THE EFFECT OF PROFILIN ON BREAST CANCER CELL MOTILITY

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INTRODUCTION

Breast cancer is the most prevalent cancer in women, accounting for about 30% of all new cancer cases in women. [1] Currently, the most common treatment method is surgery. However, this is often not successful because cancer cells have a strong tendency to metastasize. Metastasis is when cancerous cells from the primary tumor detach and enter the blood stream. While in the blood stream, the immune system tries to rid the body of these cells. Most of these cells are killed off but the most aggressive cells often survive. Since the circulatory system transverses through the whole body, these cells have the potential to establish themselves in any location. These secondary tumors are generally more detrimental than the primary tumors because of the natural selection-like process that occurs in the blood stream. Although all of the primary tumor might be able to be removed by surgery, it is practically impossible to do the same for the more aggressive secondary tumors scattered throughout the body.

profilin is an actin binding protein that regulates the rate of actin polymerization and depolymerization in the cell. There are two main variants of the protein, profilin-1 and profilin-2. Profilin-1 is expressed at relatively equal levels all throughout the body while profilin-2 is expressed at high levels in neuronal cells and lower levels everywhere else. It is well established that profilin affects cell motility: lower profilin-1 levels correspond to higher cell motility [2].

OBJECTIVE

The primary objective of this research was to determine the effects of overexpression of profilin-1+2 on the average speed of motility of breast cancer cells. The secondary objective was to determine the effects of cell confluency, also known as cell density, on profilin levels.

HYPOTHESIS/SUCCESS CRITERIA

Previous research has shown a strong negative correlation between profilin-1 and cell motility [2]. Therefore, it was assumed that overexpression of profilin-1 would hinder the cell’s motility. Due to the lack of prior studies done on profilin-2, no hypothesis was made. Since it has been long known that profilin affects cell movement, there needed to be a statistically significant difference between the control and overexpression groups for this research to be considered successful.

METHOD

The day before the cells needed to be transfected with the plasmids for overexpression, 300,000 MDA-231 cells were plated in a 6-well dish. There were four wells plated – one for the control group and three for each of the overexpression groups. These cells were then allowed to incubate overnight at 37°C.

The following day, 1 µg of DNA was initially added to 500 µL of serum-free OPTI-MEM media in an Eppendorf tube. For the control group, pcDNA was used as the plasmid. For the three overexpression groups, one was transfected with profilin-1 plasmid, the other with profilin-2 plasmid and the third was transfected with a 50-50 mixture of both. Next, 1 µL of LipoFectamine LTX reagent was also added. It was then well mixed and allowed to sit at room temperature for five minutes. 2.25 µL of Plus Reagent was then added to the tube and allowed to sit for fifteen minutes. Then, the mixture from the Eppendorf tube was added directly onto the cells that were plated the day prior. These were then put back in the incubator overnight.

Two days after the transfection, the cells of each well were replated on a 24-well dish. Each of the wells contained 8,000 cells. This low count was necessary in order to ensure that the cells were not touching and influencing the movement of each other. Two wells per group were plated to ensure that there were enough transfected cells for each of the groups. The cells were allowed to incubate overnight at 37°C.

The movie was taken the next day. Two fields of view were selected for each of the wells. Each field had to have the greatest number of individual cells possible. If these cells were touching each other, it would be very hard to quantify and the results would be skewed. After each field of view was chosen, a fluorescent image was taken. This image is necessary to only quantify the movement of the transfected cells. The microscope then took one image per minute of each field over a two hour time period.

Once the movies were done, they were uploaded on ImageJ. Using the fluorescent images as a guide, all of the transfected cells were manually tracked.

For the confluency experiments, different quantities of MDA-231 cells were plated on the 6-well dish. The goal of the plating was to have a plate at about 20%, 50% and 100% confluency ready to be extracted the next day.

Before the cells were extracted, the number of cells in each well was counted. This would be useful to control for the amount of protein extract that needs to be added to each of the lanes while running the Western Blot. The extraction was performed using a lysis buffer. This buffer consisted of a detergent to lyse the cells and protease inhibitors to prevent the degradation of the proteins in the cells. A western blot was then performed on each of the extracts to determine their profilin levels.

RESULTS

After repeating the experiment eight times over the course of two weeks, 70 cells were quantified for the control group, 40 for the profilin-1, 22 for the profilin-2 and 35 for the combined profilin-1+2 group.
The average speed for the control group was about 1.3 \( \mu \text{m/min} \) while the average speeds for the P-1, P-2 and P-1+2 groups were 0.85, 1.4 and 1.63 \( \mu \text{m/min} \) respectively.

The western blots for the confluence experiments are shown below. Tubulin was used as the loading control to confirm that equal amounts of protein was added to each lane.

**DISCUSSION**

The results from the motility experiments initially appear to agree with the hypothesis. There is a slight decrease in average speeds from the control group to the profilin-1 overexpression group, which is what was expected. However, it is important to notice that there was a huge range for the speeds of the profilin-2 group and that the ranges of the average speeds of all of the groups overlap significantly. As a result, nothing can be concluded from the motility experiments as the differences between each group was not significant enough. There are many possible sources of this error.

Since the quantification of the cells were done by hand, there was a lot of variability and guessing involved. This might have skewed the results ever so slightly, which could explain the small differences between each group.

Another plausible source of error is different number of cells quantified for each group. There were a lot of issues getting the profilin-2 transfection to work properly. Therefore, only about 1/3 as many cells were quantified for the profilin-2 group compared to the control group. This explains the extremely wide range of speeds for the profilin-2 group as the presence of a few outliers could have had a large effect on the average speed of the whole group.

The low profilin-2 transfection rate also brings into question the cells quantified in the profilin-1+2 group. Did they overexpress both profilin-1 and profilin-2? Or did they only overexpress profilin-1? Unfortunately, since both plasmids had the same fluorescent tag, it was not possible to determine what was overexpressed in those cells. In the future, it is worthwhile get different tags on the plasmids so that it is possible to differentiate between the two.

Although all of the issues stated above were fixable, it would have taken far too long. Therefore, the project was slightly modified. Instead of altering profilin levels and observing its effects on the cells, the profilin levels were measured in response to changing cell confluency.

Due to the nature of the confluency experiment, there was a lot of issues getting the western blot to work correctly. In order to properly compare protein levels in two different lanes, it is necessary to load the same amount of protein into each lane. This is very difficult to accomplish for this experiment because each well has a different number of cells. The extract from highly confluent cells will have a larger concentration of proteins than those of lower confluence. One of the methods to control for this is by measuring protein concentrations of the extracts. This was tried multiple times but did not produce correct results. The second option, which was utilized for the blots shown above, was to count the number of cells before they were lysed and to only load a specific number of cells into each well.

As shown by the tubulin bands, this method worked, but was not perfect. All of the tubulin bands were equally dark, but the lanes for the lower confluencies were longer than higher ones. This meant that the more protein was being loaded for the lower confluencies.

From the blots, it appears that the profilin-2 levels increase at higher confluencies and that the profilin-1 levels remain relatively constant. However, it is necessary to fix the loading errors before coming to a definitive conclusion.

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**REFERENCES**
