ECM effects on integrin-mediated signaling in breast cancer cells

Sondoss Hassan

Professor Mohamed Bouaouina

Abstract

Integrins are heterodimeric transmembrane adhesion receptors bind different extracellular matrix (ECM) protein and transduce outside-in signaling[1]. Integrins are heterodimeric adhesion receptors responsible for attachment and migration of adherent cells. Integrins-mediated adhesion and signaling in health and cancerous cells can control different cell processes and behaviors such as cell's migration, adhesion, differentiation, ability to survive and more. Different integrins and the presence of different ECM proteins can transduce different signaling in adherent cells. The relevance of the project is to investigate the specific effect of ECM proteins on cancer cell signaling. The research question is whether exposing breast cancer cells to different ECM proteins can change their signaling. To investigate this question, the cell lines used are MDA-MB-468 and MCF7. MDA-MB-468 cells are epithelial, human breast cancer cells that are invasive and differentiated triple-negative[2][3]. The MCF7 cells are also epithelial, human breast cancer cells[4] but are non-invasive and non-metastatic. The MCF7 cell-line would be used as a control. By using the 2 cell lines, we can link the different cells' behaviors to specific ECM-mediated signaling. The methods used are cell culture, cell-adhesion assay and Western blot. The specific matrices used in the cell adhesion assay would be fibronectin, fibrinogen, collagen, BSA and serum. The signaling in the cells would be compared in different ECM proteins. Moreover, the same cell line would be exposed to different ECM proteins and test for different signaling. The Western blot analysis would provide data about the phosphorylation of the following kinases: AKT, p38, ERK and FAK. These kinases would be targeted using antibodies against them. The Western blot analysis would inform about differences in the 2 cell lines that affect different behavior. Due to the current situation, I am here presenting data on MDA-MB-468 cells as I could not finish the remaining experiments with MCF7 control cell line.

Introduction

Cells in cancerous tumors behave differently than normal cells. The change in cell characteristics are caused by mutations in the cancer cells which can lead to an increase in proliferation, migration, invasiveness and more. Different integrins bind to different proteins in the ECM, transducing different signaling pathways. In this research project, 2 breast cancer cell lines (MDA-MB-468 and MCF7) were used. The ECM proteins investigated were fibronectin, fibrinogen, serum, collagen, BSA and cells in suspension. The intracellular signaling investigated was the phosphorylation of AKT and p38 kinases. The relevance of this project is to investigate the different ECM-mediated signaling in cancer cells and how it links to the cells' behaviors.

Methods

The concentrations of the matrices used in the cell adhesion assay were 1µg/mL of fibronectin, fibrinogen and collagen. Serum was not diluted. Cells in suspension was prepared by pipetting cells in a microcentrifuge tube. The cell concentration pipetted per well was 100,000 cells/mL. The cells were grown in complete DMEM and incubated in 37°C + 5% CO2 incubator. The cells were pipetted in 200uL volume. The cells pipetted in the microcentrifuge tube represent the cells in suspension control. After incubating the cells with the matrices for 3 hours, the cells were lysed using Buffer X. The protein mass was determined using BCA assay with 0.0, 0.025, 0.125, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/mL standards. The samples were then pipetted in 15.0% SDS-PAGE gel. The amount of sample pipetted was 50ug. The SDS-PAGE gel was running at 70V for 35 minutes. After running the gel, the gel was transferred to a nitrocellulose gel overnight. The primary antibodies used were rabbit and mouse antibodies to detect total and phosphorylated AKT and p38. The secondary antibodies used were Goat anti-rabbit and anti-mouse.

Data

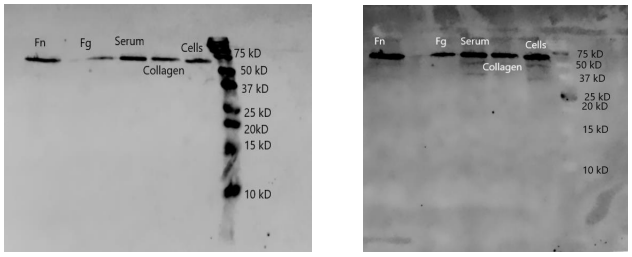

Figure 1 A representative Western-blot for the AKT kinase. (left) The channel used to detect total-AKT was 800nm. The primary antibody used was mouse anti-total AKT. (right) The channel used to detect phosphorylated-AKT was 700nm. The primary antibody used was rabbit anti-phosphorylated AKT.

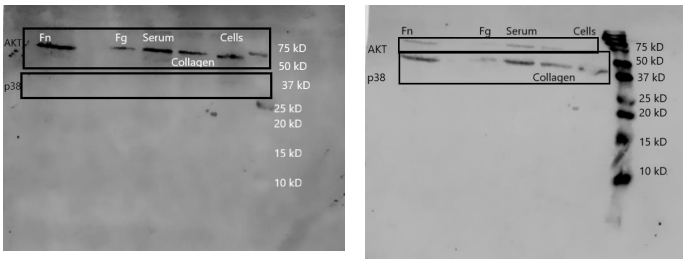

Figure 2 A representative Western-blot for p38 kinase. The bar on top is AKT and the bottom bar is p38 (left) The channel used to detect total-p38 was 700nm. The primary antibody used was rabbit anti-total p38. (right) The channel used to detect phosphorylated-p38 was 800nm. The primary antibody used was mouse anti-phosphorylated p38.

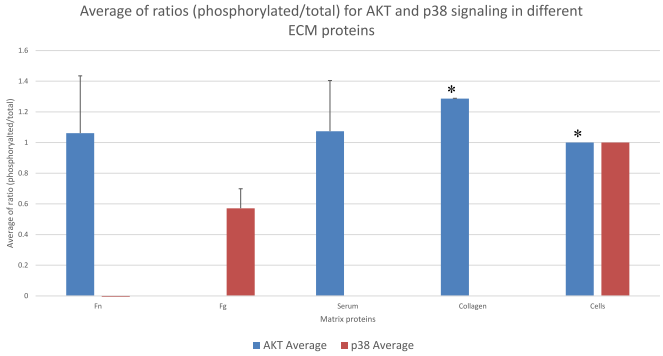

Figure 3 Bar chart of average of ratios (phosphorylated/total) for AKT and p38 kinases in different matrices. The t.scores are the error bars calculated using the ratio values. The ratio values were calculated by dividing the intensity of the Western blot band of phosphorylated/total protein. The asterisks determine the significant result ($p<0.05$) ($n>3$).

Discussion

According to Figure 1, there were bands present between the 50 kD and 75 kD. The molecular weight of AKT is 60 kD, which is present between the 50 kD and 75 kD. According to Figure 2, there were bands in the phosphorylated p38 but no bands in the total p38. The bands in the total-p38 image were the bands of AKT as the same membrane was used to probe for both kinases. However, due to technical errors, there was no detection of the total p38 bands despite detecting phosphorylated p38 bands. The ratios and t-scores were not calculated for this reason. According to Figure 3, the only statistical result (marked with an asterisks) was the collagen result compared with the cells in suspension for AKT. This means that there is a significant change in the phosphorylation of AKT when cells bind to collagen. The p38 results were inconclusive as no bands in total p38 were recorded and no t.scores were calculated.

Conclusion and Future work

The conclusion is that integrin-mediated adhesion to fibronectin and serum does not change AKT phosphorylation in MDA-MB-468 cells compared non-adherent cells. This indicates that AKT signaling is independent of integrin-mediated outside-in signaling. Integrin-mediated adhesion to collagen increases AKT phosphorylation compared non-adherent cells. This shows that collagen transduces outside-in signaling. We can not conclude about p38 signaling. The future work for AKT signaling will be to experiment on integrins that adhere to collagen. The receptors that adhere to collagen are β1-integrins.The inhibition of the integrin can be done and compare cell characteristics such as cell adherence and AKT signaling. The future work for p38 is to load a positive control and increase protein mass loaded in the SDS-PAGE. The experiments will be replicated for the MCF7 control cell line.

References

1. Cooper, G., 2000. The Development And Causes Of Cancer. [online] Ncbi.nlm.nih.gov. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK9963/> [Accessed 28 April 2020].
2. Seguin, L., Desgrosellier, J., Weis, S. and Cheresh, D. (2015). Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. Trends in Cell Biology, 25(4), pp.234-240.
3. Atcc.org. (n.d.). MDA-MB-468 ATCC ® HTB-132™ Homo sapiens mammary gland/breast. [online] Available at: https://www.atcc.org/Products/All/HTB-132.aspx [Accessed 20 Jan. 2020].
4. Thermofisher.com. (n.d.). mcf-7 Cells | Thermo Fisher Scientific - UK. [online] Available at: https://www.thermofisher.com/qa/en/home/technical-resources/cell-lines/m/cell-lines-detail-36.html [Accessed 22 Jan. 2020].

MEETING OF THE Minds
Undergraduate Research Symposium

Carnegie Mellon University Qatar