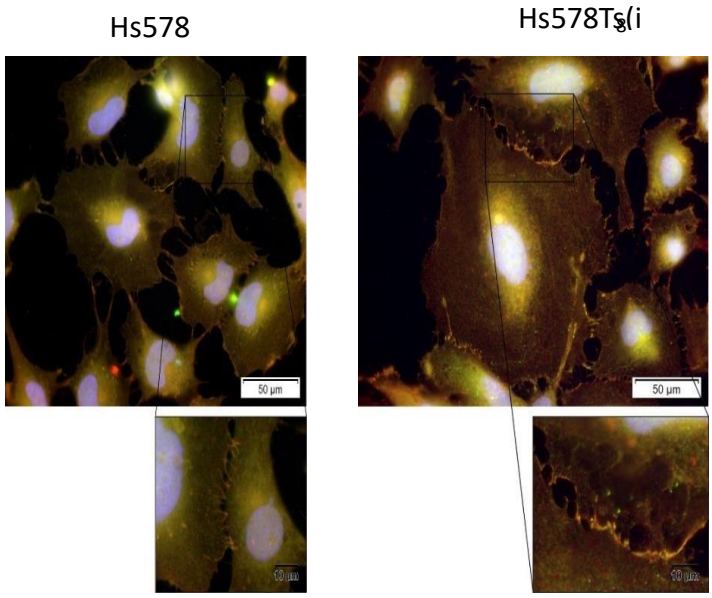

Figure 13 Dual staining of N-cadherin and Cahderin-11 on both Hs578T and Hs578Ts(i)8. Close up image of dual staining on cell-cell adhesion.

3.1.5 N-cadherin and Integrin A5B1 involved in cell-matrix interactions

Based on the collection of experiments of cadherins and their localization at non cell-junctions site, it was hypothesized that these cadherins could also be associated with cell-matrix adhesion molecules that are also a prerequisite of metastasis and invasion. Therefore to determine association between cadherin and integrins co-IP were performed as shown in Figure (14). These set of experiment not only determine that there is an association between integrin a5b1 with N-cadherin and cadherin 11 but also revealed not only that the total and cell surface expression levels of N-cadherin, Cadherin-11, A5, and B1 were higher in the parental Hs578Tcells as compared to the more invasive Hs578Ts(i)$_8$. These results support a novel idea that cadherin and integrins affect shared cellular adhesion and signaling process.

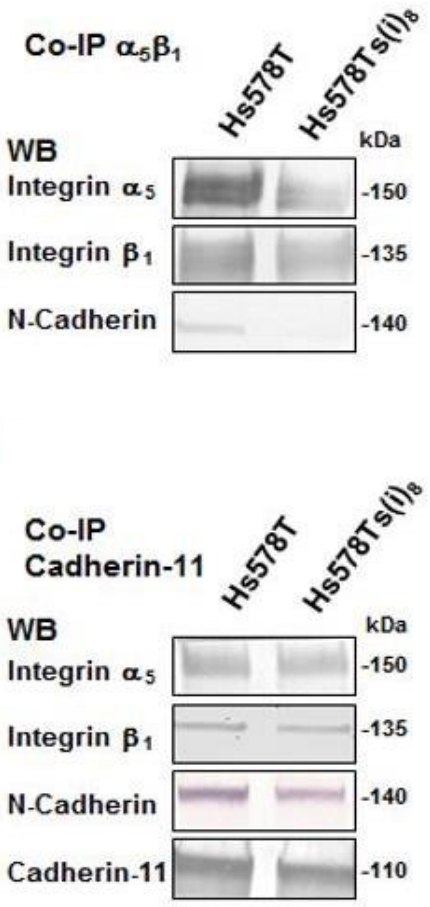

Figure 14 Co-immunoprecipitation of alpha 5 beta 1 integrin and cadherin 11. In these co-IP's you visualize that in A) An association with of alpha 5 and beta 1 with N-cadherin. B) Co-Ip of Cadherin-11 demonstrated an association with alpha 5 and beta 5 integrins and N-cadherin 11.

3.1.5 Integrin association in cell-cell adhesion

This possibility was tested by using function-blocking antibody a5b1 (clone JBS5) in cell-cell adhesion studies and AVB5 (mab 1969). A5B1 (clone JBS5) demonstrated to disrupt the cellular aggregation of Hs578T cells while not having any significant effect on the invasive Hs578Ts(i)$_8$ cell line. On the other hand, the function-blocking antibody for AVB5 (mab 1969) did not show to have any disturbance in the parental Hs578T cell line

nor in the invasive Hs578Ts(i)$_8$ cell line as shown in Figure (15). This experiment indicated that integrins association with cadherins is due to its function also being related at cell-cell adhesion sites.

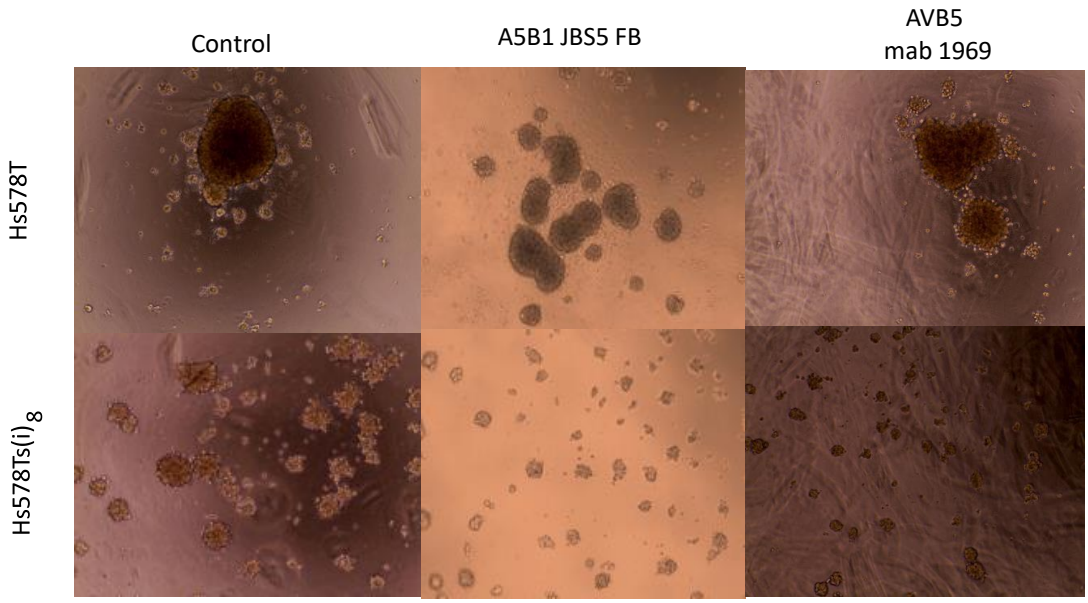

Figure 15. Cell aggregation assay with function-blocking antibodies for A5B1 and AVB5. A5B1 (clone JBS5) demonstrated to disrupt the cellular aggregation of Hs578T cells while not having any significant effect on the invasive Hs578Ts(i)$_8$ cell line.

3.1.6 Cadherins association in cell-matrix interaction

Given the ability for integrins to function at cell-cell interactions, N-cadherin and cadherin-11 function was determined in a fibronectin coated 96 well plates with function-blocking antibodies N-cadherin (JBS5) and Cadherin-11 (16G-5). The invasive variant Hs578Ts(i)$_8$ interacts with fibronectin twice in comparison to the parental cell line Hs578T. When the respective function-blocking antibodies were added the cell suspension, both

integrin a5b1 and N-cadherin significantly decreased their interaction to fibronectin by two folds. This was tested on cadherin-11 antibodies were tested on the fibronectin coated plates and no significant changes were observed (data not shown).

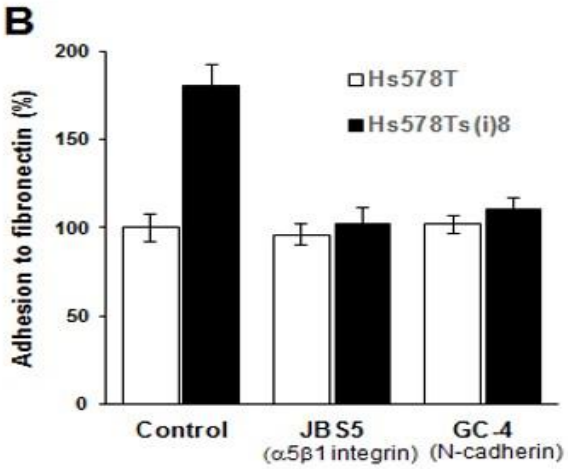

Figure 16 Graph of A5B1 and N-cadherin adhesion to fibronectin. Integrin a5b1 and N-cadherin significantly decreased their interaction to fibronectin by two folds.

3.1.6 Localization of integrins and cadherins

To further confirm the relationship between cadherins and N-cadherin immunocytochemistry followed by immunofluorescent microscopy were performed to detect possible co-localization in an episcope at 60x magnification. Integrins are cellmatrix protein and are anticipated to be seen at the bottom surface of the cell, but it is hypothesized that a5b1 and cadherins can be associated at the cell-cell junction site. As shown in images (A) merged channels between A5B1 integrin and N-cadherin in the episcope show a possible co-localization between these proteins as indicated by the white bold arrows at cell-cell adhesion while no such effect was observed in the invasive cell like Hs578Ts(i)$_8$.

This experiment was duplicated with staining for a5b1 and cadherin-11 (B) and demonstrated to co-localize at cell-cell adhesion sites as well.

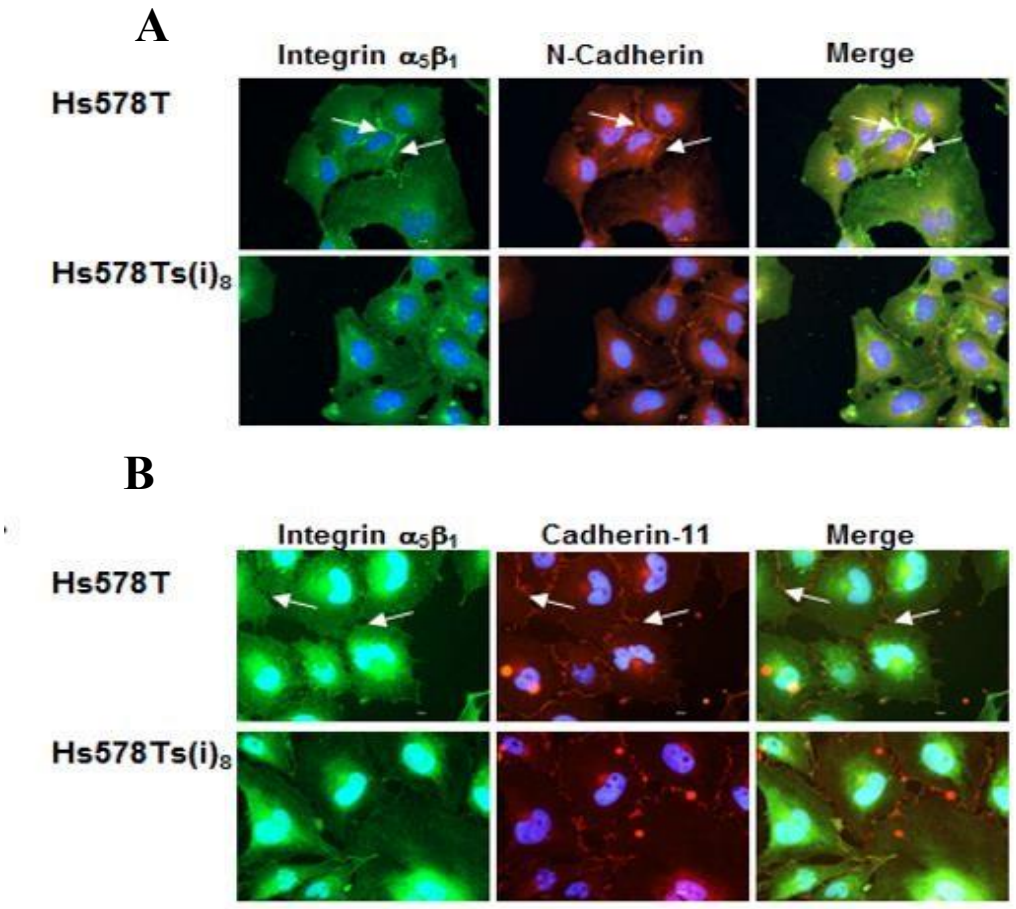

Figure 17 Dual staining of Integrin alpha5 beta 1 with N-cadherin and alpha 5 beta 1 with cadherin 11. Integrin was fluorescently tagged with a mouse 488 secondary antibody and shown in the green channel, while N-cadherin/Cadherin-11 can be seen in the red channel and was fluorescently tagged with a secondary rabbit 594 antibody.

3.1.7 Replicating result in other TNBC cell lines

Treatment of function-blocking antibodies (GC-4, JBS5, and 16G5) were tested on other TNBC cell lines on cell-cell aggregation, adhesion to fibronectin and protein

immunoprecipitation. The cell-cell function integrins were tested on both the MDA-MB231 and MDA-MB-436 through the use of function-blocking antibody JBS5 and it demonstrated to disrupt the adherence of cadherin complex in the MDA-MB-231, while no such effect was observed in the MDA-MB-436 Figure 18. Since MDA-MB-436 does not express cadherin-11, cadherin-11 function as cell adhesion sites were tested on the MDAMB-231 TNBC cell line and there were no observed significant changes. On the other hand, both cell lines adhesion to fibronectin decreased almost by half when treated with function-blocking antibody (JBS5) Figure 18. Cadherin-11 effect on fibronectin adhesion was tested but it did indicate any conclusive results that it effected cell-matrix adhesion (data not shown). Moreover, to further prove the association n between a5b1 and cadherin11 in MDA-MB-231 a co-IP was performed and demonstrated a positive association as shown in Figure 19.

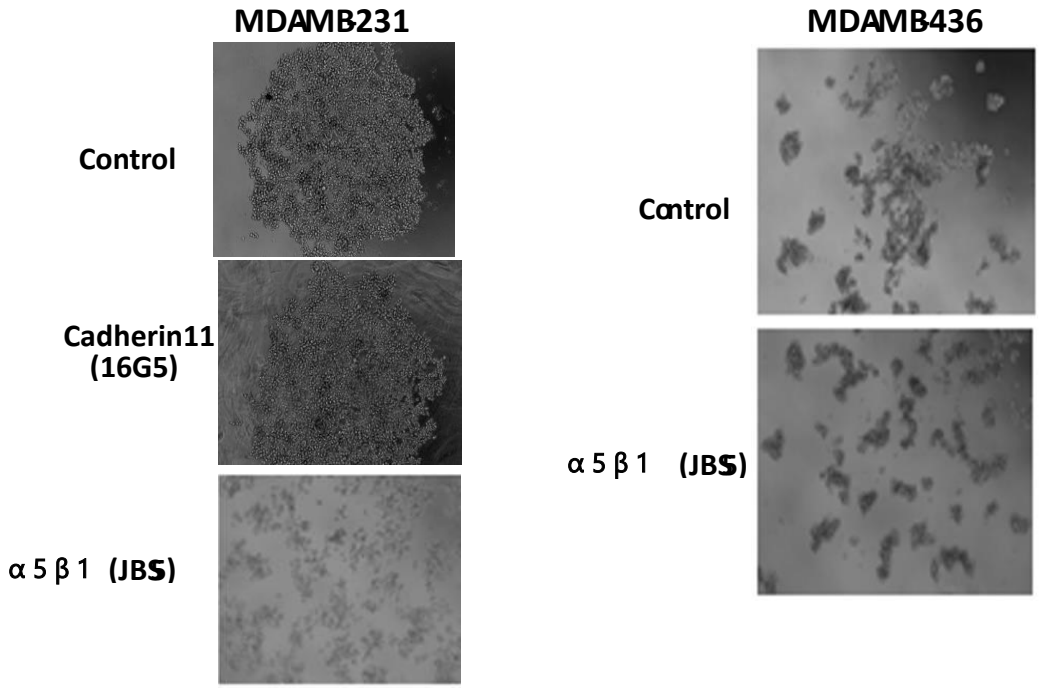

Figure 18 Cell aggregation assay with function-blocking antibodies for A5B1 and Cadherin-11. The function-blocking antibody JBS5 and it demonstrated to disrupt the adherence of cadherin complex in the MDA-MB-231, while no such effect was observed in the MDA-MB-436.

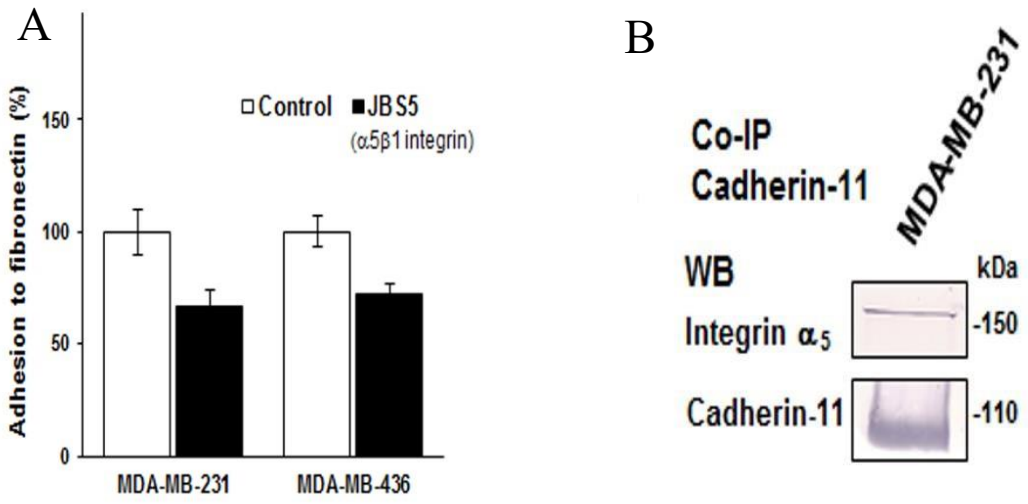

Figure 19 A) Fibronectin Adhesion of MDA-MB-231 and MDA-MB-436 with function-blocking antibody JBS5. B) Co-immunoprecipitation of Cadherin 11 showed association to integrin alpha 5.

3.2.1 RTKs' novel orphan receptors involvement in migration and invasion

Receptor Tyrosine Kinases are important participants in cancer progression and behavior. RTKs are responsible for responding to environmental cues that initiate and influence various signaling pathways that are responsible for cell migration; involving small GTPases kinases, cytoskeleton modifying proteins, and motor proteins (Welf and Haugh 2012). In the present study, the common RTKs such as epidermal growth factor (EGFR) and platelet-derived growth factor receptor (PDGFR) were barely detected, instead three novel orphan like receptors were identified with in the isogenic cell model Hs578T/Hs578Ts(i)$_8$: Ryk, Axl, and ROR2. As shown in Figure 20, the phosphorylation of these kinases was detected through a receptor tyrosine kinase human proteome array kit on the isogenic cell model and revealed higher tyrosine phosphorylation of these kinases in the parental Hs578T cell line in comparison to the invasive Hs578Ts(i)$_8$. EGFR and

PDGFR lightly was detected in the parental cell line but was not detectable in the invasive cell line. These studies led to investigate the role these kinases play on the migratory and invasive behavior of these cell lines.

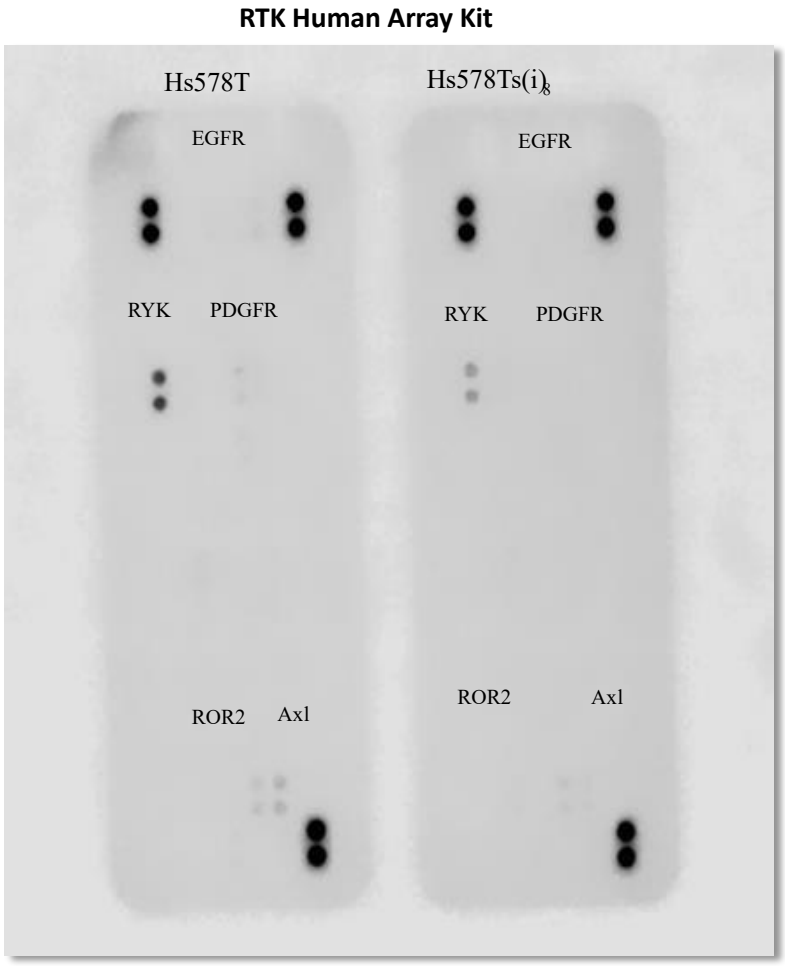

Figure 20 Receptor Tyrosine Kinase Human Proteome Array Kit on the isogenic cell model and revealed higher tyrosine phosphorylation of these kinases in the parental Hs578T cell line in comparison to the invasive Hs578Ts(i)8.

3.2.2 Confirmation of Orphan receptor by P-tyrosine-100 co-IP

To further confirm these finding a co-Immunoprecipitation (co-IP) of phosphorylated-tyrosine 100 was performed to detect the presence of these receptors. As demonstrated in Figure 21 the co-IP of p-tyrosine-100 confirmed higher detection of Ryk, Axl and Ryk on the parental Hs578T cell line in comparison to the invasive variant Hs578Ts(i)$_8$.

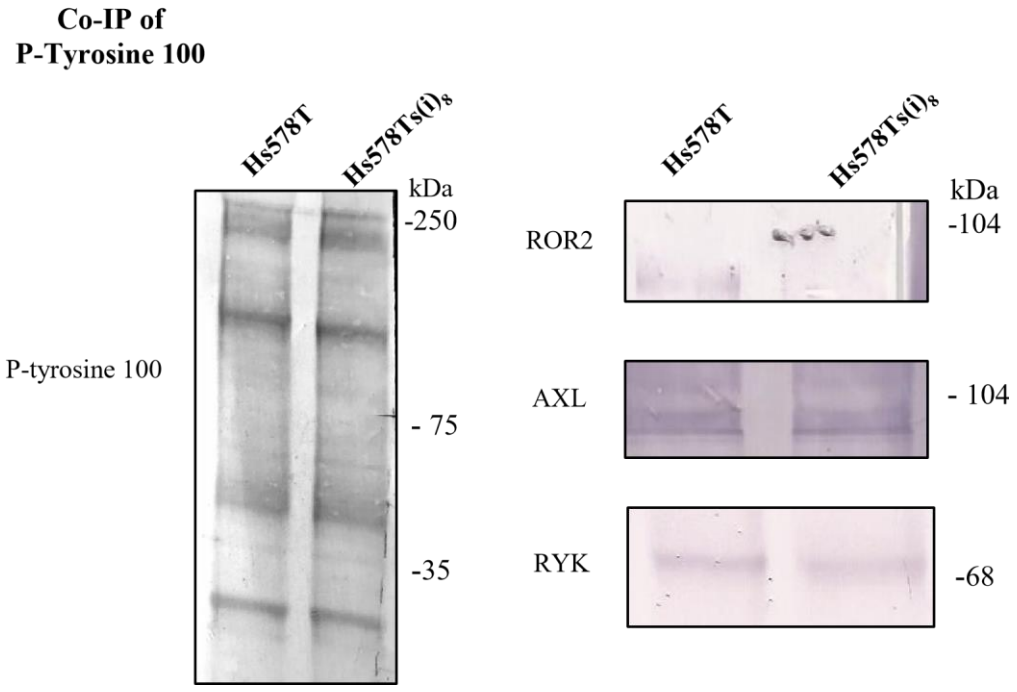

Figure 21 Co-Immunoprecipitation (co-IP) of phosphorylated-tyrosine 100 was performed to detect the presence of these receptors- ROR2, Axl and RYK. The presence of these receptors confirmed higher on the parental Hs578T cell line.

3.2.3 Localization of these RTKs in the isogenic Hs78T/Hs578Ts(i)$_8$

Due to the difference phosphorylation activity of these RTKs in these cell lines, it led us to investigate their localization in the cells, to visualize a correlation between their

spatiotemporal localization and its activity in these cells. Immunofluorescent images were taken in an epi scope following immunocytochemistry fluorescent techniques to visualize the spatial localization of these molecules: Ryk, Axl and ror2, respectively. As observed in in Figure 22: a) Ryk was observed a cell-cell junction sites as well as blebbing areas in the cell membrane in the parental cell line, meanwhile in the invasive cell line Ryk appeared more organized in the lines at the leading edge and in area of cell-cell contact. In Figure 22:

b) Axl was observed to be around the nucleus and also at cell-cell contact site, however, it was very interesting to observe less of Axl in the invasive cell line, and lastly c) ROR2 seem to cluster around the nucleus and be present at the leading edge of polarized cells, while in the invasive its seem more concentrated in the front of the cell and is less puncta in comparison to the parental cell line. The antibodies used for fluorescent immunostaining were not specific to the observed phosphorylation in the RTK array. These images demonstrate a global signaling of both active and inactive form of these orphan receptors.

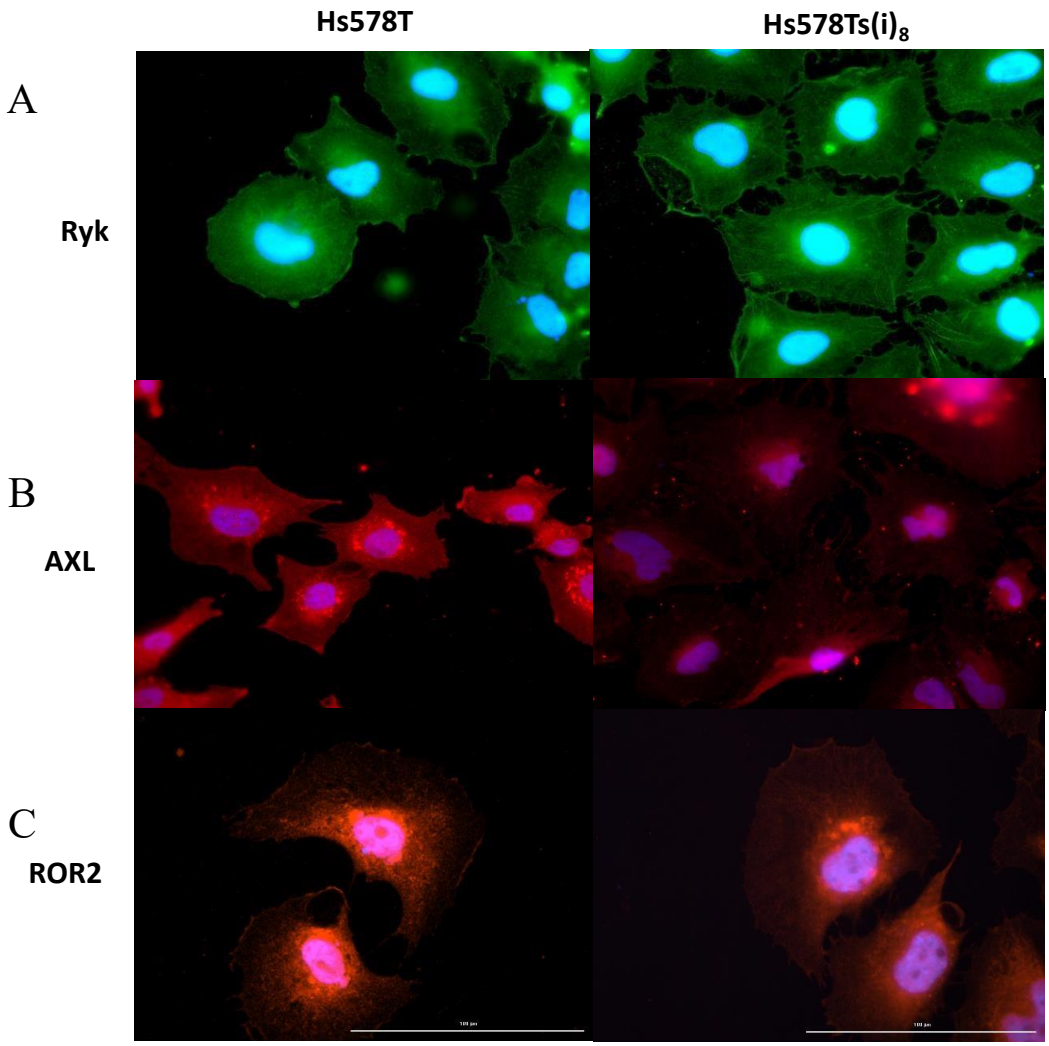

Figure 22 Single staining of RYK, AXL and ROR 2 on Hs578T and Hs578Ts(i)8.

3.2.3 Understanding Ryks role in the isogenic model Hs78T/Hs578Ts(i)8

Due to limited availability of antibodies at the time, orphan receptor tyrosine kinase Ryk became the main focus in this study. The research does not reveal significant data correlated between Ryk and TNBC migration and invasion, however, studies revealed an association between Ryk and WNT5a and prostate cancer (Koushyar, et al. 2022). This involved led the research to investigate Ryks association with the Wnt/B-catenin pathway. A co-IP of Ryk was performed and positively expressed an association with WNT5a. As

show in Figure 22 Ryk protein expression was observed slightly higher in the parental cells Hs578T while the protein expression wnt5a in association with Ryk ws slightly higher in the invasive variant Hs578Ts. These results not show that Ryk in TNBC is involved in the WNT/B-catenin pathway but it's phosphorylation can be associated with wnt5a function. Based on the results from the RTK human proteome array, Ryk tyrosine phosphorylation in the parental cell line is higher in the parental than in the invasive cell line and this correlation may be correlated to higher expression of wnt5a in a co-IP of Ryk.

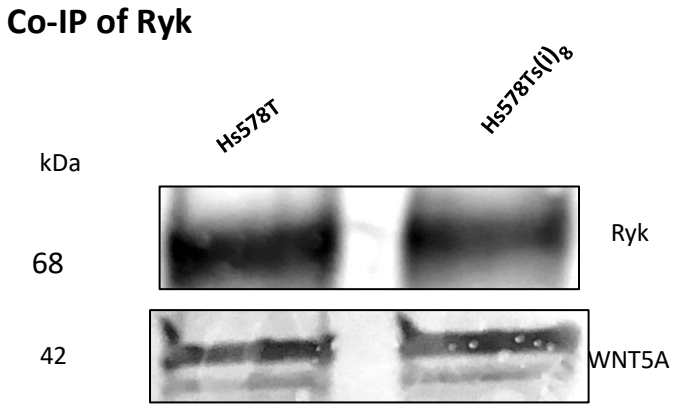

Figure 23 Co-immunoprecipitation of Ryk on Hs578T/Hs578Ts(i)$_8$ and its association with Wnt5a confirmed with the western blot.

3.2.4 Crosstalk between Ryk and Wnt5a

This hypothesis was further analyzed with immunofluorescence experiments that are seen in Figure (24) Ryk is observed in cell-cell contact and and throughout the cell membrane, wnt5a is expressed at cell-cell contact and near the nucleus in the parental cell line, co-localization of these molecules is not as evident in comparison to the invasive cell line. Wnt5a and Ryk seem more organized and puncta in the invasive cell line, they appear closely together at cell-cell junction, lamellipodia membrane extensions. These images

along with IP indicate that Ryk may be involved with the Wnt/B-catenin pathway and can influence on migration and invasion.

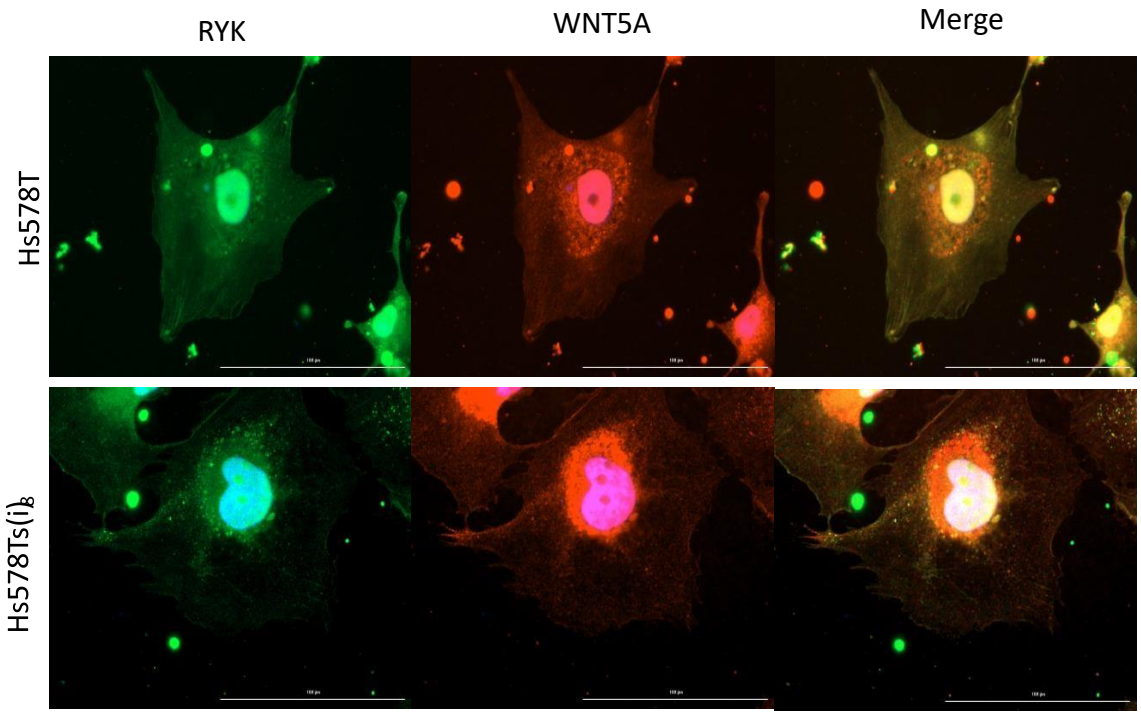

Figure 24. Dual Staining between Ryk and WNT5a on Hs578T/Hs578Ts(i)8. Ryk is shown in green channel *and WNT5a is shown in the red channel. When merged association on Wnt5a and Ryk seem more organized and puncta in the invasive cell line, they appear closely together at cell- cell junction, lamellipodia membrane extension.*

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3.2.5 Ryk siRNA knockdown

To further investigate Ryk's function in the Wnt/B-catenin pathwy as well as its role in invasion and migration, siRNA knockdown of Ryk was attempted multiple times and it

was not successful Figure 25. As it can be observed in Figures A & B applying sirna knockdown using lipofectamine 300 at different concentration and it was not successful. Western blot and cell-aggregation assay showed there were no differences in protein expression after a sirna knockdown. These experiments were done at different time points and sirna concentration. Due to limited funds and resources this level of experimentation was not continued.

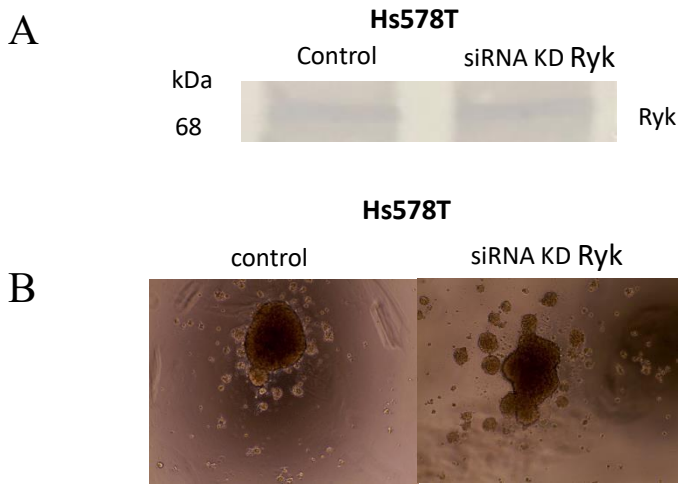

Figure 25 A) Western Blot of siRNA knock down of Ryk on Hs587T. B) Cell aggregation of siRNA knock down of Ryk.

3.2.5 Ryk's involvement with CAMs

Despite not being able to successfully knockdown Ryk protein expression and asses it's function within the Hs578T/Hs578Ts(i)8 cell model, Ryk's preliminary spatiotemporal localization indicates it's involvement in cell-cell junctions sites and lamellipodial

extensions of migrating cells. Consequently, co- immunoprecipitations, immunofluorescence imaging, cell-aggregation experiments were performed ot evaluate possible crosstalk between Ryk can migratory molecules like cadherin-11, N-cadherin and cell-matrix integrins a5b1 and avb5.

3.2.6 Ryk's involvement cell-cell adhesion isogenic cell model Hs578T/Hs578Ts(i)$_8$

Primarily cells were treated with a panel of Ryk antibodies and then seeded to agar 96 well plate, from all the antibodies polyclonal Ryk PA-5 antibody showed to inhibit the binding of Ryk and caused loose compact aggregates in the parental cell lines, while on the invasive cell line no significant changed occur. These results, as shown on Figure 25 demonstrated that indeed Ryk is involved in cell-cell junctions and inhibition of this protein can alter its phenotype behavior and promote invasive like behavior.

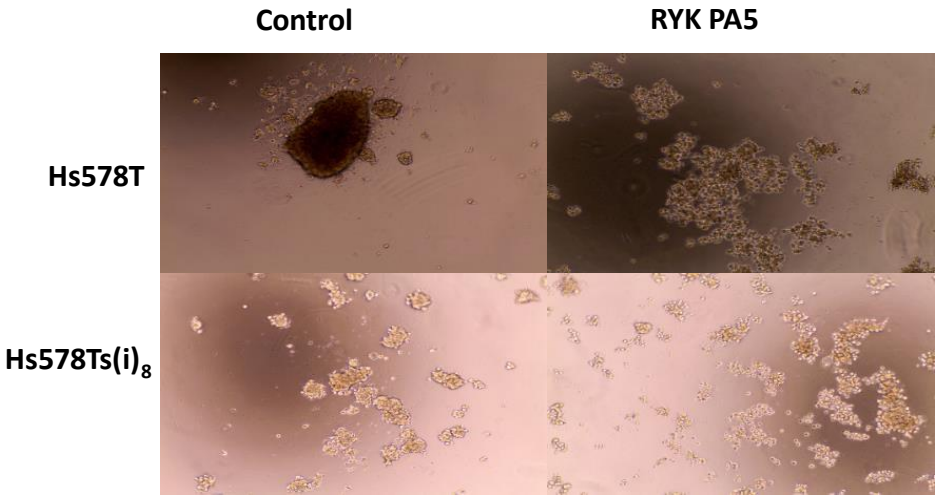

Figure 26 Cell aggregation of function-blocking antibody of RYK PA5 on Hs578T/Hs578Ts(i)$_8$.

3.2.7 Ryks crosstalk with cadherins

This was further tested by performing a series co-IP as shown in Figure 27 of RYK, Cahderin-11 and Ncadherin; all these experiments were done three times each. As observed in Figure 28 B these molecules are all closely associated with one another, this further illustrated an illustration that this orphan receptor Ryk is involved cadherins and it can hypothesize that Ryks interaction in cell-cell junction maybe involved with its phosphorylation, which cannot be directly tested due to limited commercial antibodies at the moment. However, dual immunostaining of Ryk with cadherin-11 and N-cadherin further visually show the relationship between them.

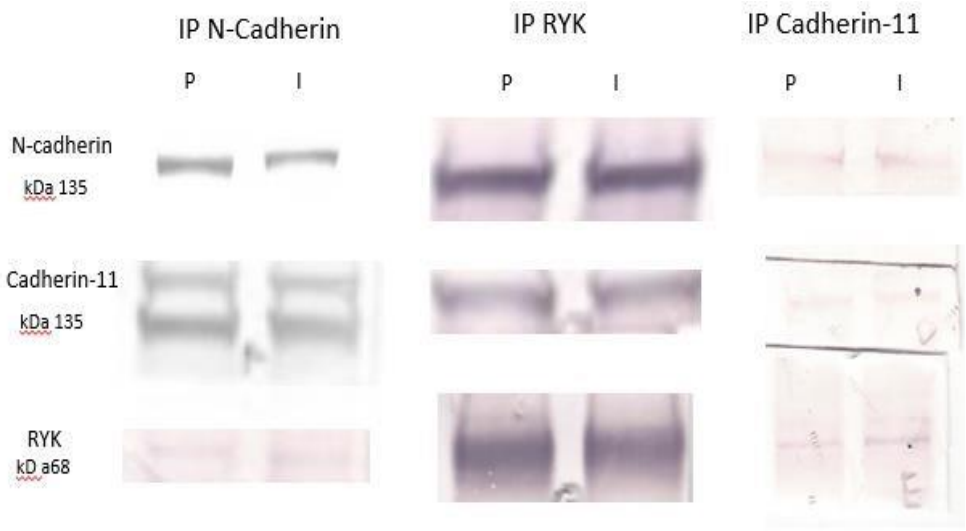

Figure 27 Co-immunoprecipitation N-cadherin, RYK and Cadherin-11 to determine crosstalk's between each other on Hs78T and Hs78Ts(i)8 cell lines.

As shown in Figure c dual immunostaining was performed in Ryk is visualized in the green channel and cadherin-11 in the red channel, when images are merged (can be visualized in a larger scale in Figure 28 C the co-localization is observed in both cell line but the spatiotemporal localization and visual concentration of these molecules differ in each cell line. Ryk is seen a cell-junction sites but it does not co-localize with cadherin-11 it shows to cluster right behind the adhesion but close enough to be working together. There is some indication of co-localization of cadherin-11 and Ryk at membrane extensions (lamellipodia). While in the invasive cell line Ryk appear to be very organized almost like in cargo across actin filaments, and more co-localization between cadherin-11 and Ryk at cell membrane extension. Taking a closer look to image d in the invasive Hs578Ts(i)$_8$ Ryk does not co-localize with cadherin-11 at cell-cell junction but does collocalize near the edge of cell-cell junction.

As for Ryk and N-cadherin the same is observed, as shown in the solid white arrow on image d Ryk and N-cadherin appears to co-localized at membrane extension, but the cell-cell connections are made N-cadherin in the parental cell line. When observing ncadherin and Ryk in the invasive, Ryk is continuing to be seen in these tracts, but is lees associated with N-cadherin at cell-cell junction sides in comparison to the parental cell line. Based on these findings, Ryk is close enough to cadherin-11 and N-cadherin in the parental cell line to cause a disturbance in cell-cell adhesions. This novel orphan receptor Ryk shows indication to be involved in cell-cell adhesion and may be participate as a facilitator to membrane extension needed for cadherins to make connection with neighboring cells.

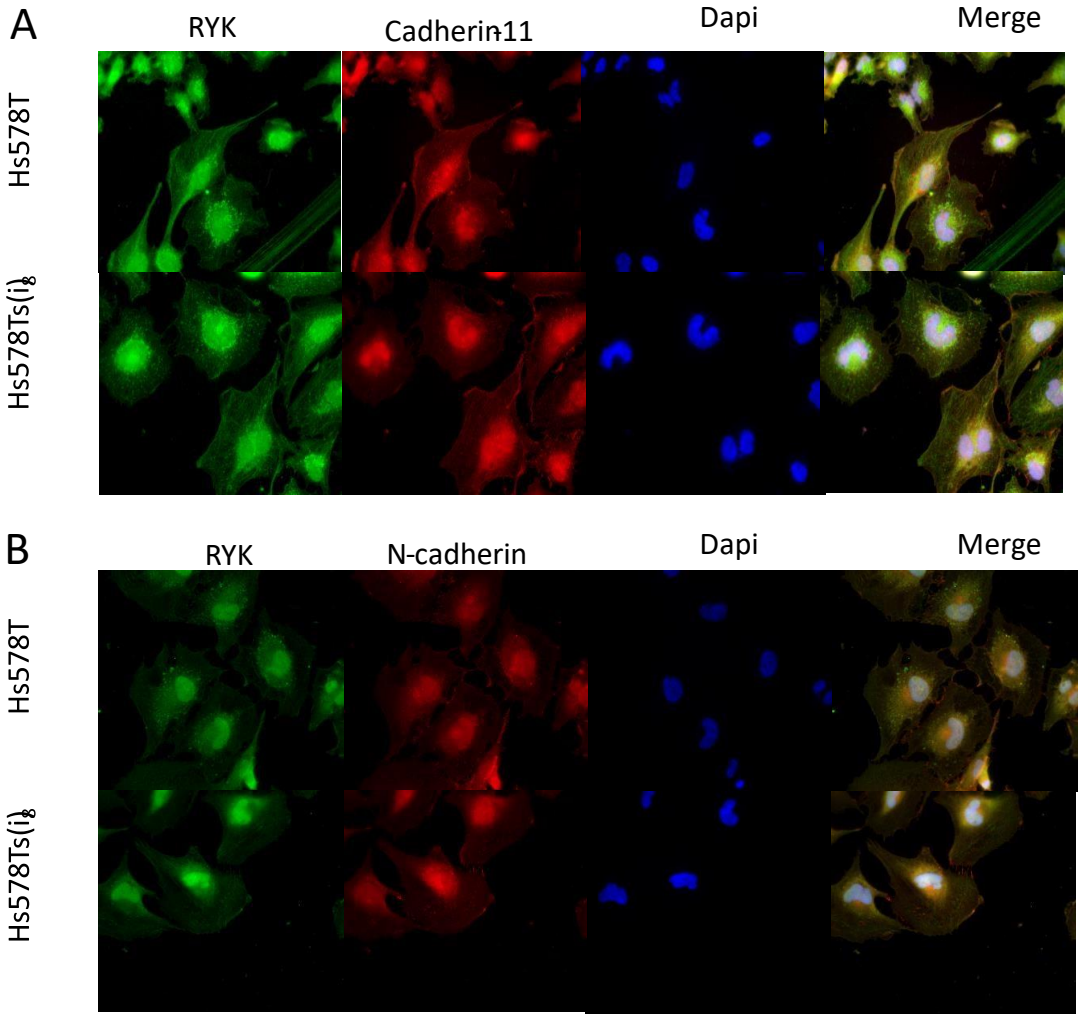

Figure 28 Dual immunostaining was performed in A) Ryk is visualized in the green channel and cadherin-11 in the red channel. B) Ryk is visualized in the green channel and N-cadherin in the red channel.

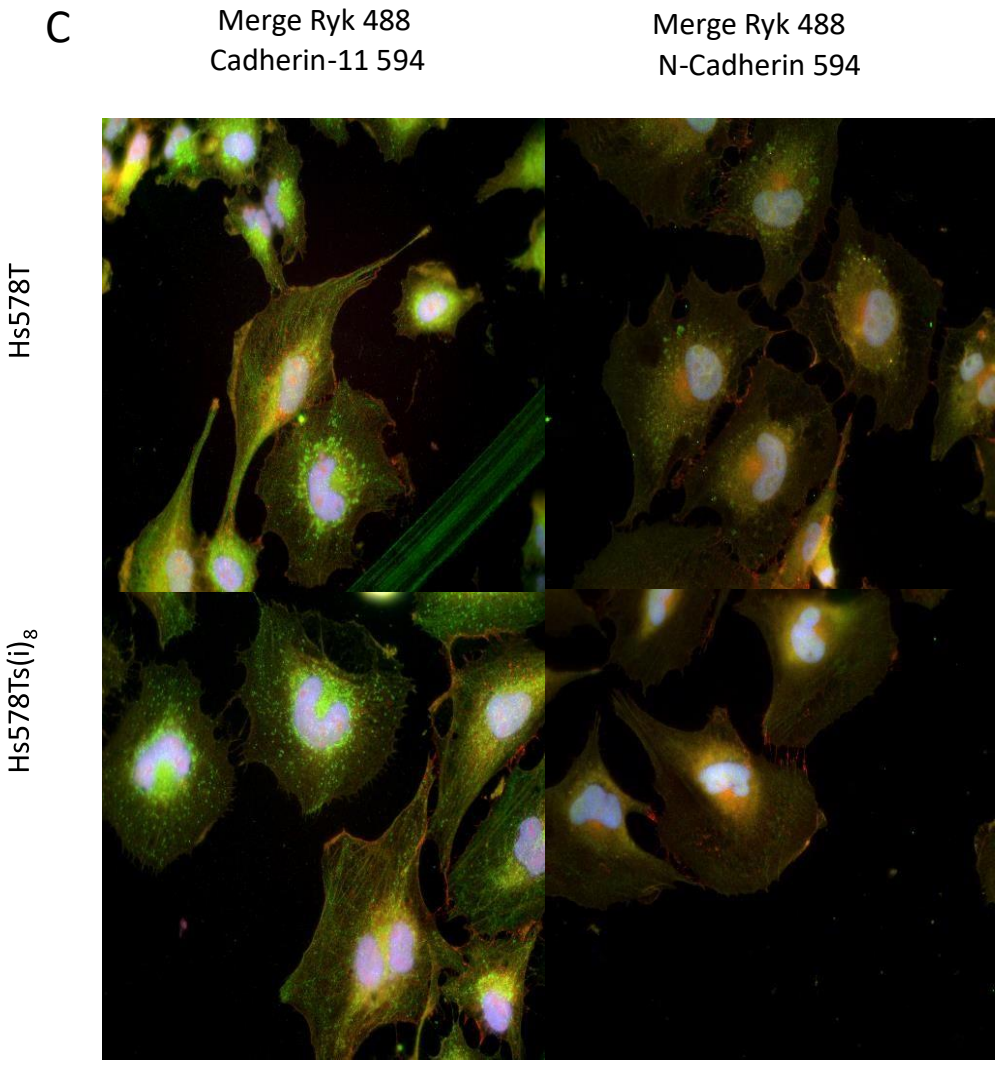

Figure 29 Close up of the dual immunostaining was performed in A) Ryk is visualized in the green channel and cadherin-11 in the red channel. B) Ryk is visualized in the green channel and N-cadherin in the red channel.

3.3.1 Migration and Blebbistatin Inhibitor on TNBC

Wound heal assay were performed on HS578T/Hs578Ts(i)$_8$ and it demonstrated that the invasive cell line Hs578Ts(i)8 cell line was twice as migratory than the parental cell line as shown in Figure (30). This focused the attention to actin cytoskeleton complex to determine any significant difference between the TNBC isogenic cell lines. Blebbistatin, an inhibitor of myosin-II-specific ATPase, has been used to inhibit contraction contains non-muscle myosin (Liu, et al. 2010) and it is being used to determine potential effects of on the adhesive and migratory behavior of the Hs578T and Hs578Ts(i)$_8$. To determine the effect this inhibitor on these cells 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromidete trazolium reduction (MTT) assay were performed with concentration ranging between 10-100uM (data not shown) and showed no effect on the
cell viability.

A wound heal assay was performed on the Hs578T/Hs578Ts(i)$_8$ and demonstrated to affect the migratory effect on the invasive cell line. As shown in Figure (30) the addition of 10uM Blebbistatin increased the invasive Hs578Ts(i)$_8$ cell line by 30% but even more effectively increased the parental Hs578T cells with a 47% as compared to the DMSO controls. This experiment was replicated on the MDA-MB-231 cell lines, and it doubled it migratory rate when added 10uM concentration of blebbistatin in comparison to the DMSO controls as shown on Figure 31.

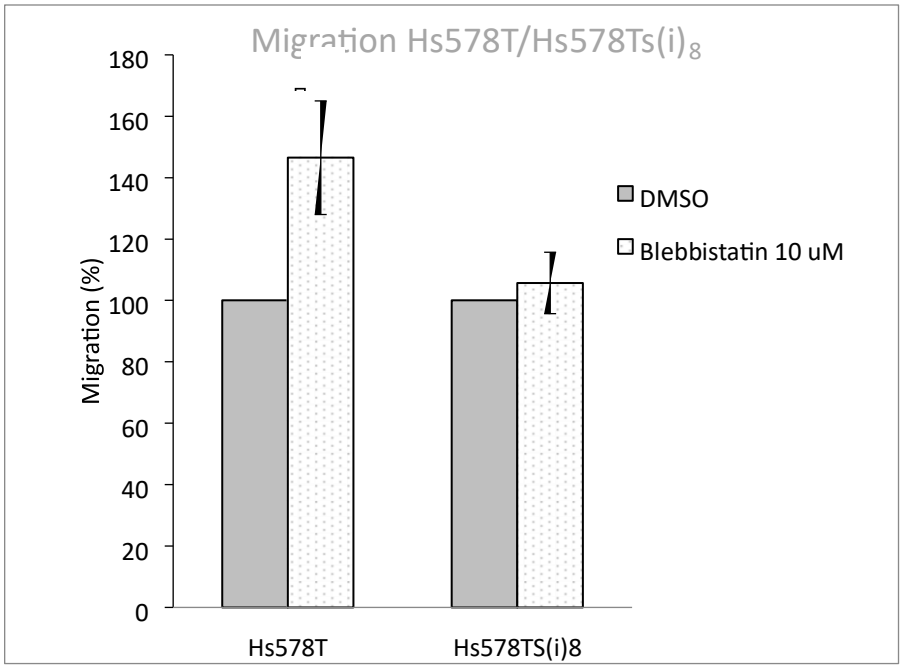

Figure 30 A wound heal assay was performed on the Hs578T/Hs578Ts(i)8 and demonstrated to affect the migratory effect on the invasive cell line with or without blebbistatin 10uM.

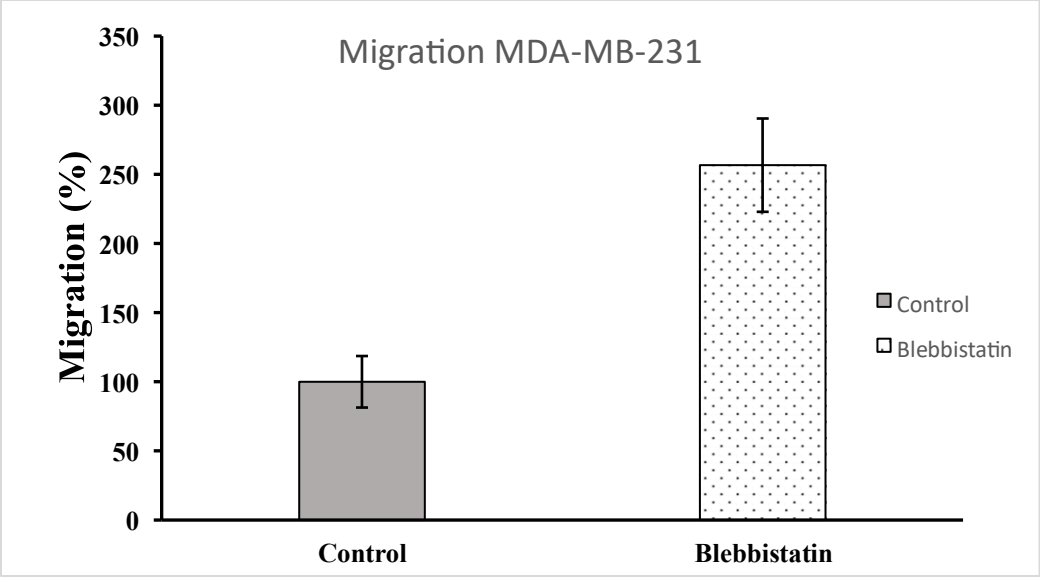

Figure 31 A wound heal assay was performed on MDA-MB-231and demonstrated to affect the migratory effect on the invasive cell line with or without blebbistatin 10uM.

3.3.2 Blebbistatin cell-cell adhesion effect on Hs578T/Hs578Ts(i)$_8$

Due to the affect blebbistatin to increase migration, it can be hypothesized that the inhibition of the ATPase site on Myosin II can affect the cell-cell adhesion sites. Therefore, treatment of 50uM of Blebbistatin resulted in a significant loss of compact cell-cell aggregation and the formation of smaller, loose aggregates in the Hs578T cells and further disrupted the Hs578Ts(i)$_8$ cell aggregates as shown in Figure 32.

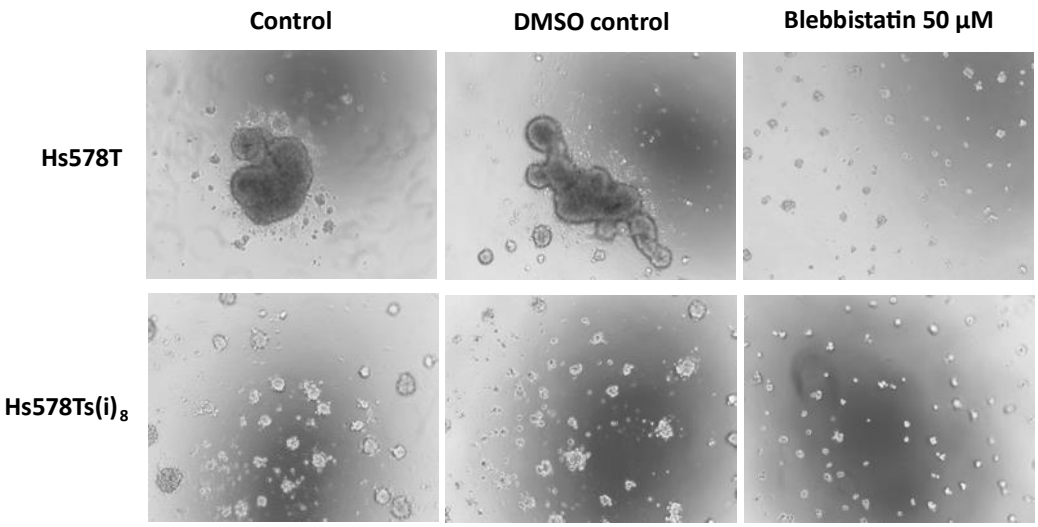

Figure 32 A cell aggregation assay was performed on MDA-MB-231and demonstrated to affect the migratory effect on the invasive cell line with or without blebbistatin 10uM.

3.3.3 Visualization of effect of Blebbistatin treatment

Imagining the effects of blebbistatin treatment (50uM for 10 mins) on the Hs578T cells showed more organized NMII-A and filamentous patterns as compared to the punctate and less filamentous pattern in the untreated and solvent-treated Hs578T cells. The Hs578T blebbistatin treated cell's structure are comparable to the invasive Hs578Ts(i)$_8$ that were grown under normal growth conditions as shown in Figure 33.

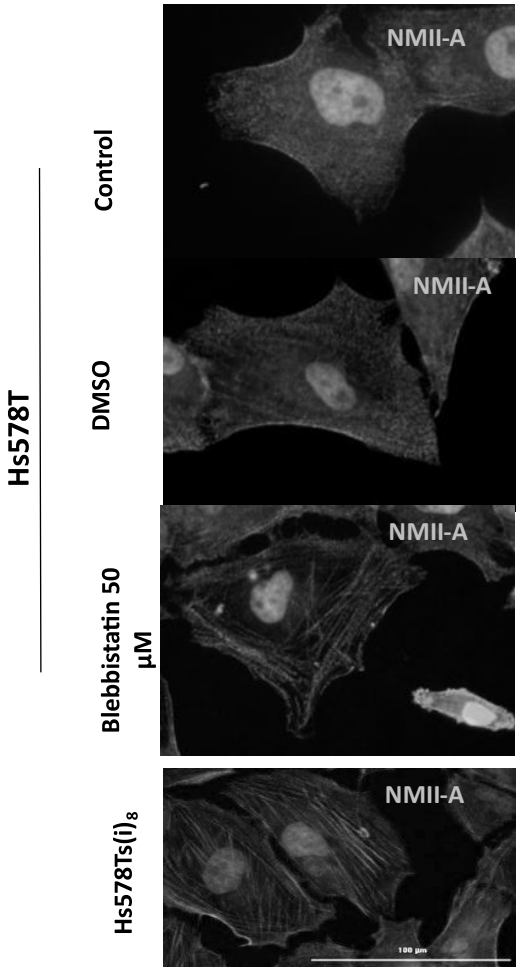

Figure 33 Imagining the effects of blebbistatin treatment (50uM for 10 mins) on the Hs578T cells showed more organized NMII-A and filamentous patterns as compared to the punctate and less filamentous pattern in the untreated and solvent-treated Hs578T cells.

3.3.1 Blebbistatin effect on F-actin

The changes that were observed in the NMII-A filament formation treated with Blebbistatin were further examined in view of the actomyosin cytoskeletal organization in Figure (34). These studies showed a pattern of reorganization in the parental (Hs578T) cells when treated with 50uM of Blebbistatin for 10 mins. This can be seen close detail in fig 34 DMSO solvent treated shows to have F-actin formation but not as filamentous as the invasive variant (Hs578Ts(i)$_8$) and 50uM Blebbistatin treated cells. These studies were also performed on the MDA-MB-231 cells and it also changes the filamentous patterns of F-actin when treated with 50 uM concentration of Blebbistatin for 10 mins in comparison to solvent-treated (MDA-MB-231 DMSO). These studies suggest that Blebbistatin induces reorganization of the cytoskeleton effect causing a disruption at cell-cell adhesion and migratory properties.

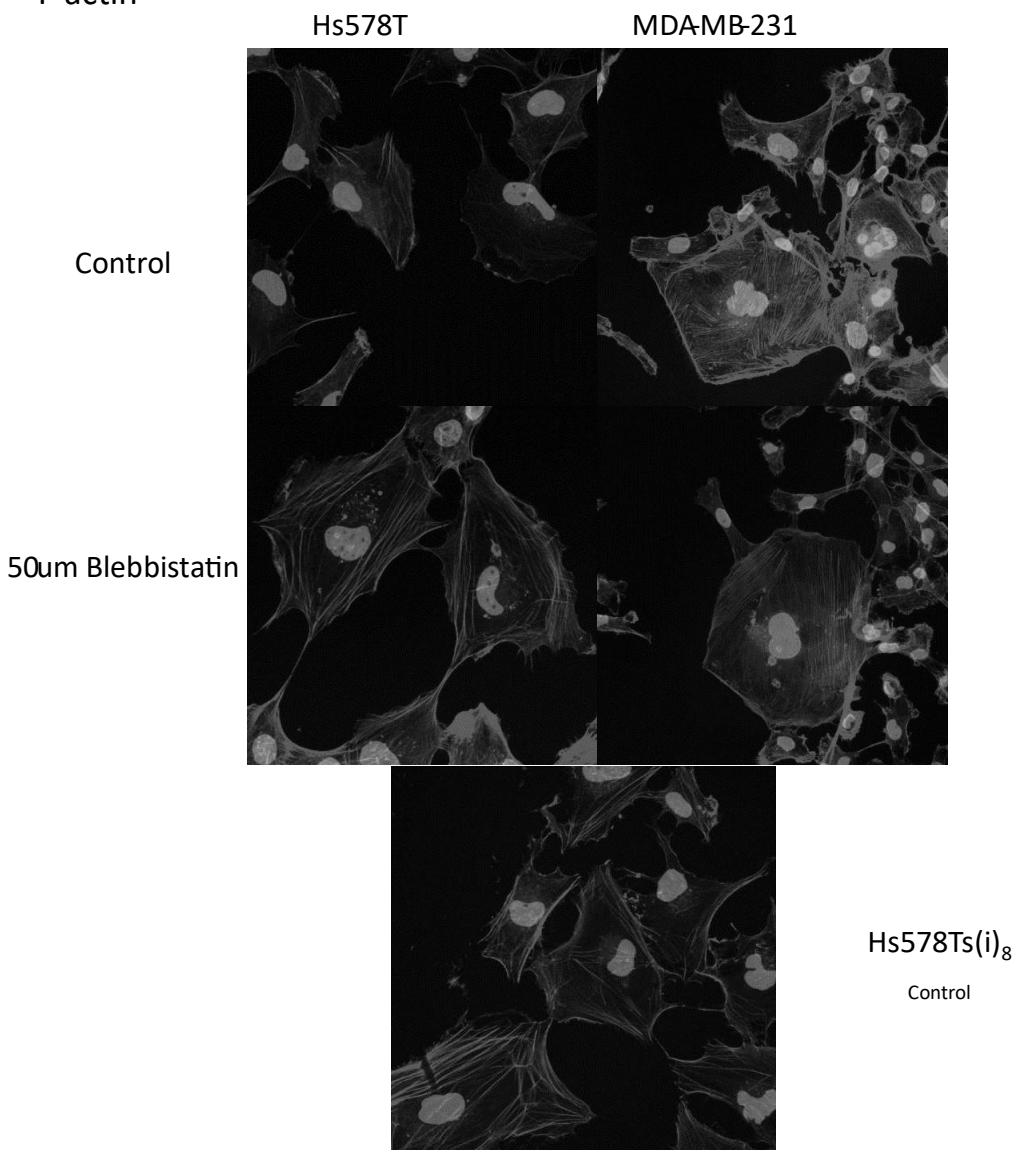

Figure 34 Blebbistatin treatment examined on the actomyosin cytoskeletal organization on Hs578T/Hs578Ts(i)8.

3.3.4 Co-IP NMIIA and NMIIB with cell adhesion molecules

Based on previous results suggests that blebbistatin has a major effect on Hs578T and MDA-MB-231 TNBC cells stimulating an invasive phenotype like the Hs578Ts(i)$_8$ cells. Co-IP of both NMIIA and NMIIB were formed, and they expressed N-cadherin which higher expressed in the parental Hs578T, and receptor tyrosine kinase orphan receptor Ryk with equal protein expression was detected. Cadherin-11 was hardly detected, to, to confirm these results an immunoprecipitation against antibody cadherin-11 was performed to detect NMIIA and NMIIB and as shown in Figure 35 it was detected, and protein association was higher in the parental Hs578T. This pattern was also observed in a co-IP of Ryk where both NMIIA and NMIIB association were highly expressed on the parental cell lines.

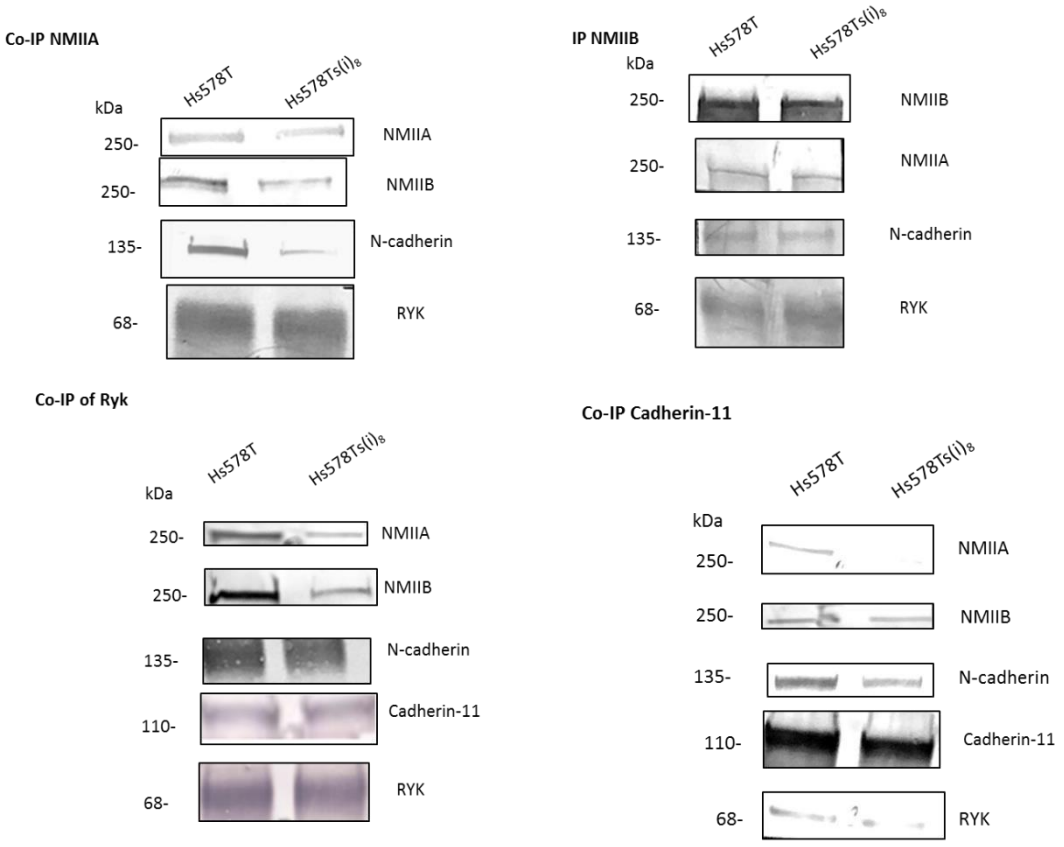

Figure 35 Co-immunoprecipitation of NMIIA, NMIIB, RYK and Cadherin-11

3.3.5 Confocal Imaging of CAM molecules with Blebbistatin treatment

Given these results, non-muscle myosin proteins are associated with these cellcell adhesion molecules and inhibition of NMII cause a re-organization of patter similar to the invasive subtype Hs578Ts(i)$_8$. It is hypothesized that blebbistatin treated cells causes a reorganization of structure in the cell's phenotype and the association of these cam molecules with NMII will cause a change localization and function. Therefore, confocal imaging was done on these cells with 50uM blebbistatin treatment after 10 mins. All images were taken on the confocal microscope in Bio stress at South Dakota State University, all experiments had positive and negative controls.

3.3.5.1 Spatiotemporal Images of Ryk treated with blebbistatin

Primary antibody RYK (PA5) was used along with secondary rab594 and imaged (fig 36) shows Ryk punctate throughout the cell with a higher concentration towards the back of a polarized cell. When these cells were treated with 50uM blebbistatin for 10 mins, this molecule was shown to be in the leading edge in a polarized cells and had the punctate appearance towards the back and this resembles what is observed in the invasive solvent-base coverslip. This was also performed on MDA-MB-231 cells and the opposite was observed, Ryk was punctate and scattered through the cells and the highly concentrated towards the back of a polarized cell.

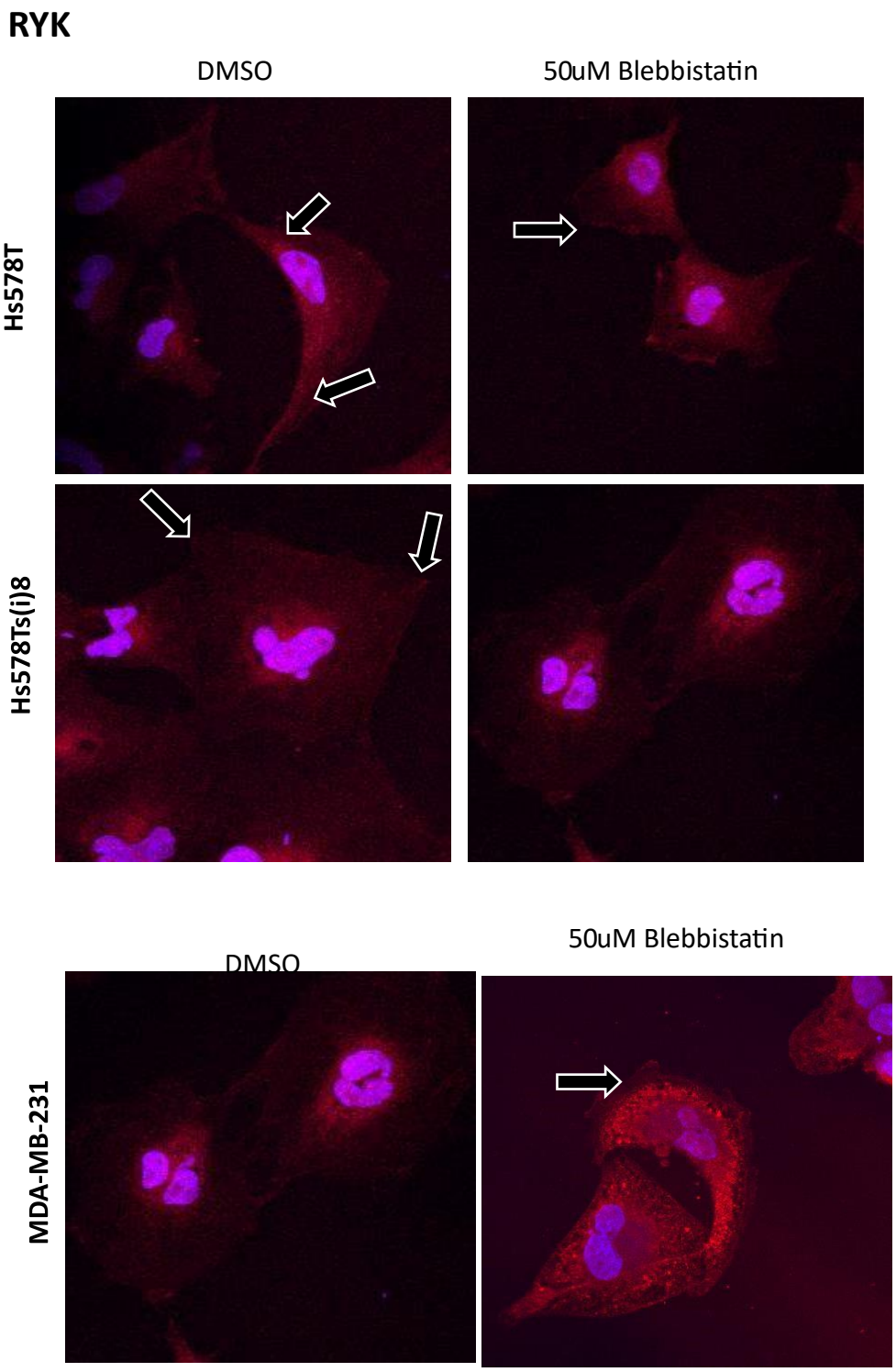

Figure 36 Confocal Images of Ryk treated with blebbistatin on Hs58T/Hs578Ts(i)$_8$ and MDA-MB-231.

3.3.5.2 Spatiotemporal Images of Cadherin--11 treated with blebbistatin

Confocal imaging of Cadherin-11 was also performed, and we observed positive signals at cell-cell adhesion sites and some member extension while 50uM treated blebbistatin, cluster cadherin-11 to fore-front of cells and less on the cell-cell adhesion site similar to invasive Hs578Ts(i)$_8$ as indicated with solid white arrows Figure 36. These results were also performed on the MDA-MB-231 cell line were Ryk is punctate in the solvent-treated coverslip, in comparison to when is treated with 50uM blebbistatin for 10 mins, more clustering occurs in the forefront of the cell on Figure 37.

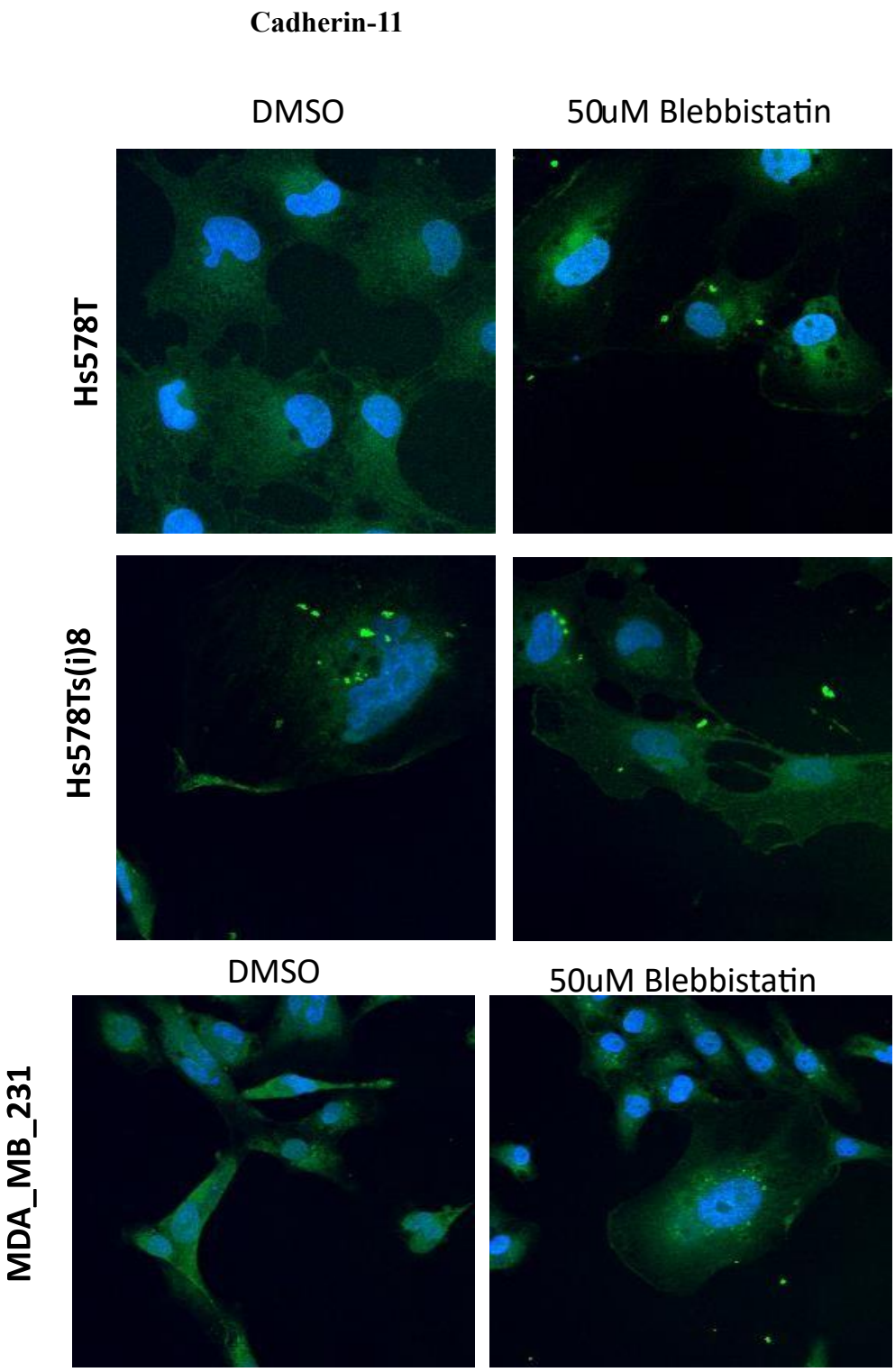

Figure 37 Confocal Images of Cadherin-11 treated with blebbistatin on Hs58T/Hs578Ts(i)$_8$ and MDA-MB-231.

Chapter 4: Discussion

Cancer metastases require the loss of cell-cell adhesion and the ability of the cell to migrate. For decades research has attempted to understand this phenomenon in greater detail and this study provides an elegant in vitro cell model system to study metastatic TNBC by using the isogenic cell line Hs578T/Hs578Ts(i)$_{8.}$ This isogenic cell pair resembles what is occurring in the human body when primary tumor cells undergo biochemical changes, and it migrates to different organs and metastasizes. Understanding the differences between the parental Hs578T cells and it invasive Hs578Ts(i)$_8$ provides new insight in understanding the cell-cell and cell-matrix disturbances for invasion and migration to occur. It's hypothesized that these molecules have functional differences between the isogenic cell line Hs578T/Hs578Ts(i)$_{8.}$ These molecules were studied using various techniques and methods such as behavioral assays, western blots, co-immunoprecipitation, and immunofluorescence microscopy. After analyzing the results, the data suggest that these molecules have different function between the parental cell vs the invasive cell line.

TNBC cell lines do not express the typical E- and P-cadherin, as illustrated in Table 1. However, these cells still retain weak calcium-dependent adhesion, which indicates the presence of another cadherin member, leading to the search of other cadherin family, specifically as investigated with TNBC cell lines Hs578T/Hs578Ts(i)$_8$, MDA-MB-231 and MDA-MB-436, which confirmed no detection of TNBC. Moreover, the isogenic cell line had higher protein expression of N-cadherin and Cadherin-11 in the parental Hs578T cell line in comparison to the invasive cell line Hs578Ts(i)$_{8.}$ The

difference between expression was further tested in various experiment to demonstrate the interruption at cell adhesion sites. Treatment with function-blocking antibodies of N-cadherin and Cadherin-11, JBS5 and 16G5 respectively, in cell aggregation is shown in Figure 10: the parental Hs578T formed loose disperse aggregates while the invasive sub-clone showed no significant change in the in invasive Hs578Ts(i)$_8$.

Consequently, the localization of the cadherins is not only seen in the cell-cell junction sites but also in lamellipodia-like projections, which is clearly appreciated in the invasive variant Hs578Ts(i)$_8$. Cadherin-11 is considered to be a molecule for invasive TNBC and N-cadherin is associated with the loss E-cadherin in the EMT theory. Both of these proteins are highly expressed in the parental Hs578T, in comparison to its invasive variant Hs578Ts(i)$_8$ that is three time more invasive and two-and-half times more migratory.

The series of experiments conducted on cadherin and their respective localization, they possess the ability to interact with the matrix, which came into question and steered the investigation in determining possible associations between integrins and cadherins through immune detection, immunoprecipitation, and fluorescent techniques. Previous investigation serving as aide to this research determined that the invasive cell line that invasive sub clone Hs578Ts(i)$_8$ interact significantly more with several other extracellular matrices including collagen I and IV, fibronectin and laminin and of these extracellular matrices function-blocking a5b1 on fibronectin matrices reduced the interaction significantly in the invasive subclone Hs578Ts(i)$_8$ (Payan, et al. 2016). Furthermore, function-blocking integrins a5b1 and avb5 affected the overall migratory effect of the invasive cell line. In order to further this point, co-IP results demonstrated

an association between integrins a5b1 and cadherin -11, namely that disruption of a5b1 in cell aggregation assays promotes loose cell aggregates in the invasive cell line, and that N-cadherin inhibition reduces the interaction with the cell-matrix. Therefore, that integrins and cadherin work closely together as a key catalyst in cancer migration.

Many studies have shown that the behavior of cells within tissues is regulated by adhesion receptors and by growth-factors receptors such as RTK's (Chiasson-Mackenzie 2018). The phosphorylation promoted by RTK on cadherin complexes weaken adhesion by disrupting the association between cadherins complex and actin cytoskeleton (McCrea, Maher and CJ 2015). Based on these experiences, the investigation took a prudent turn to explore the behavior of receptors tyrosine kinases on these cell lines by using a Receptor Tyrosine Kinase Human Proteome array kit, shown in Figure 20. This series of assays revealed three novel orphan-like receptors in the isogenic cell model Hs578T/Hs578Ts(i)$_8$, namely Ryk, Axl and ROR2. These novel tyrosine kinases exposed higher tyrosine phosphorylation in the parental Hs578T cell line in comparison to the invasive Hs578Ts(i)$_8$, as they were tested through the use of immunofluorescence, western blots, co-immunoprecipitation. However, due to the lack of antibodies and commercial availability of these novel RTKs the study focused mostly on Ryk. This kinase expressed an association to adhesion molecules cadherin-11, N-cadherin as and integrins as shown Figure 27. By using function-blocking antibodies in a cell aggregation assay, Ryk's function may be involved in cell-cell junction and inhibition of this RTK can alter its phenotype behavior and promote it to have invasive-like behavior as shown in Figure 26. To further to measure the effect Ryk on the isogenic cell line, an

siRNA knockdown was performed but no valuable data was extracted. Further experimentation is warranted.

Lastly, Ryk's association to the actin cytoskeleton complex was explored through immunocytochemistry by using blebbistatin, an inhibitor of myosin-II-specific ATPase. Due to the lack of commercial availability of such antibodies, it made it difficult to detect how the phosphorylation of Ryk behaved in the cell line. However, the experimentation showed Ryk's phosphorylated protein expression on Hs578T is higher than the Hs578Ts(i)$_8$ and has shown to have an association cell adhesion molecules like cadherin-11 and n-cadherin. This preliminary study also dove into demonstrating Ryk localization changed location when inhibiting NMIIA. The possible rearrangement of the actin-myosin complex may change the function of cell- adhesion molecules while the receptor kinases phosphorylation found in these cell line may be responsible for the differences in Hs578T/ Hs578Ts(i)$_8$. It is the opinion of this researcher that further experimentation could be pivotal.

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Chapter 5

Student Perception of Authentic Research: A Triple Negative Breast Cancer Case Study

5.1 Introduction

3.1.1 Research Question:

1) How do student interpret concepts that require higher critical thinking?
2) How does their interpretation affect their confidence on the subject?

5.1.2 Personal Reflection:

The reason for investigating my students' perceptions on authentic research is because I want to increase my students' confidence on interpreting scientific data when taking the Advanced Placement (AP) Biology exam. It was apparent that my students had learning gaps in their scientific reasoning and inquiry as I observed their participation in school science fair projects and during my AP biology course. Several examples include students not able to distinguish the dependent or independent variables in a graph, analyze data and connect it with a biological concept.

Students that have taken my AP biology course have come with strong content knowledge in biology and are able to answer higher-order thinking questions. However, scientific inquiry and interpreting data remain something to which they struggle. If students were exposed to more scientific questions and authentic data analysis, students

could improve their ability to understand the questions scientist face when trying to answer a research question. Therefore, exposing students to thinking like a scientist, which includes experimental setup and data interpretation, the results might instill confidence in their scientific thinking.

To make this possible, I decided to create my own Case Study on Triple Negative Breast Cancer using my data from research at South Dakota State University (SDSU). I chose this topic because first- and second-year AP biology students have shown to have a challenging time understanding cell communication and its association with biotechnology. Therefore, I created a worksheet that provided background information with guided questions as you can see on (Figure 4). These guided questions were meant to resemble questions a scientist-in-training would ask while investigating a topic. Moreover, the worksheet provided a cartoon illustration of what was occurring to provide a visual aid for students (Figure 5). Lastly the case study included a breakdown of the data allowing students to interpret and present a conclusion (Figure 6). This allowed students to summarize their conclusions using the analytic questions (Figure 7) provided in the case study. To assess the students' confidence level on this research, students took a pre- and post-survey. (Figure 2 and 4 respectively).

5.1.4 Significance:

Students are generally first introduced to the scientific method concepts in their K-12 education. As students enter undergraduate level only 40% are intent on pursuing STEM degrees and less than 16% of students are awarded a degree in a STEM field (Woolley, 2018). Researchers found that the two main reasons are due to students' disappointment with the curriculum and a loss of academic self-confidence in STEM

related majors that demand highly competitive environments. This research will investigate a tool to help students increase their self-confidence regarding scientific skills in reading and interpreting data from research articles by using the Triple Negative Breast Cancer case study. Despite the importance of incorporating literature in STEM curricula, reading and interpreting data from scientific sources continues to be a daunting task for most students. This can be due to the dense material, unfamiliar terminology, complex Figures, and technical details that can overwhelm a novice reader (Campbell, 2013). This can induce stress and anxiety in students that have not been taught how to read a scientific paper and this can hamper their learning process and confidence.

My research involves students taking AP biology which is equivalent to two college biology semesters. It has been observed that most students at this level often approach a research article as they would a textbook, only focusing on the narrative of the paper as fact, with Figures secondary to the text. Even then, students who are asked to read, underline, and highlight their weekly reading often are unable to describe the reasoning behind an experiment or know how to interpret data presented in the Figures. What many students fail to recognize is that the approach to reading research articles must be different from reading a textbook. Research articles contain elements of persuasion to emphasize new findings which may be contrary to other articles. Student must examine this new information with critical eyes (Gillen, 2006). Therefore, it is important for students to be engaged in reading primary literature and so that they can learn specific strategies to analyze, interpret and evaluate research papers critically (Campbell, 2013). Moreover, in this study we evaluated how implementing a research-

based learning using the scaffolding approach toward a research paper improved the self-confidence and decreased primary literature reading resistance.

5.1.5 Literature

Constructivism Theory

Constructivism theory suggests that learners actively build their own knowledge rather than just passively absorbing information through their own experiences (Bada and Steve n.d.) (Cite Constructivism Learning Theory: A Paradigm for Teaching and Learning). While it is a theory that is not specific to pedagogy it is a learning theory found by psychology that *explain how people are able to acquire knowledge and learn*. Providing this theory to be a direct application to education in which a student can learn by relying on past knowledge to make connection with new information. For students to become active creators of their own knowledge they must be able to ask questions, explore and asses what they know. In the classroom this theory is to be put into practice through real-world-problem solving, experiments, hands- on activities, and creative play. The teacher takes the role of the facilitator in this practice. They are responsible to individually assess the student's pre-existing conception and guide them in the activity to ensure a positive outcome.

Knowing about the constructivist theory is not suffice when implementing in education. Educators must know how to properly apply it in their classroom by creating an environment that promotes active engagement in learning by allowing students to work in small groups that leads to collaboration, and elaborate discussion led by the facilitator (teacher). However, educator must rely on other essential components to assist

with the constructivist approach such as scaffolding, research base learning (RBL) and activity base learning (ABL). These approaches facilitate scenarios for educators to guide their student to engage in active learning.

Scaffolding/Scaffolds

Scaffolding methodology is commonly used as a classroom technique in which the instructors break up a lesson or concepts into two smaller units, while the educator's role is to decrease that support as students' progress through various lessons. For this to be achieved the educator must know how to provide the appropriate support. The support given from the educator can be provided in different ways this could be simply helping a student through content, or guiding a student through their thinking process or simply embedding learning strategies that will facilitate students in active learning. Clearly this could only be effective through careful plannings, performing initial assessments of student's prior knowledge and monitoring students' progress to determine which type of support the student's needs *(fig 1)* Monitoring the students' progress is key in helping

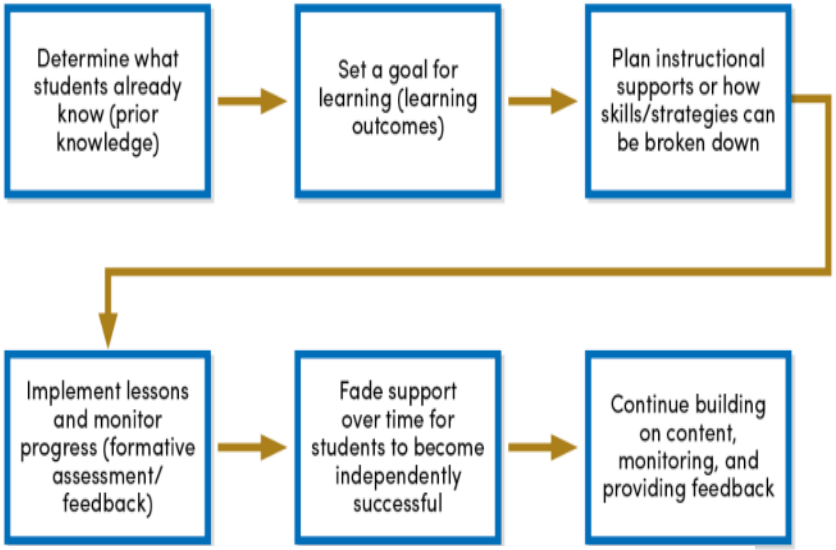


Figure 38: This is an illustration from the University at Buffalo's Office of Curriculum, Assessment and Teaching Transformation. This image demonstrated the process of using the scaffolding method overtime.

students to not only become independent thinkers but to also increasing students' proficiency and lifelong learners (Scaffolding Content , n.d.).

Moreover, is essential for the educators to motivate and encourage the student by providing them with the right amount of support for them to complete the task but also be able to support the students by using modeling, highlighting, providing hints and asking guided questions (D wood, 1976) This is done through the following six steps: 1) engaging the students intertest 2) simplifying the task 3) maintaining focus 4) highlighting key points 5) managing students' frustration 6) demonstrating paths to solve problems. This provides the students with the opportunity to complete a new task but also provides the student the opportunity to generalize a understanding to similar task. Providing reasons as to how scaffolding methods is a a pedagogical tool that follows the constructivist views.

However, over the last few decades the definition of scaffolding has evolved from educator and students and has introduced other methods that provide support to the students such tools, curricula and technology. Moreover, there has been a distinction between scaffolding process and scaffold. "Scaffolding refers to the process marked by dialogue and a sensitive adaptation of support so that the learner internalizes the necessary skills and knowledge" (Relser, 2004). Theoretically is grounded on the sociocultural approach that provide temporary graduated assistance, adult-child communication, and eventually removal of support. While scaffolds, is the tool that provides support to complete a specific task. These tools include technology, artifacts, curricula, and routines that can serve serval functions in the classroom. Technology allows us to create assignments that break downs student knowledge by implementing

the six steps to scaffolding, you can engage the students by proving real world scenarios or problems, provide guidance by providing guided questions and space for student to reflect on their understanding, encourage communication with their peers. This a form of structuring the information to disguise a complex task to a simpler form.

I decided to use this methodology in my research when creating the Triple Negative Breast Cancer article and questionnaire for my students. Prior to this activity students had studied on cell-cell communication and biotechnology through other pedagogical approaches. Due to students lack confidence in biotechnology, cell-cell communication, and scientific thinking I chose to use the scaffolding approach to provide the initial help students need to interpret scientific information. This approach broke down the background/introduction, data, and results – each section's valuable information being facilitated by providing additional support by cartoons and guided questions in the reading. All parts of the research article were included except the conclusion. This allowed students to deduce and summarize the data producing their own conclusion about the research. The result was creating *their own sense of autonomy regarding scientific reasoning.*

Research Base Learning activity (RBLA):

Research-based learning (RBL) is a constructivist-based approach because students learn through the research process— exploring, investigating, processing, and creating. In the classroom the facilitator is responsible to present the topic in a manner that promotes students to investigate and research the topic in question. The advantages in using this method in the classroom is to encourage participation, enable collaborative

learning, promote independent problem-solving seekers, and critical thinkers (Research-based learning, 2023).

This is based on the idea that students that learn through researching go through stages of open-ended research process that develop their academic and professional competences and allowing their knowledge to expand independently. Allowing for students to develop an inquisitive mind because they have learned to think beyond the curriculum and question established knowledge (Research-based learning, 2023). Research has demonstrated that RBL is a learning method that involves: contextual learning, authentic learning, problem-solving, cooperative learning, hands-on learning and inquiry discovery approach (Suntusia & Hobri, 2019). This method promotes higher-order thinking, increased motivation to learn, improved problem-solving skills and dealing with complex problems.

This approach allows students to actively explore information for important and relevant questions or challenges. Through this process students are discovering how to process, organize, and evaluate information. They are actively learning how to interpret, develop, and evaluate hypothesis and to think critically and creatively. Moreover, students develop problem solving skills as they share through verbal and nonverbal discussions, increasing their communication skills (Suntusia & Hobri, 2019).

Activity Based Learning

Activity-based learning (ABL) theory is a cognitive learning theory similar to the constructivist learning theory (Hein, 1991). Recalling that the constructivist view recognizes that each learner constructs their own knowledge and learning process based

on their previous experience. This asserts that the learning takes place when psychological environments of the students interact with a particular structure. ABL has shown to be an effective method to promote creative and critical thinking skill enhancement and it can be accomplished in four ways, engage, exploration, experimentation and expression (Anwer, 2019) (How Activity-based Learning can Help in Knowledge Retention?). By engaging the students in ABL they are involved in activities that are designed to participate students in their own learning process. Experimentation allows for students to arrive to their own solution to a given task, allowing for a broad answer. Exploration promotes student curiosity that drive students to investigate and provide information on a problem. Lastly, expression, when students are given the opportunity to be involved in the learning process, they become more confident in their findings or understanding (How Activity-based Learning can Help in Knowledge Retention?).

In my study, this approach was used in the form of an activity, in which the educator was the facilitator guiding students through the learning process. In the case study TNBC activity, students were allowed to investigate any words or concepts they did not understand as they read the articles creating an opportunity to act as an independent researcher on a topic that we had reviewed and discussed in class prior to the activity. Allowing for a deeper and more meaningful learning opportunity that required students to practice what they had learned in class and apply it to real life research. This promotes engagement and active participation in class and usage of online resources. As the facilitator in this approach, I provided students with just-in-time

guidance to interpret the information they had found helping them to make connections to content learned in class.

5.2 Method

5.2.1 Pre-Survey Questionnaire

Students will be given a survey question before starting on the case study activity. Students answered seven survey questions independently about their confidence level on cell communication and biotechnology. They were asked to rate their confidence level in answering AP Biology questions on these topics 1-5 (1 being not confident and 5 being very confident). Below you will see the questions provided to students. They were given 15 minutes to answer these questions and then submitted them to the instructor. Questions were designed to assess students' confidence prior to the research activity and gather insight on how much they knew on these topics and whether they could apply that knowledge to real world scenarios.

1) On a scale 1-5, how confident do you feel about answering the following cell communication questions? (1- not confident and 5- very confident)

Research

The epinephrine signaling pathway plays a role in regulating glucose homeostasis in muscle cells. The signaling pathway is activated by the binding of epinephrine to the beta-2 adrenergic receptor. A simplified model of the epinephrine signaling pathway is represented in Figure 1.

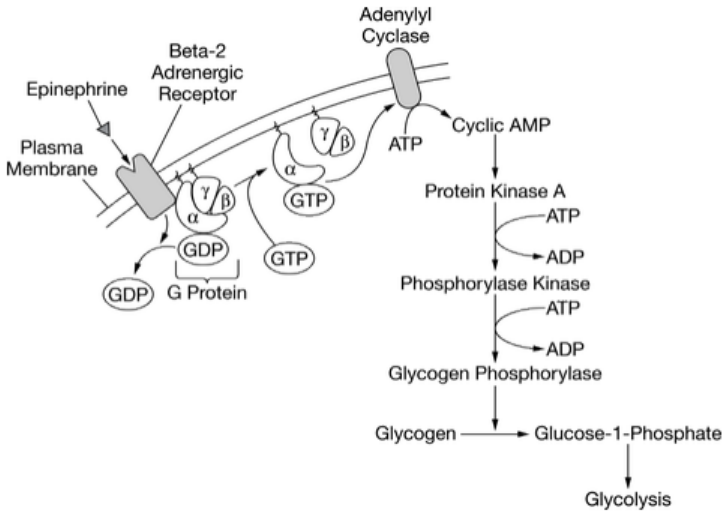


Figure 1. A simplified model of the epinephrine signaling pathway in muscle cells

1. Based on Figure 1, which of the following statements best describes the epinephrine signaling pathway?

(A) It involves the opening and closing of ion channels.
(B) In involves enzymes activating other enzymes.
(C) It involves changes in the expression of target genes.
(D) It involves protons moving down a concentration gradient.

Figure 39. Pre-Survey Question 1 illustration: The question used was taken from College Board AP Biology question bank on cell communication.

2) Briefly describe what makes you feel confident (or not) about this topic. If you feel confident, what preparation did you use to make you feel confident in this topic? On a scale 1-5, how confident do you feel with questions about Biotechnology?

Students subjected three samples of five different molecules to gel electrophoresis as shown in Figure 1.

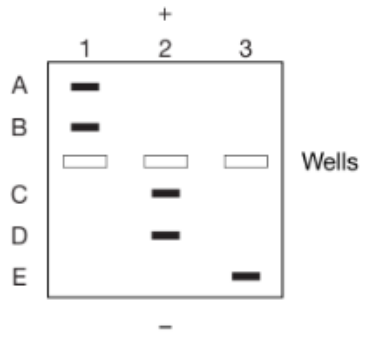


Figure 1. Gel electrophoresis of three prepared samples

Which of the following statements best explains the pattern seen on the gel with regard to the size and charge of molecules **A** and **B**?

(A) Molecules **A** and **B** are positively charged, and molecule **A** is smaller than molecule **B**.

(B) Molecules **A** and **B** are positively charged, and molecule **A** is larger than molecule **B**.

(C) Molecules **A** and **B** are negatively charged, and molecule **A** is smaller than molecule **B**.

(D) Molecules **A** and **B** are negatively charged, and molecule **A** is larger than molecule **B**.

Figure 40.Pre-Survey Question 2 illustration: The question used was taken from College Board AP Biology question bank on biotechnology.

3) How do Biotechnology and Cell Communication relate to each other? What would be an example of this relationship?

4) In your own words, describe the importance of understanding how cell communicate. How do researchers use knowledge about cell communication and biotechnology to advance their ideas in science?

5.2.2 Assessment Design

Case Study: I used my Triple Negative Breast Cancer research and scaffold method to facilitate student understanding of the data and background information using guided questions and cartoons. First, I created the background along with guided questions to help students decipher words they may not have heard before. The background section provided information on triple breast cancer, metastasis, and protein receptors HER2, PR, and ER. Students at this level understand most of these terms but are not fully aware of the name and functions of the protein receptors involved. Therefore, having guided questions where they look up explanation of these molecules allowed them to make a connection to what they learned in cell communications on protein receptors and apply it to a real-world scenario.

In addition, to the background I also provided a cartoon version of background information showing a visual understanding on terms and concepts that were difficult to comprehend for the students. As shown in (page where a sample of worksheet), the cartoon demonstrated two cartoon cells holding hands and how that represented the role of a particular protein called cadherins. Another representation was made with the feet of the cells which represented another protein named integrins. These images allowed students to understand that the behavior of these proteins were associated with metastasis. The cartoon clearly explained how a cell must let go of their hands

(cadherins) and their feet must have the ability to walk (integrins) in order to invade resulting in metastasis in another region of the body.

The third page on this case study was occupied with my data from prior research which provided the reader with an understanding of the biotechnology used in order to obtain these results. In this case study there was no conclusion section as the student had to come up with their own analysis with the guided analysis questions on the last page. The overall goal was helping students break down a research paper and build their confidence in reading the data and being exposed to various experimental designs in biotechnology.

The final page provided students with the opportunity to analyze the researcher's findings through using their critical thinking instead of passively reading the researchers conclusion. In the end, the most important stage of reading a research article is being able to question data and confidently agree or disagree with another researcher's arguments. The ability to provide evidence using the data given and to agree or disagree with the results of a researcher is an important skill to develop and obtain for the AP Biology exam. On page 4, I provided questions that allowed students to formulate a conclusion by summarizing the findings.

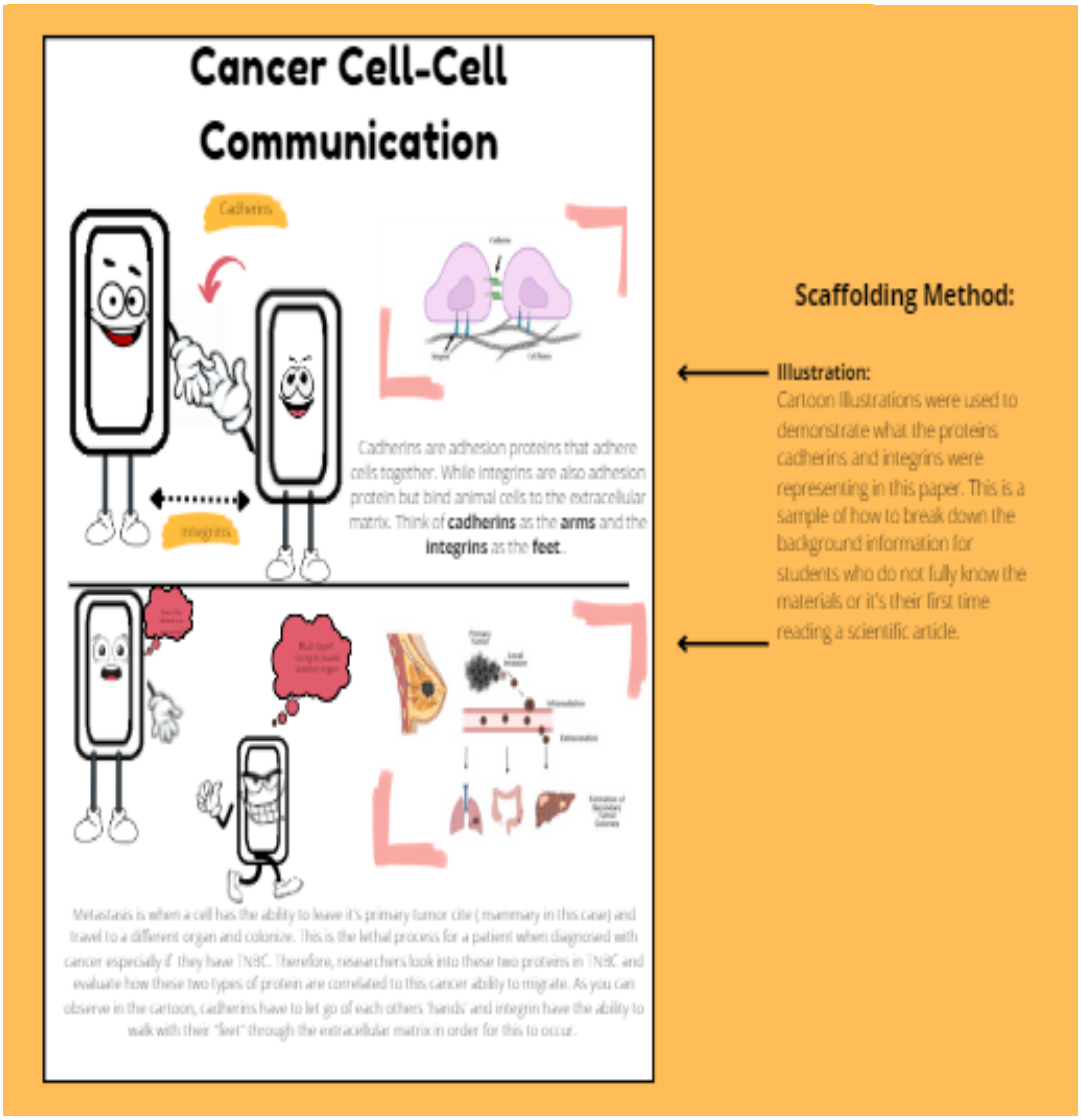
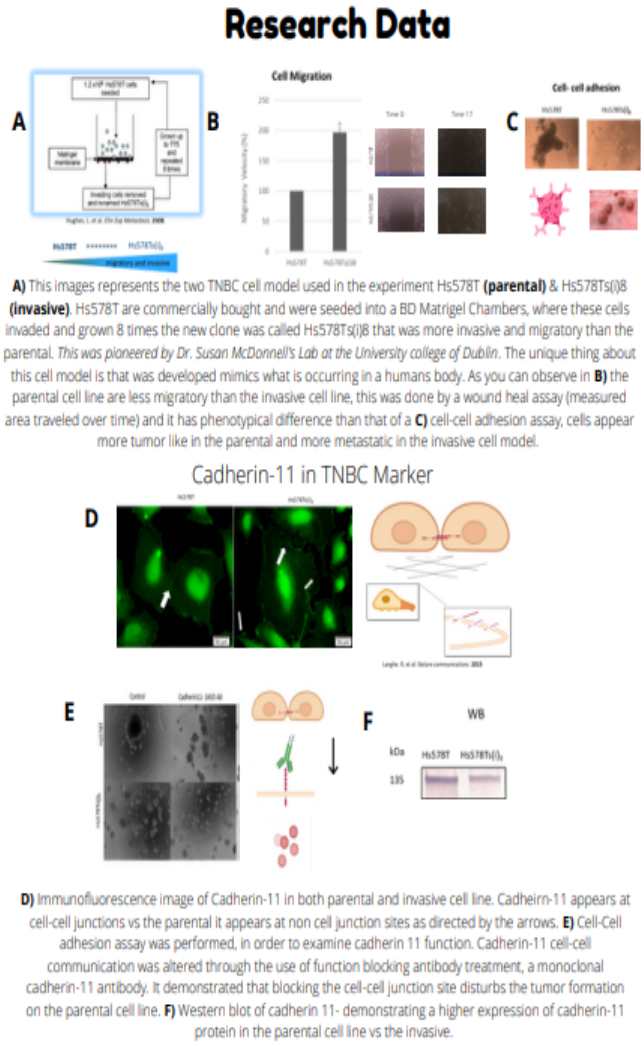


Figure 42. Triple Negative Breast Cancer Research Study Worksheet: Second page of the case study.

This provides illustrative information, and you can observe the use of scaffolding method.

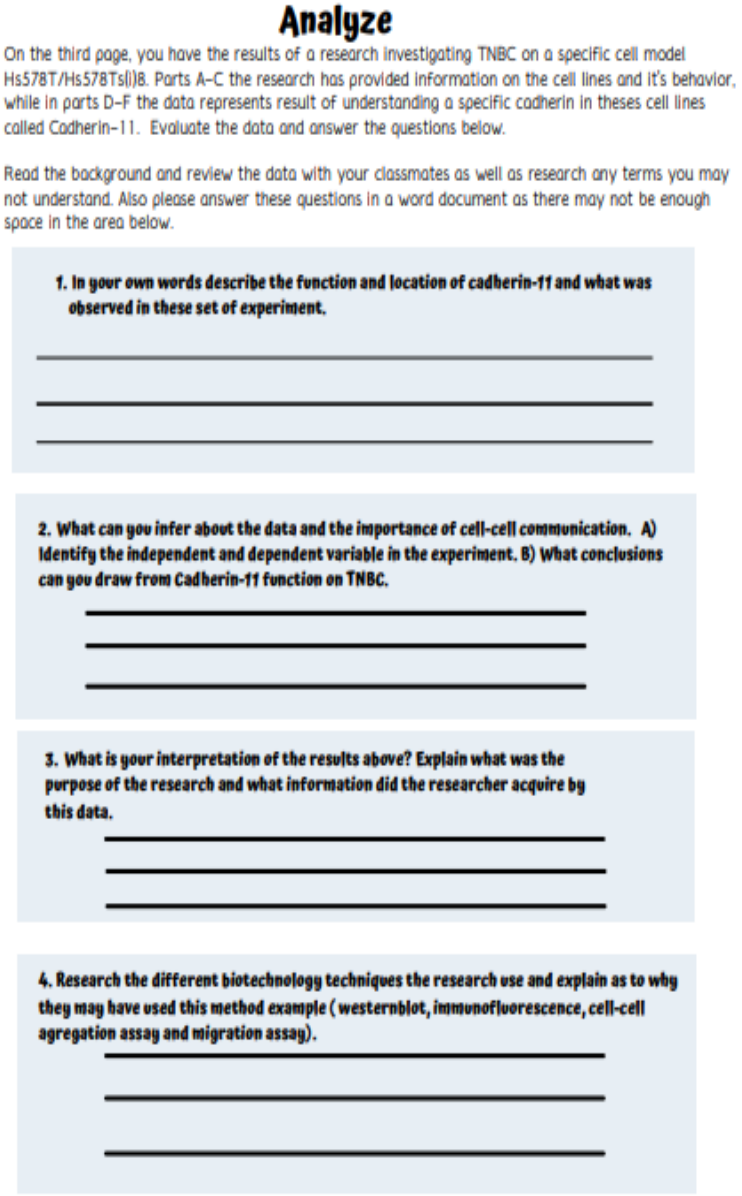


Scaffolding Method:

Research Data

Sample Data from the research that includes detailed caption to aid students in reading the data and allow them to come up with their own conclusion on the results. In this data students learned how to read

Figure 43. Triple Negative Breast Cancer Research Study Worksheet: Third page of the case study. This provides authentic research data. In this page you can observe scaffolding method.



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A-C the research has provided information on the cell lines and it's behavior, while in parts D-F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin-11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

Scaffolding Method:

Analyze:

Provided guided questions to help students organize and summarize their thoughts. Using Bloom taxonomy questions and scientific methods students were able to describe, infer, identify and draw conclusion on the research article.

Figure 44.Triple Negative Breast Cancer Research Study Worksheet: Four page of the case study. This the analysis question page that will allow students to engage with the data.

5.2.3 Post Survey Questions:

Soon after students completed the TNBC activity they received the post survey questions which required 15-20 minutes to complete. These questions were designed to review if student confidence level on cell communication or biotechnology increased, decreased, or stayed the same. The context of the 5 questions included: 1&2) the first two questions required students to rank their confidence level, 3) question three asked students to describe their self-confidence, 4) question four asked students about the relationship between these two topics and if their confidence level has changed, and 5) lastly, the final question asked if the activity was helpful in any way. These questions provided insights as to their confidence.

1) On a scale 1-5, how confident do you feel with questions about Cell Communication? (1- not confident and 5- very confident)

2) On a scale 1-5, how confident do you feel with questions about Biotechnology? (1- not confident and 5- very confident)

3) Briefly describe what makes you feel confident (or not) about this topic. If you feel confident, what preparation did you use to make you feel confident in this topic?

4) After completing the Triple Negative Breast Cancer Case Study, do you feel more confident about the relationship between cell communication and biotechnology?

What reason do researchers use cell communication and biotechnology to further develop therapies and close gaps. Please explain in 250 words or less.

5) On a scale 1-5, did you find this case study helpful? Please provide any additional comments and feedback:

Implementation in the classroom

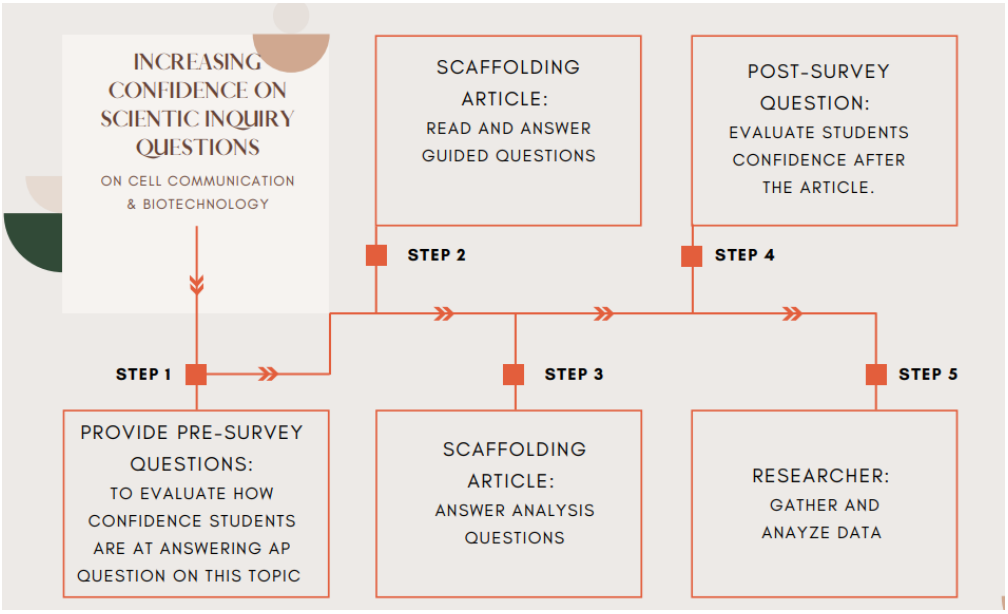


Figure 45. Overview of the steps taken on "Increasing Confidence on Scientific Inquiry Questions on cell Communication and Biotechnology."

5.2.4 Participants

The students that participated in this study were in the age group 15-16 years (9th and 10th grade) and were enrolled an AP biology course. A total of 11 students have

participated in this study over two years. Students that have taken this course also completed General Biology and passed their end of course exam with a passing score between 3-5. Every student cohort enrolling in the AP biology course had a biology foundation on macromolecules, photosynthesis, cellular respiration, central dogma, and other topics ideal for AP Biology curriculum.

5.2.5 Role as a researcher

My role in this study was to assure that the students understood the assignment on Triple Negative Breast Cancer by clarifying information on the assignment. I did not interfere in their data analysis or development of conclusions; my only role was to facilitate a technical question they were not able to research on their own. As the person who is conducting this research, I was responsible for interpreting their assignment and survey questions. In this qualitative research I carefully considered any personal biases when interpreting their results.

5.3 Data:

5.3.1 Likert Scale

The Likert scale is the most widely used scale since the 1930's for surveys. For this work, it was used to measure attitudes and is a five-point scale which allows a person to express how much they agree or disagree with a particular statement (Mcleod, 2023). This rubric makes the assumptions that the strength of a person's attitude is linear from no confidence to high level of confidence along the following scale: 1- No confidence, 2- low level of confidence, 3- Neutral, 4- Confident, 5-High level of confidence (table 1).

1 No Confidence	2 Low Level of Confidence	3 Neutral	4 Confident	5 High level of Confidence
Student does not feel confident in answering questions on the topic. Needs to be retaught the concepts. Has no idea what the concepts about. No connection to BIG IDEA.	Student has a general idea of the concept. Can answer superficial questions but it not able to explain their answer choice. No connection to BIG IDEA.	Student understands the concepts and question being asked but can answer a question that key steps in a problem and produce a conclusion. No Connection to Big IDEA	Student is confident they completed questions accurately and answered fundamental questions. However, does know how to relate concepts with other BIG IDEAS.	Student has mastered content. Student feels they can relate concepts with other BIG IDEAS and feels confident in teach other on this content.

Table 2 BIG IDEA: The AP Biology exam has 4 BIG Idea concepts (Evolution, Energetics, Information storage & Transmission, and System Interaction) that allow students to create meaningful connection among course concepts.

Information Processing	Evaluating, interpreting, and manipulating or transforming information.					
Category	**0**	**1**	**2**	**3**	**4**	**5**
Evaluating		**Minimally** determined the significance or relevance of information/data needed for the task		**Partially** determined the significance or relevance of information/data needed for the task		**Completely** determined the significance or relevance of information/data needed for the task
Interpreting		**Inaccurately** provided meaning to data, made inferences and predictions from data, or extracted patterns from data		Provided meaning to data, made inferences and predictions from data, or extracted patterns from data **with some errors**		**Accurately** provided meaning to data, made inferences and predictions from data, or extracted patterns from data
Manipulating or Transforming (Extent)		**Minimally** converted information/data from one form to another		**Partially** converted information/data from one form to another		**Completely** converted information/data from one form to another
Manipulating or Transforming (Accuracy)		**Inaccurately** converted information/data from one form to another		Converted information/data from one form to another **with some errors**		**Accurately** converted information/data from one form to another
Comments:						

Table 3 Rubric for assessing information process. (Gil Reynders, 2020)

5.3.2 Analysis

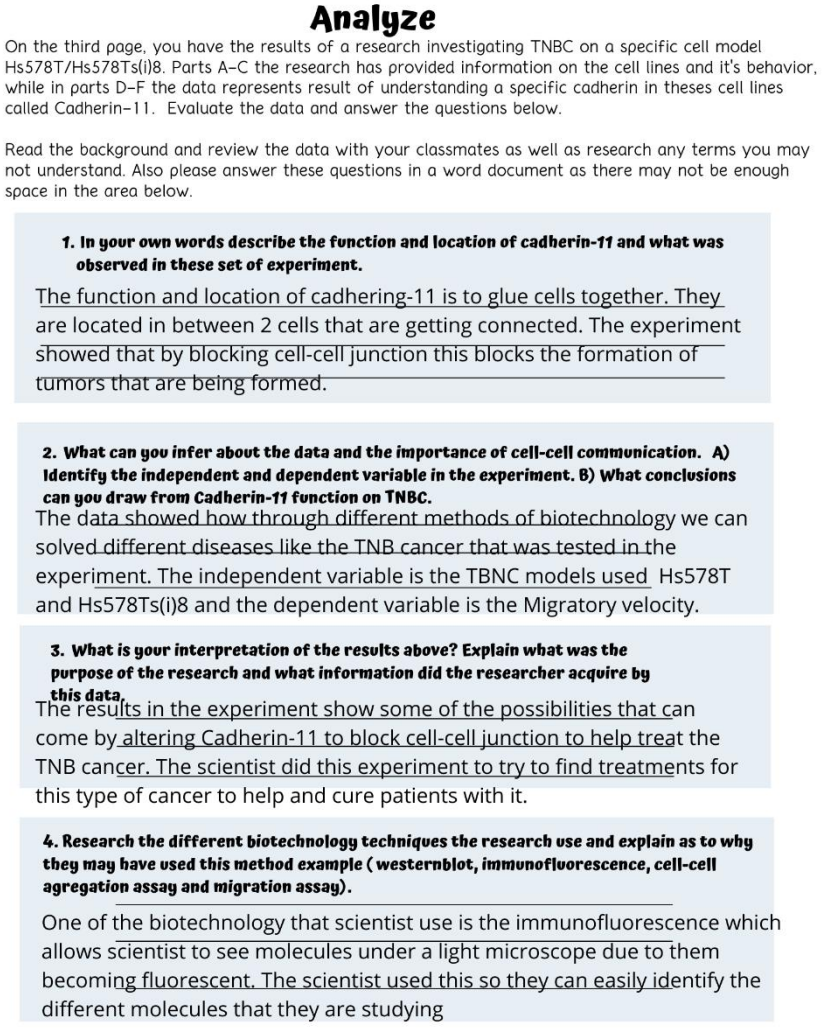
Cohort 1:

Milan

Pre-case study: This student described their confidence in terms of cell communication as a 3 and biotechnology as a 4 (1 being not confident and 5 having high level of confidence). He felt confident because he reviewed the information a lot because he was initially struggling with the topics. He described that his method of study was using the practice test from the college board and reviewing some videos. This method of study demonstrated that he was a studious student that learned best through trial and error and through visual learning. Based on my observations, Milan is a very dedicated and responsible student. He is the kind of student that benefit from understanding concepts and grasp them very quickly. On average, he can answer higher level critical thinking questions I place this student between a 3.5 in information processing. He is able to completely determine the significance or relevance of information/data needed

for the task, provide meaning to data, make inferences and prediction from data with some errors, partially convert information/data from one form to another and with some errors.

Activity Review:



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A–C the research has provided information on the cell lines and it's behavior, while in parts D–F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin–11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

The function and location of cadhering-11 is to glue cells together. They are located in between 2 cells that are getting connected. The experiment showed that by blocking cell-cell junction this blocks the formation of tumors that are being formed.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

The data showed how through different methods of biotechnology we can solved different diseases like the TNB cancer that was tested in the experiment. The independent variable is the TBNC models used Hs578T and Hs578Ts(i)8 and the dependent variable is the Migratory velocity.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

The results in the experiment show some of the possibilities that can come by altering Cadherin-11 to block cell-cell junction to help treat the TNB cancer. The scientist did this experiment to try to find treatments for this type of cancer to help and cure patients with it.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

One of the biotechnology that scientist use is the immunofluorescence which allows scientist to see molecules under a light microscope due to them becoming fluorescent. The scientist used this so they can easily identify the different molecules that they are studying

Figure 46 Milans analysis question answers.

Based on Milan's answers he demonstrated an overall understanding of the research. In reviewing question 1 Milan responded efficiently and was able to explain his understanding of the cadherin role in cells and research. The student explained: "The function and location of cadherin-11 is to glue cells together. The[se]sic are located in

between 2 cells that are getting connected. The experiment showed that by blocking cell-cell junction this blocks the formation of tumors that are being formed." Milan expressed how cadherin-11 was involved in connecting cells together – this was a vital point in the article that he clearly understood. The location of cadherins was where they were connected which was in the cell membrane. Milan clearly comprehended the experiment by stating that "blocking cell-cell junction this blocks the formation of tumors that are being formed." The only caveat to his answer is not being able to state that the comparison in the experiment was between the parental vs invasive cell online. However, based on his response on question one he clearly demonstrated that not only did he understand cell-cell communication he was also able to apply the information to applied research. This is a clear indicator that the student can make connection with big ideas from college board AP biology exam. Moreover, based on the information analysis, I'd score Milan from 5 in evaluating information and 3 in interpreting the results. Student was able to completely determine the significance or relevance of information/data needed for the task and provide meaning to data. Made inferences from data or extracted patterns from data with some errors.

Moving on to question two, Milan demonstrated his data analysis skills as he answered the independent and dependent questions correctly. Specifically, the student stated that "the data showed how through different method biotechnology we can solve different diseases like the TNB cancer that was tested in the experiment." Based on the information processing chart, I score this answer a 3 on manipulating and transforming (extent) as the student partially converted information/data from one form to another and

5 in evaluating the results, as the student completely determined the significance or relevance of information/data needed for the task.

On question three Milan stated his interpretation of the research in the following words “the results in the experiment show some of the possibilities that can come by altering cadherin-11 to block cell-cell junction to help treat the TNB cancer. The scientist did this experiment to try to find treatments for this type of cancer to help and cure patients with it.” Milan provided a summary sentence that displayed an understanding of the experiments in terms of why and how they were be done. In this question, student displayed accurately converted information/data from one form to another and give him a score of 5 in manipulating/transforming (accuracy).

This understanding now leads to the last question where Milan confirmed his understanding of immunofluorescence and its purpose in this experiment. Although he did not answer the question completely, Milan articulated his understanding very well by stating “one of the biotechnologies that scientist use is the immunofluorescence which allows scientists to see molecules under a light microscope due to them becoming fluorescent. The scientist used this so they can easily identify the different molecule that they are studying.” Overall, Milan’s answers demonstrated proficiency in cell communication and biotechnology in his knowledge I would score a 5 for interpreting biotechnology techniques and 3 evaluating the biotechnology techniques. The reason for this is because the student accurately interpreted the meaning of the data made inferences and predication from data or extracted patterns from it, however, since the student did answer all questions it hard to determine how well he evaluated all biotechnology practices. Non the less, student demonstrated that he can partially determine the

significance of relevance of information/data needed for the task. Moreover, he showed great strength in analyzing data and applying it to concepts which is an example of a high level of critical thinking. Based on the rubric used to assess critical thinking in the undergraduate STEM course in table 2 I would score the student with 4. Milan clearly was able to evaluate, interpret, and apply the information accurately with minimal errors.

Post-Case study: Milan continued to assign a 3 and a 4 to cell communication and biotechnology. He expressed that the case study did not help him feel more confident on cell communication, but he does feel the study was helpful toward understanding biotechnology. The student expressed that he did not feel the activity made him feel more confident in these areas, but it did expand his knowledge on biotechnologies and how they are used. Based on his pre-survey answer, Milan's confidence increased based on comparing his scores and answering test questions. However, this activity demonstrated that Milan used higher level of critical thinking and is able to use information and apply it to new information initially, but through the use of the activity he was understood more about biotechnology. He had a strong foundation and increased his mastery on cell communication and biotechnology. I believe this student's self-assigned levels of confidence should be higher, but since there is no actual numerical value to this activity to show him how much he knew and therefore he did not see the quality of his work indicated by his statements. I'm confident that the student answers a high-level critical thinking question, without any issues. Based on Milan's answers it shows that he has the ability to assess a question, analyze data and interpret with confidence and accuracy. While there is room to grow in his information processing, I

believe is more to do with his exposure to the topic. Should he decide to study in more detail about the research, I can say with confidence that the student is able to answer higher level thinking questions and accurately make connection between the big ideas presented in the AP biology exam with minimum errors. Based on this analysis, Milan found this helpful, however it did not increase his confidence level. However, I can understand how he found this exercise helpful for him to apply his critical thinking skills.

Conclusion/Reflection: Based on Milans pre-post survey and his answer on the worksheet. I strongly believe that he did benefit from this activity despite not increasing his confidence level. He has strong building block and is able to read and understand complex data with his prior knowledge. This activity gave Milan the opportunity to show case and strengthen his abilities and overall understanding of a concept he did not feel to confident in.

Keywords: self taught/practice problems/visual learning

Caleb

Pre- Survey: This student felt confident assigning a 5 on a scale of 1-5 regarding confidence on the topic of cell communication. When asked to briefly describe this confidence, he provided an interesting answer. His confidence was the result of his ability to memorize. He felt confident in the ability to answer pathway questions because he had it memorized. However, when it came down to biotechnology, he felt less confident (scored himself a 3). The reason was that it is not as easy to memorize intricate details that go into gel electrophoresis than a signal pathway. For this student it was clear that memorization played a central role in his confidence answering a question.

However, based on this student's exam scores and quality of answers I believe his confidence level should be higher. The student has demonstrated to answer questions accurately and can answer fundamental questions, and often able to connect to BIG IDEA concepts. Caleb can process and analyze concept accurately and provided meaning to data with minimal errors and is able to partially convert information. I give this student a critical thinking score of a 4.

Review of the student's activity

Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A–C the research has provided information on the cell lines and it's behavior, while in parts D–F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin–11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

The function of cadherin-11 is to allow for the adhesion and formation of a tumor. This suggests that the location of this cadherin is inside of mutated cells with uncontrolled cell growth. It is observed in the experiment that when this protein is inhibited that the formation of tumors is disturbed.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

The data call allow us to better understand how the understanding of cell communication can be in relation to diseases such as cancer. This can further allow to better control such diseases or even possibly a cure. In addition, it can also allow us to better understand the different functions of our body. The independent variable of the experiment was whether the cell in the model was parental or invasive. The dependent variable is the migratory and invasive characteristics of each model. The funstion will allow this form of cancer to more easily attach to each other and grow forming tumors.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

These results allowed researches to get a glimpse into the behavior of each of the strands. This included how fast they will spread and multiply allowing them to learn how to better predict the growth pattern and how to best counter it. In addition, scientists see how different important proteins play their role and how they can be affected. This can allow them to learn how in inhibit different aspects that can be deadly. The purpose of this research was to gather information on a certain strand and how it spreads, this can allow to development of treatments that can minimize it's growth.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

The first technique that is used in the experiment is Cell-cell aggregation assay tests the functionality of complexes in epithelioid tumor cells. This allowed the experimenters to examine the inside complexes of the invasive strands. Another technique utilized is cell adhesion assay which tests the cell binding to ligands and monolayers under static conditions. This allows them to test the capabilities of these invasive cells especially with the help of cadherin-11. These proteins were signaled out through immunofluorescence which is used to identify specific proteins. Allowing us to know that this protein is found in this invasive strands. All of the techniques chosen allowed the scientists the examine all the desired characteristics of the invasive cell model.

Figure 47 Caleb 's analysis question answers.

Based on Caleb answers he demonstrated an overall understanding of the research. In reviewing question 1 Caleb responded proficiently and described the role of cadherin properly. The student wrote the following: “The function of cadherin 11 is to allow for the adhesion and formation of a tumor. This suggest that the location of this cadherin is inside of mutated cells with uncontrolled cell growth. It is observed in the experiment that when this protein is inhibited that the formation of tumors is disturbed.” The student understood the role of cadherins and the role it was playing in forming tumor formation and provided a suggestion as to why this occurs and providing evidence from the data. This is a clear indicator that the student can make connection with big ideas from college board AP biology exam. Based on the information analysis I’d score Caleb with 5 in evaluating information’s and 5 interpreting the results. The student was able to completely determine the significance or relevance of the data needed to answer the question and was able to accurately provide meaning to the data and made inferences and predications from the data.

In question two student the student was able to answer the question proficiently. The student answered the following, “The data can allow us to better understand how the understanding of cell communication can be in relation to diseases such as cancer. This can further allow to better control such diseases or even possibly a cure. In addition, it can also allow us to better understand the different functions of our body. The independent variable of the experiment was whether the cell in the model was parental or invasive. The dependent variable is the migratory and invasive characteristics of each model. The function will allow this form of cancer to more easily attach to each other and grow forming tumors.” The student was able to answer the inference question

accurately he understood one of the reasons as to why this research can help in a larger scale. He was also able to identify the independent and dependent variable correctly and was able to draw conclusions from the data about Cadherin-11s function. The student answer: “The function will allow this form of cancer to more easily attach to each other and grow forming tumors” shows an understanding of what the cadherin-11 does however, his answer could have been complete if he explained that the opposite is true the lack of cadherin-11 attachment promotes cell invasion. Non the less the student evaluated and interpreted the answer completely and accurately (score of 5) and was able to partially manipulate the data (score of 3).

However, in question 3 the student demonstrated a deeper understanding of the data as he explained the following interpretation of the results: “These results allowed research to get a glimpse into the behavior of each of the strands. This included how fast they will spread and multiply allowing them to learn how to better predict the growth pattern and how to best counter it. In addition, scientists see how different important proteins play their role and how they can be affected. This can allow them to learn how in inhibit different aspects that can be deadly. The purpose of this research was to gather information on a certain strand and how it spreads, this can allow to development of treatments that can minimize its growth.” The student answers this question with a profound detail and understandings. He accurately provide meaning to the data and made inferences and extracted patterns from the data and provide an concrete answer (score of 5).

Lastly on question 4 Caleb state his interpretation of the research in following words, “The first technique that is used in the experiment is Cell-cell aggregation assay

tests the functionality of complexes in epithelioid tumor cells. This allowed the experimenters to examine the inside complexes of the invasive strands. Another technique utilized is cell adhesion assay which tests the cell binding to ligands and monolayers under static conditions. This allows them to test the capabilities of these invasive cells especially with the help of cadherin-11. These proteins were signaled out through immunofluorescence which is used to identify specific proteins. Allowing us to know that this protein is found in this invasive strand. All the techniques chosen allowed the scientists the examine all the desired characteristics of the invasive cell model." In the answer the student now only provided an explanation as why the scientist used these methods of research but was able to tie it in with research. In this question the student was able to evaluate, interpret and manipulate the data completely and accurately. Student was able to determine the significance of the data, accurately provide meaning to data and provide inference and predictions and lastly was able to convert information from one from to another (score of 5, all).

Post-Case Study: This student's confidence did not change after the case study. He felt more confident in cell communication than biotechnology and continued to study for this topic by memorization. However, the activity had a positive outcome for him. He felt more confident in the relationship between biotechnology and cell communication and how the use of technology led to certain pathways resulting in treatment breakthroughs. Overall, he ranked the case study a 4 in terms of helpfulness. My conclusion is that the student did not score themselves higher after the post-lab question because the case study did not fit his method of understanding-memorization. I'm confident that the student answers a high-level critical thinking question without any

issues. Based on Caleb answer it is evident that he can analyze questions and interpret with confidence and accuracy. His critical thinking level is at a 5 higher than what I hypothesized. I think the reason Caleb confidence level did not change is because the student confidence in a topic is linked with his memorization level, however, he clearly has high level of critical thinking skills that allow him to answer proficiently.

Conclusion/Reflection: Based on Caleb pre-post survey and his answer on the worksheet. I strongly believe that the student benefited from this activity although it did not increase his confidence level. Based on his answers he demonstrated to have a strong bases for scientific research and can interpret data and provide accurate inferences. This activity provided an opportunity for Caleb to exercise the ability to use his knowledge on cell communication be able to answer higher level thinking questions.

Keyword: memorization/understanding

Donald

Pre-Case Study: This student assigned a 3 in cell communication and a 4 in biotechnology. He believed he needed to study even harder and that was his approach. Student did not provide to much detail on his answer however based on his performance in class I suggest that his critical thinking level is at a 3.5. Donald is very studious and applied, however, he not only needs to study in detail it requires him more time than other to fully understand a topic.

Review activity

3. The cadherins are located on the side of the cells, which connects both cells as if they were arms. The data supports where the connections are made and when they will separate to invade other places, creating more cells in other organs.
4. If the cell communication fails, it would lead to cancer as shown in the pictures and in the research. The independent variable, in this case, is the first parental TNBC while the dependent variable is the end clone created by the failure of adhesion by the cadherin. Because of this failure to join both of them, the tumor spreads to different organs making it harder to treat.
5. My interpretation of the results above is that if we can find a way for the cadherins to not fail then we can be a step close to treating this type of cancer. At the end of the experiment, it was found that using an antibody in cadherin 11 prevented the tumor from ever developing.
6. The researchers were trying to research a specific type of protein so using the western blot was the best option because it resulted in effective expression of the protein of TNBC using the gel electrophoresis. By using the immunofluorescence approach they could clearly see the cadherin as well as the invasive protein as it was left in different places under the microscope. The cell-cell aggregation assay was a must-do in this experiment because it provided really useful finding information regarding the tumor in this situation by testing the function of the tumor TNBC. The migration assay was an effective way to show how the invasive protein would go to different places in a normal body.

Figure 48 Donald's analysis question answers

In question one the student answered the question accurately and completely. The student response to cadherin 11 function is the following, “The cadherins are located on the side of the cell, which connect both cells as if they were arms. The data supports where the connections are made and when they will separate to invade other places, creating more cells in other organs.” He demonstrated in this answer that not only he understood the role cadherins play how the loss of cadherin can lead to cells invading to other places creating more cells. Student was able to evaluate and interpret question completely and accurately (score of 5). The student completely determined the

significance of the data and accurately provided meaning to data and made inference from it.

In question two the student related his understanding of the data to cell communication. The student used the following words to answer question 2, “If the cell communication failed, it would lead to cancer as show in the pictures and in the research. The independent variable, in this case, is the first parental TNBC while the dependent variable is the end clone created by the failure of adhesion by the cadherin. Because of this failure to join both, the tumor spreads to different organs making it harder to treat.” Student tried to relate cancer promotion due to a “failure” or alteration in a cell’s mechanism, which is in fact correct. However, the student was not able to answer the question correctly for the dependent and the independent variable. In attempting to interpret the data he expressed that the failure to of cadherin to adhere to other cells causes the tumor to “spread” (invade) to other organs. The student partially determined the significance or relevance of data and provided meaning to the data with some errors (score value 3, respectively).

In question 3 the student further interpreted the data and used the following words, “My interpretation of the results above is that if we can find a way for the cadherins to not fail then we can be a step closer to treating this type of cancer. At the end of the experiment, it was found that using an antibody in cadherin 11 prevented the tumor from ever developing. Based on the student’s answer he partially determined the relevance of the data and provided meaning to data with some errors (score of 3). In his answer the students show some understanding of what is occurring in the data but fails to fully comprehend the data.

Lastly question four the student elaborately answered the questions biotechnology. The student used the following words: “The researcher was trying to research a specific type of protein so using the western blot was the best option because it resulted in effective expression of the protein of TNBC using the gel electrophoresis. By using the immunofluorescence approach, they could clearly see the cadherin as well as the invasive protein as it was left in different places under the microscope. The cell-cell aggregation assay was a must-do in this experiment because it provided useful finding information regarding the tumor in this situation by testing the function of the tumor TNBC. The migration assay was an effective way to show how the invasive protein would go to different places in a normal body.” In this section the student was able to relate the data to the biotechnology that was used. The student was able to partially determine the significance of the data and was able to provide meaning to data by making inferences with some errors (score of 3).

Post- Survey questions: This student assigned a 4 in confidence in cell communication and 3 in biotechnology. After the exam the student was not able to remember the biotechnology part. The student felt more confident in relation to cell communication and less to biotechnology. The student expressed that the case study showed him how using biotechnology could help obtain information regarding how to treat this mutation in cell communication. The student felt that the background was a huge help in understanding resulting in the assignment of a 5 in terms of the helpfulness of the case study. Overall, the student scored a critical thinking value of 3.5 in which student can partially evaluate data, interprets data with errors and partially convert information from one form to another.

Keyword: understanding

Conclusion/Reflection: Based on Donalds survey and his answer on the worksheet, I strongly believe that he benefited from this activity despite his confidence level dropping in biotechnology. The student felt he increased his confidence on cell communication. The students feel confident in cell communication as he feels he can complete questions accurately and answer fundamental questions but is not able to relate concepts with other BIG IDEAS. As for Biotechnology he lowered his score to a 3, where the student feels neutral about Biotechnology. He understands the concepts and question being asked but can answer a question that key steps in a problem and produce a conclusion. He cannot create a connection to Big IDEAS. In my opinion, I believe this to be accurate, I think the student got a sense of the questions for Biotechnology and the exposure that he needed to answer those questions and felt that he should study more on this concept.

Sabrina

Pre- Case Study: This student assigned a 2 regarding their confidence in understanding both cell communication and biotechnology. She expressed that she did not feel confident about cell communication and biotechnology because she had not "looked" over the models and vocabulary. It appears that for this student to understand refreshing the models and vocabulary would help them answer questions on this topic. Sabrina is a studious student and relies on her study methods to understand concepts. However, it does take her extra time to link concepts together and this could t attribute to her low confidence level. When give the appropriate time the student has

demonstrated to have critical thinking level of a 3. The student can partially determine the significance of the data and provide meaning with some errors.

Activity Review:

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

My interpretation of the results is that parent cells depends on cadherin-11 protein to grow and maintain a tumor formation. It also appears that subsequent generations of the cells are more invasive and can migrate to other organs causing metastatic disease as compared to the parent cell. The results also show how monoclonal antibodies disrupt the function of cadherin-11 protein in cell-cell communication and its ability cause cells to adhere to each other for both parent and invasive cells. Although cadherin-11demonstrated the ability disrupt tumor formation, it did not demonstrate the ability to stop migration of the cells that can potentially lead to metastatic disease. The purpose of this research is to find an antibody that may inhibit the cadherin protein and prevent or disrupt cell aggregation. This may be useful in the development of a medication that can decrease tumor formation in TNBC.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

In the Immunofluorescence method, an antibody that can bind to a specific protein is labeled with a fluorescent dye. This method allows easy visualization of the cadherin-11 protein under a light microscope. Western blot is used to detect the presence of a specific protein. This method was used to detect a higher expression of Cadherin-11 protein in the parent cell compared to the invasive clone. The wound heal assay is used to detect the migration of cells and the interaction between the cells. This method was used to measure the migration area traveled over time between the parent and invasive clones. The cell-cell aggregation assay is a method used to test tumor formation. It was used to evaluate the clustering of parent cells and compare it to disruption in tumor formation of the invasive clone when a monoclonal antibody was used against cadherine-11 function to inhibit cell to cell communication.

Figure 50 Sabrina analysis question answers

The student answered question 1 with the following words: “Cadherin-11 is a protein receptor that encourages cell to cell communication that leads to cell-to-cell adhesion. It is located on the cell’s membrane. In the first experiment, it was shown that the Hs578t parental TNBC were cloned in Matrigel. The clones Hs578Ts(i)$_8$ appear to be able to migrate and invade better than the parent TNBC giving them the characteristic of metastatic cells. In the cell-to cell assay, parents Hs578T shows to be a large clump of cells, whereas the Hs578Ts(i)$_8$ clones, have smaller clusters and are more diffused. The immunofluorescence uses fluorescent dyes to tag cadherin-11 to have a better visual of the cell’s boarder and its interaction with adjacent cells. Graph D shows the parent Hs578T perfectly adhered to the neighboring cell by Cadherin-11, whereas the Hs578Ts(i)8 clones have a gap between cells. Graph E shows the results of placing an antibiotic that will block the communication between cadherin-11 protein. It inhibits the communication between Hs578T parent cells therefore prevents large tumor formation. Although it was not pointed out in the description, the monoclonal antibody also decreases the adhesions between the Hs578Ts(i)$_8$ clones. The western blot shows a higher concentration of Cadherin-11 protein in the Hs578T parent and a lower concentration in the Hs578Ts(i)$_8$ invasive clones. The student response was accurately evaluated however, provided meaning to data with some errors (score of 5 & 3, respectively). Student took her time to evaluate the data but failed to provide an explanation for the data.

Moreover, in question two the student answers the following, “I can infer that functional Cadherin-11 protein is imperative for cell-cell communication, formation, and preservation of larger clusters of cells or tumors. The independent variable is the

monoclonal antibody. The function of Cadherin 11protein is the dependent variable. Disrupting the function of cadherin-11 between cells with monoclonal antibodies inhibits the formation of larger TNBC tumors." In this question the student demonstrated that she understood background information of the research however, she was not able to answer the question correctly on the independent and dependent variable. Based on this evaluated the questions accurately however, interpreted the data finding with errors (score 3, and 1 respectively).

The student answers the following to question three, "My interpretation of the results is that parent cells depend on cadherin-11 protein to grow and maintain a tumor formation. It also appears that subsequent generations of the cells are more invasive and can migrate to other organs causing metastatic disease as compared to the parent cell. The results also show how monoclonal antibodies disrupt the function of cadherin-11 protein in cell-cell communication and its ability cause cells to adhere to each other for both parent and invasive cells. Although cadherin-11demonstrated the ability disrupt tumor formation, it did not demonstrate the ability to stop migration of the cells that can potentially lead to metastatic disease. The purpose of this research is to find an antibody that may inhibit the cadherin protein and prevent or disrupt cell aggregation. This may be useful in the development of a medication that can decrease tumor formation in TNBC." The student provided an elaborate response on the research and demonstrated to have study and attempted to understand the research. The student understood the general picture of the research however provided a few inaccurate findings. The over goal of the research was to understand the behavior of Cadherin 11 in the research and be inhibiting the parental with monoclonal antibodies it made it behave more like the

invasive cell line. Overall, I gave the students response a score on 3 evaluating and interpreting the results and she had some errors in her understanding.

Lastly on question the student answers the following answer, “In the Immunofluorescence method, an antibody that can bind to a specific protein is labeled with a fluorescent dye. This method allows easy visualization of the cadherin-11 protein under a light microscope. Western blot is used to detect the presence of a specific protein. This method was used to detect a higher expression of Cadherin-11 protein in the parent cell compared to the invasive clone. The wound heal assay is used to detect the migration of cells and the interaction between the cells. This method was used to measure the migration area traveled over time between the parent and invasive clones. The cell-cell aggregation assay is a method used to test tumor formation. It was used to evaluate the clustering of parent cells and compare it to disruption in tumor formation of the invasive clone when a monoclonal antibody was used against cadherin-11 function to inhibit cell to cell communication. The student took her time to evaluate the methods and associate the with the research. She answers the question precisely and was able to manipulate the information and accurately associate it with the research (score of 5, respectively)

Conclusion: Overall, the student demonstrated to have taken her time to evaluate the data and provide a reasonable explanation to the data. While the answers were not always correct, she did show to use logic and her critical thinking skills to evaluate, interpret, and manipulate the data. Student score an overall score of 3.5 in her critical thinking skills.

Post-lab questions: This student scored a 4 in confidence for both cell communication and biotechnology. She expressed that she felt confident in

understanding the topic because the guided questions were helpful on the cell-communication worksheet. It also helped her to understand women bodies when expressing breast cancer. She agreed that she felt more confident about the relationship between cell communication and biotechnology. She therefore scored the worksheet a 5 as being helpful. In addition, I had predicted that Sabrina would score a 3 in her critical thinking skills and she was able to score higher to 3.5. I believe the reason for this because she spent time on the worksheet and was able to research extensively and study the data. If this would have been a time assignment, we may not have the same about of information as we did.

Keyword: models/vocabulary/understanding

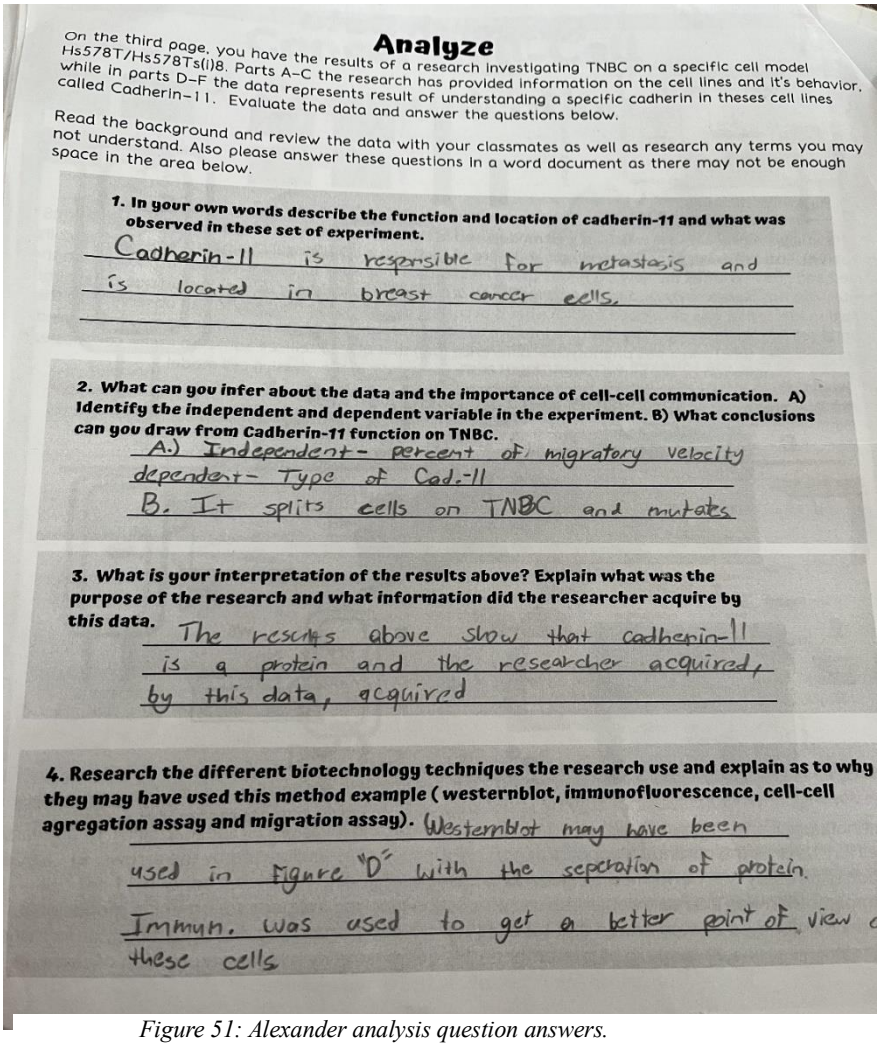
2nd Cohort

Alan

Pre-Survey: This student did not feel confident in answering a cell communication question (2/5) or a biotechnology question (2/5). He explained feeling confident in using his common sense to his advantage, however, he was aware that studying was required to fully answer these questions. Based on my observation of this student in the classroom, the student is inquisitive and hardworking, however, he required extra time to read and answer questions and sometimes certain questions were extremely difficult to comprehend. Overall, I would score this student 2.5 in evaluating information as the student could partially determine the significance or relevance of the information/data needed for the task and he was able to provide meaning on the data,

but made some errors while trying to manipulate the data and could extend the information only minimally.

Review of Activity:



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A-C the research has provided information on the cell lines and it's behavior, while in parts D-F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin-11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Cadherin-11 is responsible for metastasis and is located in breast cancer cells.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

A.) Independent- percent of migratory velocity
dependent- Type of Cad.-11
B. It splits cells on TNBC and mutates

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

The results above show that cadherin-11 is a protein and the researcher acquired, by this data, acquired

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay). Westernblot may have been used in figure "D" with the seperation of protein. Immun. was used to get a better point of view these cells

Figure 51: Alexander analysis question answers.

In the analysis portion of the activity the student demonstrated difficulty with vocabulary as shown in question one of the analysis portions of the activity: **"In your own words describe the function and location of cadherin-11 and what was observed in these set of experiments**." The student answered the following: "Cadherin-11 is responsible for metastasis and is located in breast cancer cells." The student was

not only answered this question incorrectly but did not fully answer the question. As explained in the activity Cadherin 11 is a transmembrane protein found in the cell membrane and it is responsible for cell-cell adhesion in mammalian cells. Based on the processing chart the student minimally determined the significance or relevance of the information/data needed for the task (score value 1) and inaccurately provided meaning to the data, made references and predictions from data (score value1).

In question two when the students were asked to A) Identify the independent and dependent variable in the experiment and B) to explain what conclusion can be drawn from cadherin-11 function on TNBC student partially answered the question and continues to demonstrate vocabulary deficiency. Student answered that "the independent variable was the percent of migratory velocity and dependent the type of cadherin". The independent variable was the type of TNBC, and dependent variable was the % migratory velocity. As for Part B student answered: "It split cells on TNBC and mutates." This answer demonstrates that student was not able to comprehend the background information and was not able to use prior knowledge to draw a proper conclusion. Again student was not able to evaluate, interpret or manipulate information (score value 1). Moreover, in the particular question the student inaccurately converted the information/data from one form to another (score value 1).

Question three asked for student interpretation of the results and to explain the purpose of the research and what information the researcher could have acquired from the data. Student answered: "The results above show that cadherin-11 is a protein and the researcher acquired, by this data, acquired." Student did not know how to answer this question correctly, reworded the question and provided a definition like answer to a

critical thinking question. Student was not capable of minimally converting information/data from one form or another (score value 1).

Lastly, question 4 the question asked about the different biotechnology techniques the research use and explain as to why they may have used that method and student answered: “Western blot may have been used in Figure “D” with the separation of protein, immun. Was used to get a better point of view on these cells.” Student answered western blot correctly and provided ambiguous answer for immunohistochemistry, however, I can infer he understood that this technique is used to provide a visual to better understand the cells behavior. In the question the student minimally determined the significance/relevance of the information and inaccurately provided meaning to the data (value score 1)

Post- Survey- This student remained the same with respect to confidence toward cell communication (2) but increased in confidence toward biotechnology (3). He felt this case study was helpful overall (3). Vocabulary was the challenge for this student which increased his difficulty in understanding and therefore addressing the question. After evaluating the students pre/post survey and activity answer, I understand how the student confidence increased from a 2 to 5 in biotechnology since he was exposed to various methods in the study, and out of the analysis question he answered question 4 best. The student showed clear deficiencies in the topic and was not able to grasp information from the article. The vocabulary on this activity may have been too advanced and the student did not have a good understanding of base cell communication. Based on these results, the student scored a value of 1 when evaluating and processing information in this topic.

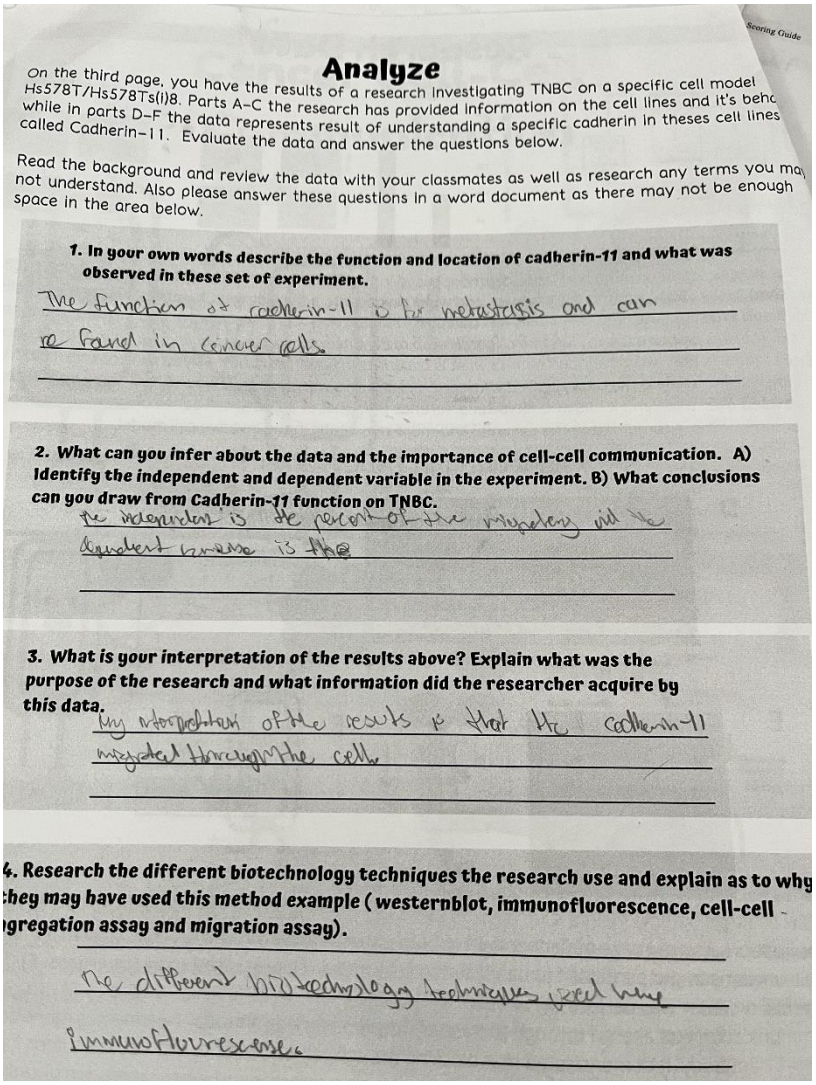
Conclusion/Reflection: In evaluating all this information, I think that Alan did not truly benefit from this activity. For him to have the full experience there are certain skills he needed in order properly evaluate, interpret, extended and accurately manipulate data. In this activity the student would need to go back and study the basic information on cell communication to fully increase his critical thinking abilities in this activity. Overall, a student whose critical thinking at this level will not benefit from this activity.

Key Words: Vocabulary/studying/common sense

Sawyer

Pre-survey: This student indicated moderate confidence by assigning 3/5 in both cell communication and biotechnology questions. His confidence level was connected to his memory, which determined whether he remembered the details on the topic. Based on my observation, the student relied more on his memorization rather than understanding concepts, and although he was a studious and responsible student, he did not have a high level of critical thinking. I would score this student a 2 in processing information, because he does know how to memorize information and therefore identify low tier questions. However, the student struggled to answer the big idea question on the AP Biology exam.

Review of Activity



Scoring Guide

Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A–C the research has provided information on the cell lines and it's behc while in parts D–F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin–11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you ma not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

The function of cadherin-11 is for metastasis and can be found in cancer cells.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

the independent is the percent of the migratory and the dependent variable is the

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

My interpretation of the results is that the cadherin-11 migrated throughout the cell.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell aggregation assay and migration assay).

the different biotechnology techniques used were immunofluorescence.

Figure 52. Sawyers analysis question answers.

As you can see in Figure (11) student 2B was not able to fully answer the analysis portion of this activity. The student's answers were incomplete and incoherent. In question one when asked, in your own words describe the function and location of cadherin-11 and what was observed in these sets of experiments, the student answered: " The function of cadherin-11 is for metastasis and can be found in cancer cells." The student attempted to answer a definition question but failed to answer an observation question on the experiment. Based on the processing chart the student minimally

determined the significance or relevance of the information/data needed for the task (score value 1) and inaccurately provided meaning to the data, failing to reference and make predictions from data (score value1).

In question 2 the student was asked to A) Identify the independent and dependent variable in the experiment and B) what conclusions can you they draw from Cadherin-11 function on TNBC. The student only partially answer question A, " The independent is the percent of the migratory and the dependent is the.." The student failed to answer any data inquiry or critical thinking questions. This answer demonstrates that the student was not able to comprehend the background information and was not able to use prior knowledge to draw a proper conclusion. Again, the student was not able to evaluate, interpret or manipulate information (score value 0.5). Moreover, in the particular question the student inaccurately converted the information/data from one form to another (score value 0).

In question 3 and 4 the student gave brief answers that did not provide any evidence of understanding the material. In question three the student was asked to interpret the results above and to be able to explain the purpose of the research. The student was only able to answer, "My interpretation of the results is that the cadherin-11 migrated through the cell." Clearly the student did comprehend the data and much less understand what conclusion could be drawn from the data. Student did not know how to answer this question correctly, reworded the question and provided a definition like answer to a critical thinking question. The student was not capable of minimally converting information/data from one form or another (score value 1).

Question four asked the student to research the different biotechnology used in the research and to explain why the research used this method to study this cell line. The student answered: “The different biotechnology techniques use have immunofluorescence.” The student either did not research the different biotechnology techniques or did not understand their application to fully respond in this answer. In the question the student was not capable of minimally determining the significance/ relevance of the information and inaccurately provided meaning to the data (value score 0)

Post-survey: His confidence in cell communication stayed the same but increased by 0.5 in biotechnology. For this student memorization was important and not being able to remember the different methods /technologies and or pathways was extremely important for determining his confidence level. The explanation from the case study helped with biotechnology because it provided real life examples which was described as profound levels of information. The student scored the case study a 4/5 in terms of being helpful. The student scored a value of 1 in processing, evaluating, and extending information. I strongly think that the student’s confidence may be preventing the student from trying and showing what he knows. Based on his answers, (short and incomplete) it demonstrated that the student was frustrated by not understanding the activity and decided not to give it an honest try. However, it does demonstrate that the student had deficiencies in the topic but maybe in other skills like interpreting data.

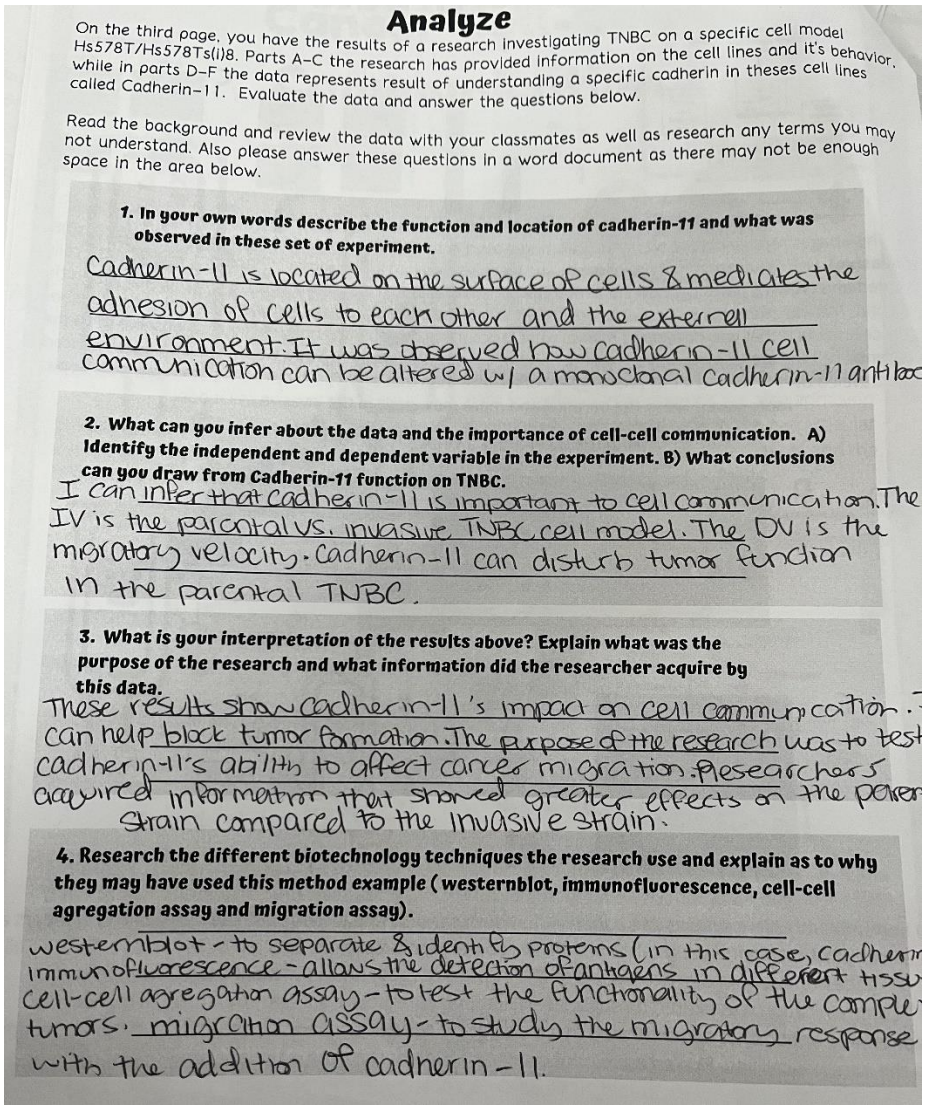
Conclusion/reflection: The student my find that the activity helps as it may be a point of reference if the student decided to study on this topic later. However, it is clear that the student relies on memorization as a study tool and it has created a struggle when analyzing information. In my opinion this activity was not very helpful to the student since he was not able to pull from prior experience. His confidence was not boosted and he did not develop the necessary building blocks to analyze and interpret data.

Keywords: memorization

Kadence

Pre-survey: This student scored a 2/5 in both cell communication and biotechnology indicating she was not confident because she did not feel strong about the topic. Kadence was very studious and relied on understanding and analyzing information allowing her to properly connect big ideas from college board. When analyzing her critical thinking level I would score her a 4/5. The student could process, evaluate and extend information accurately with minimal errors.

Review of Activity:



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A-C the research has provided information on the cell lines and it's behavior, while in parts D-F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin-11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Cadherin-11 is located on the surface of cells & mediates the adhesion of cells to each other and the external environment. It was observed how cadherin-11 cell communication can be altered w/ a monoclonal cadherin-11 antibo

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

I can infer that cadherin-11 is important to cell communication. The IV is the parental vs. invasive TNBC cell model. The DV is the migratory velocity. Cadherin-11 can disturb tumor function in the parental TNBC.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

These results show cadherin-11's impact on cell communication. can help block tumor formation. The purpose of the research was to test cadherin-11's ability to affect cancer migration. Researchers acquired information that showed greater effects on the paren strain compared to the invasive strain.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

westernblot - to separate & identify proteins (in this case, cadherin immunofluorescence - allows the detection of antigens in different tissu cell-cell agregation assay - to test the functionality of the comple tumors. migration assay - to study the migratory response with the addition of cadherin-11.

Figure 53 Kadence's analysis question answers.

Question one asks students to describe the function and location of cadherin-11 and what observed in these set of experiments and student answered: “Cadherin-11 is located on the surface of cells and mediates the adhesion of cells to each other and the external environment. It was observed how cadherin-11 cell communication can be altered with a monoclonal cadherin-11 antibody.” This answer demonstrated that the

student had a clear understanding of the proteins being investigated and was able to provide an appropriate answer from the data provided on cadherin-11. The student showed she could read and analyze data and be able to apply it to new information. I would place a value of 5 in evaluating and interpreting data as she could completely determine the significance and relevance of the information/data and accurately provide meaning to the data.

In question two, the student was requested to A) identify the independent and dependent variable in the experiment and B) to draw conclusions on cadherin-11 function on TNBC . The student responded: " I can infer that cadherin-11 is important to cell communication. The IV (independent variable) is the parental vs. invasive TNBC cell model. The DV (dependent variable) is the migratory velocity. Cadherin-11 can disturb tumor function in the parental TNBC." The student answered the question correctly and demonstrated an understanding of the function on cadherin in the TNBC cell line. In this answer the student was able to interpret the data accurately and manipulate the data by completely converting the information/data from one form to another (value score of 5, both interpreting and extending data).

In question three, the student was able to successfully explain his/her interpretation of the results above. The student explained with the following: "These results show cadherin-11's impact on cell communication. It can help block tumor formation. The purpose of the research was to test cadherin-11's ability to affect cancer migration. Researchers acquired information that showed greater effects on the parental strain compared to the invasive strain." This was a great response and scored a value of 5 in manipulating data accurately.

Lastly, in question four the student was able to describe and explain the purpose of each experimental approach using various methods of biotechnology. The student explained: “western blot -to separate and identify protein (in this case, cadherin-11). Immunofluorescence- allows the detection of antigens in different tissues. Cell- cell aggregation assay- to test the functionality of the complete of tumors. Migration assay- to student the migratory response with the addition of cadherin-11.” Based on the student’s answer it was evident that the student was able to use prior knowledge learned from class on cell communication and was able to build new understanding using this research article. Again in this question the student demonstrated the ability to evaluate and interpret the questions completely and accurately and manipulate the questions by extending and accurately explaining the data (score value 5).

Post- survey: This student scored their confidence level at 3.5/5 in cell communication and 4/5 in biotechnology. She said the information on cadherin protein and the various technologies used for this case study made her more confident in the topic. After this case study she made a stronger connection between cell communication, biotechnology, and researchers, stating that “researchers use these to understand diseases, develop drugs and to close gaps in healthcare.” The student scored this case study a 4.2/5. The student was clearly shown to have strong critical thinking skills with a score of 5/5. I can understand how the student scored herself higher in confidence in both cell-cell communication and biotechnology.

Conclusion/Reflection: Based on the information above, it is evident that this activity benefited the student. She was using her knowledge on cell communication and biotechnology although she felt she did not know enough. However, because she had a

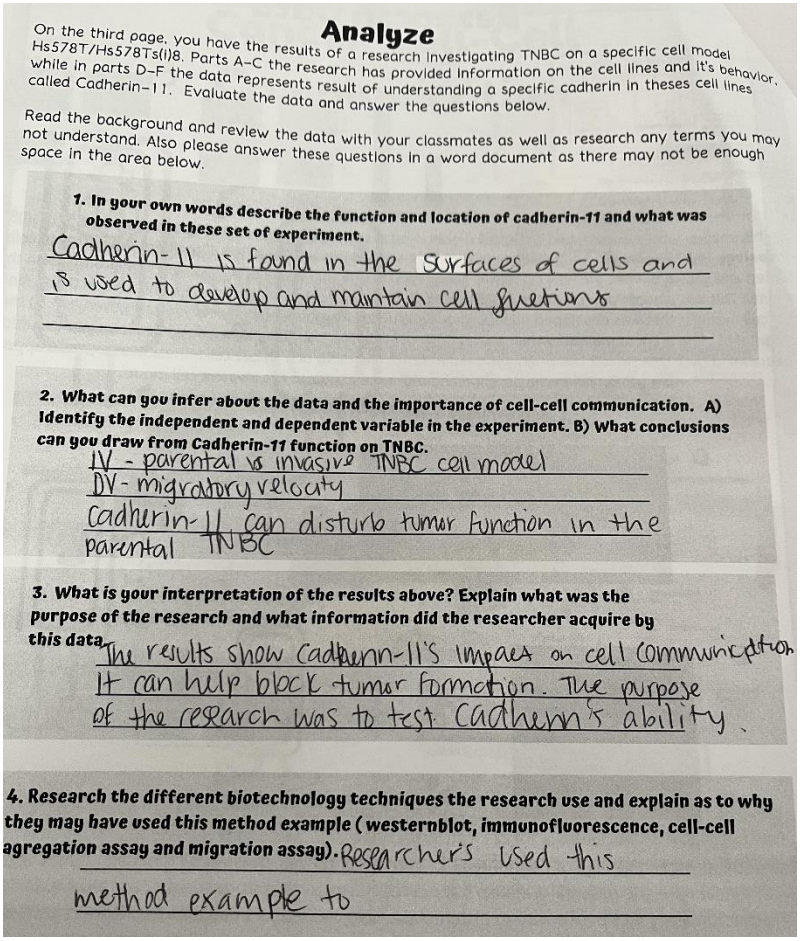
high level of critical thinking skills, it was easy to navigate through the activity. The student clearly could make connections with the big ideas in the AP biology exam and was able to interpret and evaluate data accurately and precise.

Keyword: topic information/unfocused

McKenzie

Pre-survey: This student scored their own confidence level at 4/5 in cell communication and 3/5 in biotechnology. She was more confident because she understood the concept of biotechnology, but she could not implement them into a problem or scenario. Based on my observation in class, McKenzie was studious and placed maximum effort in studying but at times could be idle in her study methods or answering questions. However, her methods of understanding and learning was by writing and visualizing concepts. If for any reason the student missed a lesson or did not study for a topic, future lessons could become difficult for her. I'd score her a 3 in evaluating, interpreting, and manipulating/transforming information.

Review from Activity



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(I)8. Parts A–C the research has provided information on the cell lines and it's behavior, while in parts D–F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin–11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Cadherin-11 is found in the surfaces of cells and is used to develop and maintain cell- juctions

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

IV - parental vs invasive TNBC cell model
DV - migratory velocity
Cadherin-11 can disturb tumor function in the parental TNBC

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

The results show Cadherin-11's impact on cell communication. It can help block tumor formation. The purpose of the research was to test Cadherin's ability.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay). Researcher's used this method example to

Figure 54. McKenzie 's analysis question answers.

On question one of this activity the student was able to successfully describe the function and location of cadherin-11: "Cadherin-11 is found in the surfaces of cells and is used to develop and maintain cell- junctions." However, the student failed to answer what was observed in these sets of experiments. In this question the student minimally determined the significance or relevance of information, and was scored a 1 when evaluating information. In question two student was able to identify the independent and dependent variable correctly as shown in the Figure () "IV- parental vs invasive TNBC

cell model and DV- migratory velocity. The student was able to successfully answer part b in question two as well. The student concluded that "cadherin-11 can disturb tumor function in the parental TNBC." In question two the student partially determined the significance and relevance of the question (score 3- evaluation) and was able to partially convert information from one form to another (score 3- manipulating data).

When asked to interpret the results discussed in question two the student's response provided a strong explanation: "the results show cadherin-11 impact on cell communication. It can help block tumor formation. The purpose of the research was to test cadherin ability." The answer could have been more elaborate however, the student provided enough information to allow the teacher, in this case myself, to infer that the student was able to make a connection by using prior information learned in class. Again in this particular question, the student was able to interpret the question and provide meaning to the data, then make inferences and predictions from data with some errors (interpreting score-3) and partially converted information/data from one form to another (manipulating data – 3). However, on question four involving biotechnology, the student provided an incomplete and answer and no data could be taken from this question resulting in a score value of 0.

Post-survey: The student stayed the same in confidence level in cell communication but felt more confident in biotechnology 4/5. The reason for this was because the scenario helped her break down the topic and understand the connection. She gave a 4/5 on the usefulness of the case study. I do not agree with student's

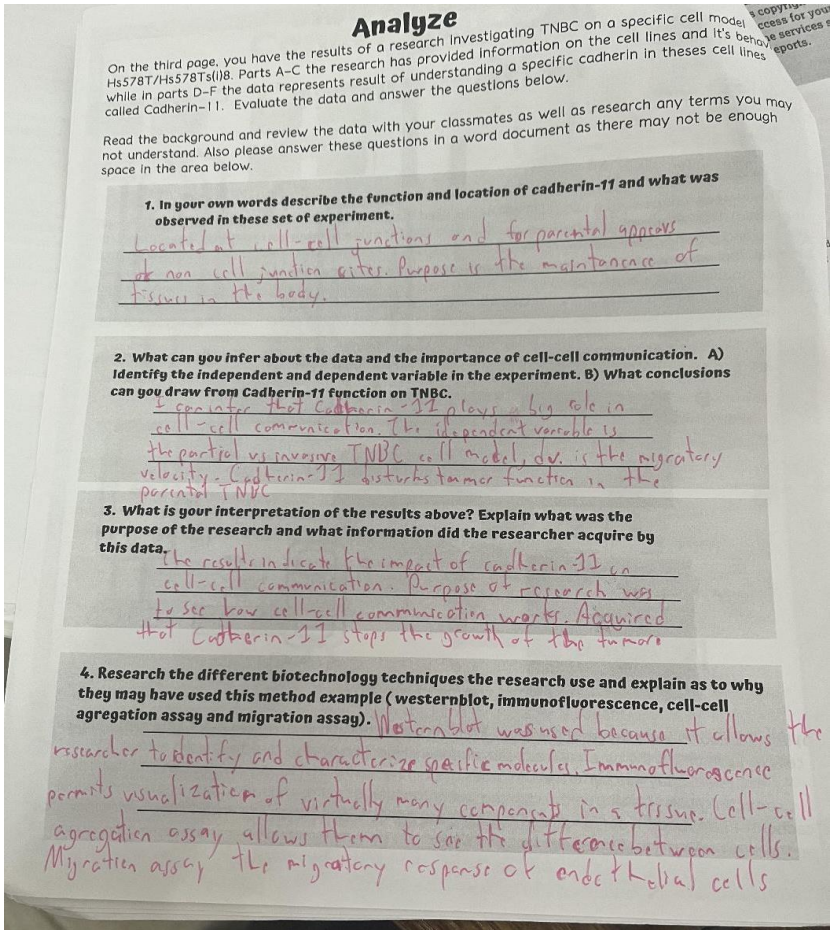
confidence assessment. Her work reflected a score of 3 in which a student was able to understand the concepts and questions being asked, answer a question about the key steps in a problem, and produce a conclusion. No connections to Big ideas were needed for the AP biology exam. Based on the student's evaluation on information processing student scored at a critical thinking level of 2. While the student answered most questions at a 3 in critical thinking, the student's score was lowered due to incomplete answers and or answering a question incorrectly. I believe the reason the student scored lower than a 3 was because the student was not invested in the assignment. In addition, I believe the student's confidence level did not correspond with her answers on the question or critical thinking questions. The student said her confidence increased in biotechnology although she did not answer the biotechnology question. Nonetheless, the student must have believed that evaluating and reading the graphs were helpful enough to increase her confidence in biotechnology.

Conclusion/Reflection: Based on the student answers and evaluation, I believe the student did not truly try on this assignment and scored a critical thinking skill of 2/5 in this assignment. The reason for this could be that the student did not have a strong basis on cell communication/ biotechnology and evaluating the answer was too complex and required higher critical thinking than she was invested in for in this activity. I do believe, that the assignment helped the student observe different method biotechnology that were used to answer a scientific question.

James

Pre-survey: This student scored their confidence level at 3/5 in cell communication and 4/5 in biotechnology. The reason biotechnology was higher was because charts and graphs increased his/her confidence in this topic as they provided enough information for them to understand. James was studious and very serious about his education. He relied on multiple forms of studying such as taking notes, practice problems and visual aids. He incorporated all these techniques into his studying. He paid attention in class and asked question when he did not fully understand a topic. Based on his class performance I placed James on 4/5 in critical thinking process.

Review of Activity:



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A-C the research has provided information on the cell lines and it's beha while in parts D-F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin-11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Located at cell-cell junctions and for parental approxs at non cell junction sites. Purpose is the maintenance of tissues in the body.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

I can infer that Cadherin-11 plays a big role in cell-cell communication. The independent variable is the partial vs invasive TNBC cell model, dv. is the migratory velocity. Cadherin-11 disturbs tumor function in the parental TNBC

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

The results indicate the impact of cadherin-11 on cell-cell communication. Purpose of research was to see how cell-cell communication works. Acquired that Cadherin-11 stops the growth of the tumors

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

Western blot was used because it allows the researcher to identify and characterize specific molecules. Immunofluorescence permits visualization of virtually many components in a tissue. Cell-cell agregation assay allows them to see the difference between cells. Migration assay the migratory response of endothelial cells

Figure 55 James's analysis question answers.

In question one the student described the function, location and provided and connection of the protein to the experiments provided. The student answered: "located at cell-cell junction and for parental appears at non cell junction cites. Purpose is the maintenance of tissues in the body." The student provided a definition of cadherin-11 and was able to connect its location to the parental and invasive cell lines and make a comparison, which demonstrated preliminary understanding of cadherin-11 in this research. The student evaluated, interpreted, and extended the information completely and accurately (score 5). The student completely determined the significance or relevance of information/data needed, accurately provided meaning to data, made inferences and predications from data, and completely converted information from one form to another.

The student further developed his understanding in question two when he successfully answered (part A) identify the independent and dependent variable and (part b) what conclusion can he/ she draw from the experiments. The student answered: " I can infer that cadherin-11 plays a big role in cell-cell communication. The independent variable is the parentall vs invasive TNBC cell model, the dependent variable is the migratory velocity. Cadherin-11 disturbs tumor function in the parental TNBC." The student once again demonstrated he could evaluate and interpret accurately (score 5). The student partially converted the information or data, but he could have elaborated and explained the conclusion more extensively (score of 4).

In question 3 the student continued to develop his understanding on the research has he/she responded: *" the results indicate that he impact of cadherin-11on cell-cell*

communication. Purpose of research was to see how cell-cell communication works. Acquired that the cadherin-11 stops the growth of the tumor." Cadherin-11 does not stop the growth of tumors as the student expressed but it does have an influence on cell migratory process. Although the student did not answer the question on what information the researcher acquired out of this data correctly, the student demonstrated high levels of critical thinking to answer this question. His train of thought was in the right place, and I highly believe if the student spent more time with the data and or the process the student could reached the correct conclusion on cadherin-11. Based on the student's answer he evaluated completely and determined the significance or relevance of information (score 5), provided meaning to data, made inferences and predications from data with some errors (score 4) and partially converted information from one form to another with some errors (score 4).

Lastly, the student researched the different type of technology used in the research and explained why the research used that method example. The student responsed: "Western blot was used because it allows the research to identify and characterize specific molecules immunofluorescence permits visualization of virtually many components in a tissue. Cell-cell aggregation assay allows them to see the difference between cells. Migration assays the migratory response of the endothelial cells." The student overall demonstrated mastery on data analysis and an ability to interpret experimental results without being part of the process. It could also be safely inferred that student used prior knowledge and skills to successfully identify and reason through the experimental data. The student used all information processing category and scored 5 in evaluating, interpreting, and manipulating/ transforming information.

Post-survey: The student scored their confidence level for both cell communication and biotechnology at 4/5. He felt confident in doing the case study as he understood that the point of the research surrounded knowing how cadherins may be used to prevent tumors. Overall, the student felt more confident in this topic and scored the cased study a 4/5 as helpful. The student's confidence increased in cell communication which I believe had to do with using his critical thinking skills and applying them to a real-world research scenario. The student score 4.5 in critical thinking based on his answer on this activity. I agree with his confidence score since student was able to complete questions accurately and answer fundamental questions and know how it relates with other BIG Idea in AP Biology.

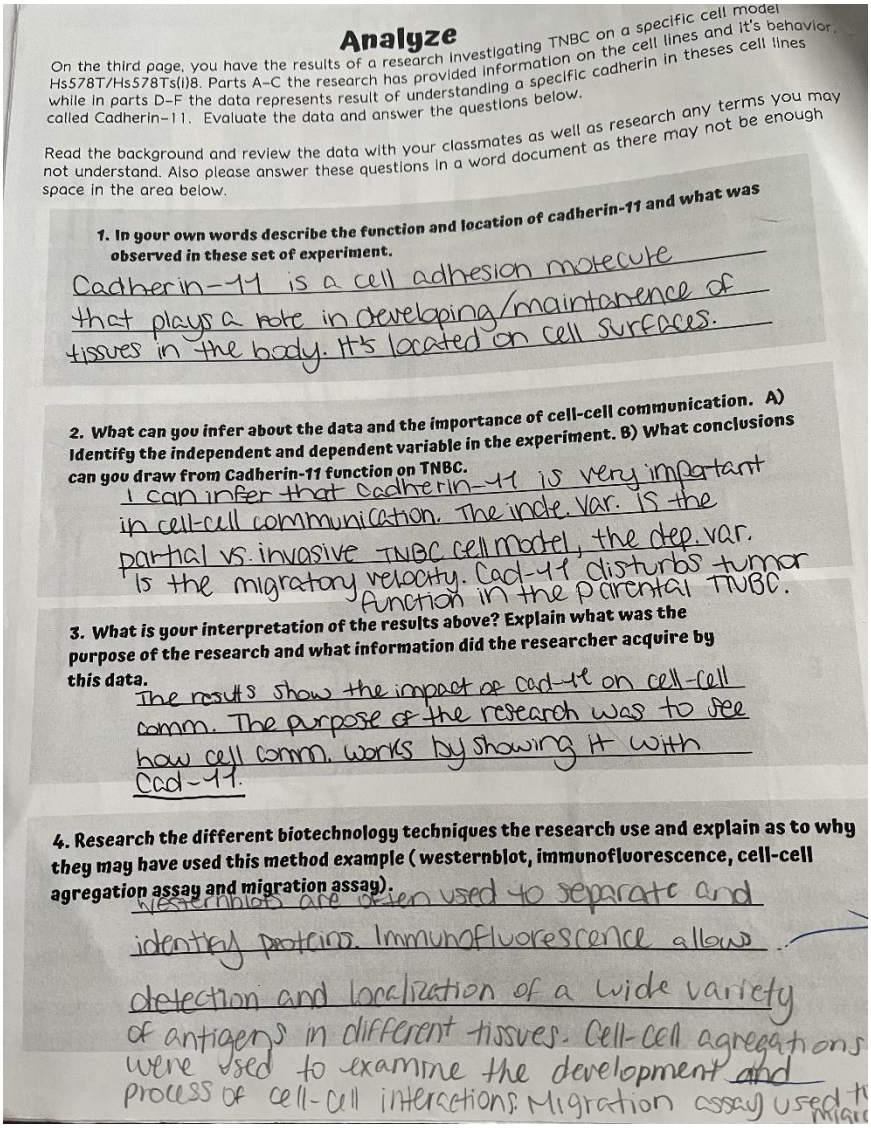
Conclusion/Reflection: I believe this worksheet was beneficial to the student because he was able to use his critical thinking and apply his knowledge on a topic (cell communication) that he did not feel particularly confident about during the lesson. The reason the student scored higher than 4 in critical thinking was because the student understood more on cell communication than he thought. I believe this exposure allowed the student to show how much he did know about the topic and how to evaluate data. The student could certainly answer more Big Idea question on the AP Biology exam, and I strongly believe he could have scored 5/5 if the student had more research-based exposure.

Abigail

Pre-survey: This student scored her confidence level at 4/5 in cell communication and 2/5 in biotechnology. She described that graphs/charts were simple to read, and she

had considerable background information about them making her more confident. Based on the student's feedback the student was confident that they completed questions accurately and answered fundamental questions. However, they did not know how to relate concepts with other Big Ideas related to AP Biology exam. Jenifer relied on visual learning and needed to break down concepts smaller chunks to understand a topic. She was very studious and very serious about her academic career. Based on my observation in class performance I gave Abigail a critical thinking score 3/5. She wais able to partially determine the significance or relevance of the data, provide meaning to the data with some errors, and partially convert information/data from one form to another.

Review of Activity:



Analyze

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Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Cadherin-11 is a cell adhesion molecule that plays a role in developing/maintanence of tissues in the body. It's located on cell surfaces.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

I can infer that Cadherin-11 is very important in cell-cell communication. The indep. var. is the partial vs. invasive TNBC cell model, the dep. var. is the migratory velocity. Cad-11 disturbs tumor function in the parental TNBC.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

The results show the impact of cad-11 on cell-cell comm. The purpose of the research was to see how cell comm. works by showing it with Cad-11.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

Western blots are often used to separate and identify proteins. Immunofluorescence allows detection and localization of a wide variety of antigens in different tissues. Cell-cell agregations were used to examine the development and process of cell-cell interactions. Migration assay used t

Figure 56. Abigail's analysis question answers.

In question one of the analysis the student partially answered the question on cadherin-11 location and function, she failed to explain her observation on the set of experiments. The student's answer was the following: "Cadherin-11 is a cell adhesion molecule that plays a role in developing/maintenance of tissue in the body. It's located on cell surfaces." The student was able to partially evaluate the significance or relevance

of the data needed for the task (score of 3) but inaccurately provided meaning to data (score of 1).

However, in question two the student was able to infer about the importance of cell-cell communication in the data provided. The student responded the following:" I can infer that cadherin-11 is very important in cell-cell communication. The independent variable is the parental vs invasive TNBC cell model, the dependent variable is the migratory velocity. Cadherin-11 disturbs tumor function in the parental TNBC." The student partially determined the significance ore relevance of the data (score 3) and provided meaning to the data with some errors (score 3) and minimally converted information from one form to another (score 1).

In addition to question three the student explained the interpretation on the results above: "The results show the impact of caderin-11 on cell-cell communication. The purpose of the research was able to see how cell communication works by show it with cadherin-11." With the combination of answers that the student provided, she showed gaps in her understanding on cell-cell communication. The answer was superficial except in identifying independent and dependent variables. This indicated that the student was not able to make connections between what they had learned with the new information with great confidence. The student minimally converted data from one form to another (score 1). On question four the student answered the following: "Western blots are often used to separate and identify proteins. Immunofluorescence allows detection and localization of a wide variety of antigens in different tissues. Cell-cell aggregations were used to examine the development and process of cell-cell interactions. Migration assay used to be migratory response." The student was able to provide a

definition for the different applied methods in the research, however failed to interpret and provide meaning to the data (score 1).

Post Survey: The student scored 4/5 in cell communication and 3/5 in biotechnology. Cell communication was an easy topic for her to grasp but the biotechnology had many components that confused her. The student scored this activity 4/5 as it made them feel more confident about the relationship between cell communication and biotechnology, as these are needed to further develop therapies and close gaps. Additionally, in question four the student provided a description on Biotechnology which could contribute to their increased confidence in the post survey. The student's critical thinking was rated at 2/5 indicating the student is in between partially evaluating and interpreting data inaccurately and minimally converting information from one form to another.

Conclusion/ Reflection: Based on the student's results it increased her confidence score in biotechnology and I believe that can be due to two factors: 1) researching about the different method and 2) having answered questions which analyzed data based on these methods. This exposure allowed the student to increase their confidence from 3 to 4, which means the student was now able to relate concepts with other Big Ideas in the AP Biology exam and was confident they could complete questions accurately with the fundamental questions answered. However, based on her answers and critical thinking score, this does not align with her confidence.

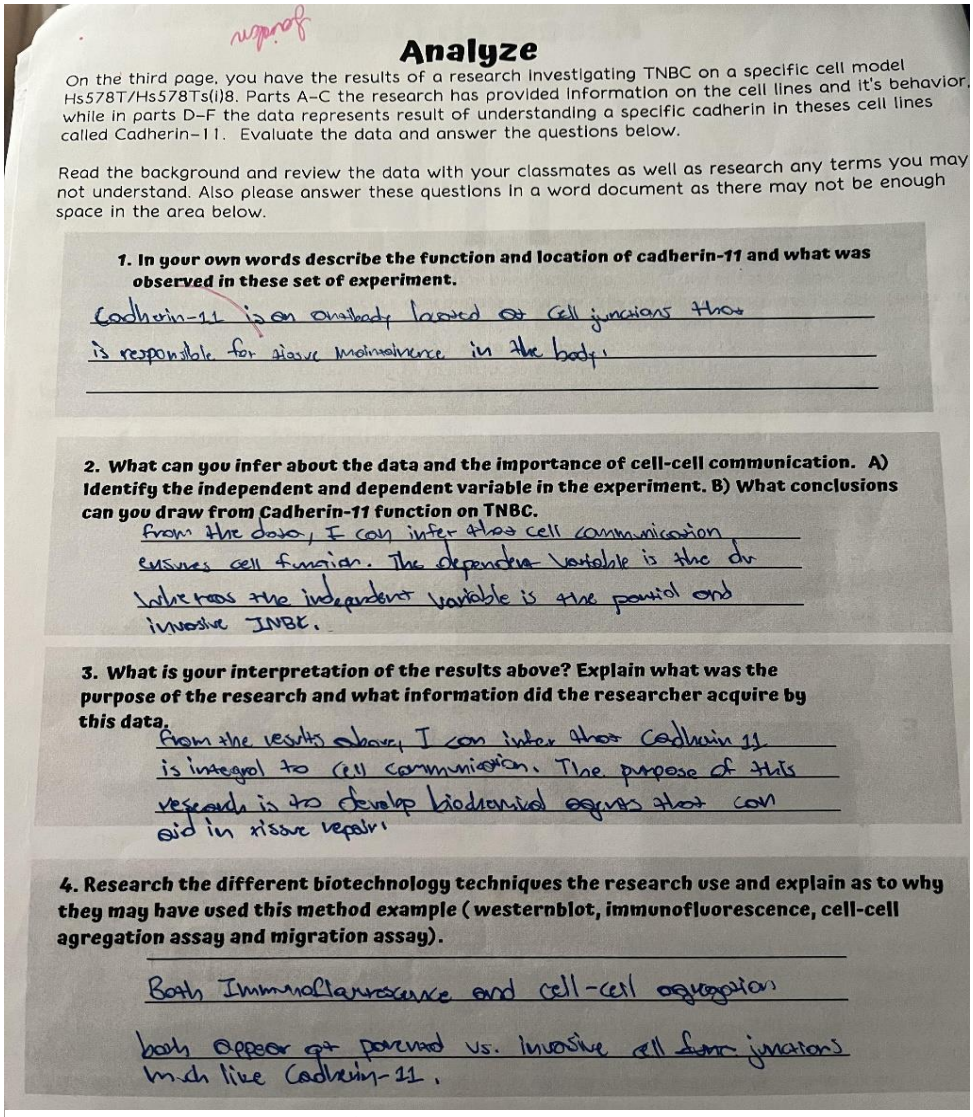
Abigail scored 2/5 in critical thinking and the reason I think this is because I suggested she has not studied in depth cell-cell communication and her ability to manipulate information was impaired because she did not fully understand the concept

itself. I do believe that the activity was helpful in her confidence because of the biotechnology exposure however, I have observed that she would need to study more on the topic to fully get the experience from the activity.

Wyatt:

Pre-survey: The student scored 2/5 in cell communication and 1/5 in biotechnology. As he explained, he did not understand how cell communication works. Student had low level of confidence in cell communication. He had a general idea of the concept but could only provide superficial answers to questions and he was not able to explain his answers. No connections were made to Big Idea questions on the AP Biology Exam. The student had no confidence in biotechnology and did not feel confident in answering questions on the topic. After the activity this student needed to be retaught the concepts. Based on in-class observations, Wyatt's had poor study habits and was very idle during class work. However, Wyatt's critical thinking was advanced, and he was very in tune with what he did or did not know. I would score him with 4/5 in critical thinking related to this activity and because he did not feel very confident in, I would score him 3/5 in terms of confidence. He could evaluate data and extract patterns from data with minimal errors and accurately converted information from one form to another.

Review of Activity:



Analyze

On the third page, you have the results of a research investigating TNBC on a specific cell model Hs578T/Hs578Ts(i)8. Parts A–C the research has provided information on the cell lines and it's behavior, while in parts D–F the data represents result of understanding a specific cadherin in theses cell lines called Cadherin–11. Evaluate the data and answer the questions below.

Read the background and review the data with your classmates as well as research any terms you may not understand. Also please answer these questions in a word document as there may not be enough space in the area below.

1. In your own words describe the function and location of cadherin-11 and what was observed in these set of experiment.

Cadherin-11 is an antibody located at cell junctions that is responsible for tissue maintenance in the body.

2. What can you infer about the data and the importance of cell-cell communication. A) Identify the independent and dependent variable in the experiment. B) What conclusions can you draw from Cadherin-11 function on TNBC.

From the data, I can infer that cell communication ensures cell fusion. The dependent variable is the dv whereas the independent variable is the ponsol and invasive INBC.

3. What is your interpretation of the results above? Explain what was the purpose of the research and what information did the researcher acquire by this data.

From the results above, I can infer that Cadherin 11 is integral to cell communication. The purpose of this research is to develop biochemical agents that can aid in tissue repair.

4. Research the different biotechnology techniques the research use and explain as to why they may have used this method example (westernblot, immunofluorescence, cell-cell agregation assay and migration assay).

Both Immunofluorescence and cell-cell aggregation both appear at paracad vs. invasive cell ~~fun~~ junctions much like Cadherin-11.

Figure 57. Wyatt's analysis question answers.

In question one the student described the function and location of cadherin-11 however, failed to explain what was observed in the set of experiments. The student provided the following answer: "Cadherin-11 is an antibody located at cell junctions that is responsible for tissue maintenance in the body." The student's answer was interesting, and it sounded like the student was trying to make connections between the definition of cadherin-11 and what was occurring in the data. Cadherin-11 is a transmembrane protein found in the cell membrane and it is important for tissue maintenance in body

however, in this research is important to TNBC migration. In the investigation the antibodies created for this protein was used to observe how it affect the behavior of both TNBC models (parental and invasive). In this case the student did not answer the question correctly but demonstrated attempts at making connections between prior knowledge and new knowledge. The student minimally determined the significance of information needed (score 1) and interpreted meaning to data, making inferences with some errors (score 3).

In question two and three the student was asked to identify independent and dependent variables and to conclude and infer cadherin-11 function on TNBC based on the data provided. The student responded to questions two and three, respectively: “From the data, I can infer that cell communication ensures cell function. The dependent variable is the dv whereas the independent variable is the parental and invasive TNBC.” “From the results above, I can infer that cadherin-11 is integral to cell communication. The purpose of this research is to develop biochemical agent that can aid in tissue repairs.” Student’s answer for independent variable was unclear however, the student showed an understanding as to why investigating on this cadherin could shed light on new discoveries. The student partially converted information from one form to another with some errors (score 3) and inaccurately provided meaning to data from other data (score 1).

Lastly, on question four the student did not answer the question fully but did try and make connections with the data: “Both immunofluorescence and cell-cell aggregation both appear at parental vs invasive cell junctions much like cadherin-11.” The students’ response was not clear but did demonstrate an attempt to reason with the

data provided. The student provided reasoning and tried to relate the information with the data the student partially determined the significance of data (score 3) but minimally converted the data from one form to another (score of 1).

Post-Survey: The student scored 3/5 for both cell communication and biotechnology. After the activity the student had a better understanding on how biochemical agents like cadherin 11 are used to carry out bodily functions. This student found this activity 3/5 helpful. The student felt he did not fully understand cell communication because there were several forms with several functions. Student increased his confidence level to 3/5, as he felt like he understood the concept and question being asked but could not answer a question that involved key steps in a problem and to produce a conclusion. No connections to the Big Idea were made. The student's critical thinking score was 2/5 and he was not able to fully evaluate, interpret and manipulate data accurately and precise.

Conclusion/Reflection: Based on the information above the student's confidence on cell communication and biotechnology increased after the assignment. This could be due to the exposure to content forcing the student to make connection and just being able to apply what he already knew. His critical thinking score was 2/5. This does not align with the score I originally gave him because he clearly did not understand the topic on cell-cell communication and biotechnology enough to answer the question appropriately. This demonstrated that for a student to fully use all of their critical thinking abilities they must know some information about concepts in order to use them.

5.4 Conclusion:

Student Perception of Authentic Research: A Triple Negative Breast Cancer Case Study provided insights on our students learning gaps in their scientific reasoning and their confidence in interpreting scientific data. If students were exposed to more scientific questions and authentic data analysis, students could improve their ability to understand the questions scientist face when answering research question. Therefore, it is hypothesized that exposing student to think like scientist, the results might instill confidence in their scientific thinking. Therefore, in this study, I wanted to evaluate *1) How do students interpret concepts that require higher critical thinking* and *2) How does their interpretation affect their confidence on the subject.* To make this possible I created my own Case Study on Triple Negative Breast Cancer using my data from my research in South Dakota State University. I created a worksheet that provided background information with guided questions that aided the reader to think like a scientist in training. The worksheet provided cartoon illustration of what was occurring to provide a visual aid for the students. As student work through the background information and data section of the worksheet they progressively building their knowledge to be able to answer the analysis question in the last section of the worksheet. The analysis questions were formulated to force student to engage with the data and make their own inferences based on their observations and knowledge on the topic. Lastly, to assess students' confidence level on the research students took a pre- and post-survey. Which led to an array of results, majority of the student's confidence increased after working through the

case study. There was positive correlation between students' confidence and critical thinking skills.

Based on the data cohort 1 and cohort 2 both have similarities and difference. In cohort 1, three out of four students confidence level increased in both cell communication and biotechnology and their critical thinking score were all higher than expected. Donald scored himself a 3 in cell communication and 4 in biotechnology on the pre-survey and scored himself in the post- survey a 4 in cell communication and 3 in biotechnology. The critical score remained the same at a 3.5. As mentioned in the data Donald is studious and applied to his studies, however, he requires to study material in detail and requires more time than other to fully understand a topic. I think Donald felt confident in both area but stronger in biotechnology. However, after fully investing his time in the case study he understood that he's much stronger in cell communication than in biotechnology. As predicted the student has critical thinking skills at 3.5/5 which means the student can partially evaluate, interpret data with errors and partially convert information from one form to another. Overall student that spent time one this activity increased their confidence in both topic or at least in one and all had critical thinking score value 3 or higher.

In cohort 2 had a different correlation than Cohort 1. Cohort 1 had 7/7 student whose confidence increased in biotechnology, 2/7 increased in cell communication and 5/7 remained the same in cell communication. Alan and Sawyer both increased their confidence in biotechnology however, had a low critical thinking score. I hypothesized based on their classroom performance for Alan to have critical thinking score of 2.5 and

Sawyer a critical thinking score of a 2 and both scored a 1 in the critical thinking evaluation. Based on their performance these students slightly benefited from this activity however, they clearly were not able to use their critical thinking skill beyond the information given on the activity. They would need to go back and learn about the topic because they were not able to build their understanding on prior knowledge. McKenzie , Abigail and Wyatt also increased their confidence score in biotechnology with the exception or Wyatt that all had an increase in confidence in cell communication. However, these three students who had an increase in confidence at least in one of the topics all scored the same critical thinking score, 2. The expected critical thinking score for all three students was 3. Based on past performance I still believe their critical thinking score is higher than a 3 but, in these topics, students did not have the fundamental knowledge to be able to take advantage of the case study activity. Lastly, Kadence and James confidence both increased in biotechnology and it also increased in cell communication for Kadence. However, they both demonstrated to have strong critical thinking skills, 5 and 4.5 respectively. This was higher than the expected critical thinking score that was given, 4. They both demonstrated to have a strong background on both topics and where able to use their critical thinking skills and be able to evaluate and interpret data with minimal errors.

This study demonstrated that students require to have prior knowledge in order to use or even demonstrate their critical thinking skills. Student who had low confidence like Savannah and Kadence on cell communication and biotechnology took the time to understand the topic by researching on the concept and then answered the analysis question confidence increased significantly. To fully evaluate a student's critical thinking

in cell communication and biotechnology they must have a baseline understanding on these topics. Moreover, when comparing cohort 1 to cohort 2 it appears as Cohort 1 had a stronger bases for both topics as they were the group the most benefited from the case study activity. Cohort 2 struggled more on cell communication but overall benefited from the biotechnology exposure from the research. I'd like to establish that the reason for these two groups to have a difference in strength has to do with the amount of workload students had the year they were taking AP Biology course. Student in cohort 1 was generally taking 1-2 upper-level courses such as Advanced Placement or Dual enrollment, while cohort 2, was taking between 3-5 upper-level courses. I feel as the study could be improved by repeating the investigation and analyze the critical thinking score before and after the activity and observe the correlation of the workload of students to determine if in fact it affected the student ability to fully benefit from the case study.

Overall, students that a had a strong foundation on cell communication and biotechnology was able show case their critical thinking skills. This study showed that even students that have high critical thinking skills need to have a foundation on the area they are studying and it only than that they can practice and expand their critical thinking. I hypothesized that if students were exposed to more scientific question and data, they will be able improve their ability to answer research questions and increase their confidence scientific thinking. This study provided evidence that my hypothesis is correct. Although not all students did not increase their critical thinking skills. All students did increase their confidence in at least one of the topic cell communication or biotechnology. Students were not only able to understand the research through scaffolding method, but they had guided question that allowed them to interpret the data

with or without errors. Regardless, they were all exposed to authentic data and were forced to engage with the data and turn increased the student's confidence. Moreover, it also provided students with the opportunity to understand where they struggle on this topic and where they need to spend more time on. I believe this research will help other educators understand the importance of context for students when reading a research paper and or scientific data. High school and undergraduate students tend to have the most difficulty in reading a scientific article and interpret the data and using this template will allow students get through a research paper. This study will continue to expand to other research paper in different courses such as chemistry, and environmental sciences topics student tend to have more difficulty and evaluate student confidence and critical thinking skills.

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