THE POTENTIAL ROLE OF PROFILIN IN METASTATIC BREAST CANCER

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INTRODUCTION

Breast cancer is the most common form of cancer among women. In fact, 1 in 8 women are to be diagnosed with invasive breast cancer, the fatal form of breast cancer. Already in 2016, there have been approximately 250,000 cases of metastatic breast cancer with a death total of around 41,000 women [1]. Clearly, there is a need for better treatment options or detection methods for this form of cancer.

The actual deaths, however, are not caused by the breast cancer itself. Rather, it is the spread, or metastasis, of cancer niches from the primary site in the breast to secondary sites such as lung or lymphatic tissue that leads to death [2]. Metastasis is characterized by significant cell migration. Such migration is regulated by the dynamic actin cytoskeleton. Actin, in turn, is regulated by a protein known as profilin (Pfn) which, under normal physiological conditions, helps to sequester free actin monomers and attach them to further actin polymerization [3]. In pathophysiological states, lower levels of profilin have been associated with more aggressive and metastatic breast cancer [4].

However, Pfn, which comes in two isomers: profilin-1 (Pfn-1) and profilin-2 (Pfn-2), regulates cancer differently depending on the stage that the disease is in. Our lab has shown that in early stages of cancer, profilin induces the migration of cancer cells to secondary locations; however, once these cells reach the new sites profilin actually impedes the recolonization of a new niche [2]. Clearly, Pfn’s function in cancer is not as cut and dry as other proteins such as Ras or p53.

Moreover, E-cadherin is a transmembrane cell-cell connecting protein which has been shown to be a tumor suppressor. Loss of E-cadherin induces what is known as epithelial to mesenchymal transition (EMT) in which cells become unbound from each other and are thus allowed to migrate [4]. EMT is a hallmark of metastatic cancers.

Structurally, E-cadherin is anchored to the cell via actin fibers [5]. Our lab has previously shown that this structural relationship also plays a role with the signaling axis of both profilin and E-cadherin. When Pfn-1 is knocked down via siRNA in MDA-MB-231 breast cancer cells, there is a knockdown of 46% cell-cell connections made through E-cadherin [4].

OBJECTIVE

Despite knowledge of Pfn’s dual role in early versus late stages of cancer, the structural relationship with E-cadherin, and the reduction in cell-cell connections from Pfn-1 knockdown, there is still a gap in the research for Pfn’s exact signaling relationship with E-cadherin. Depending on the stage of breast cancer, E-cadherin may or may not be present which therefore has the potential of dysregulating Pfn. It is because of this uncertainty, an investigation on disruptions of E-cadherin is needed. Elucidating whether or not Pfn is changing as a result of perturbed E-cadherin is the primary goal of this project.

HYPOTHESIS/SUCCESS CRITERIA

It is therefore hypothesized that perturbing levels of functional E-cadherin will alter levels of Pfn. Despite current knowledge about Pfn and E-cadherin regulation, it cannot be predicted whether Pfn will increase or decrease as a result of decreased functional E-cadherin. This is primarily due to the contrasting functions of Pfn in early and late stage cancers [2]. Nonetheless, the project will have been successfully undertaken should consistent changes be detected in either Pfn-1 or Pfn-2.

METHODS

The signaling axis was studied using an aggressive, metastatic breast cancer cell line known as MCF-7. Disruption of E-cadherin in these cells was done by modifying the density at which these cells were plated on to 10cm petri dishes. A low density plate had 15% confluency, a medium density plate had 40% confluency, and a high density plate had 90% confluency. Cells were incubated at 37°C and 5% CO₂ and placed in 10% FBS, 1% penicillin/streptomycin DMEM.

24 hours after seeding the cells into their respective dishes, the media was changed to removed non-functional and dead cells to ensure that the culture was pure. An additional 24-hour incubation period was given to ensure that the cells recognized the changes in the physical environment thus allowing for subsequent homeostatic regulation via transcriptional changes.

Next, the cells were lysed using RIPA buffer to extract the culture proteins. These lysates were loaded onto a 15% gradient electrophoresis gel in a 3:2:1 volume ratio for the low, medium, and high cultures respectively to account for variations in the protein concentrations due to differences in culture densities.

Once the gel was run, a western blot was done using a 1°/2° antibody system to check levels of Pfn-1 and Pfn-2 with a loading control of GAPDH. The GAPDH 1° antibody is optimized at a 1-2000 (v/v) concentration. Pfn-1 is at a 1-4000 (v/v) concentration. Pfn-2 is at a 1-1000 (v/v) concentration. All 2° antibodies are at a 1-1000 (v/v) concentration, and all exposure times for imaging are at .75s.

The data was analyzed by measuring band intensities of desired proteins using Image Lab software. Intensity contributed by noise was removed through Image Lab. Loading was normalized by dividing each experimental band by its corresponding GAPDH band. To compare the experimental bands to each other, the Pfn bands were normalized to their respective low density bands.
RESULTS

Upon band intensity analysis for Pfn-1, when normalized to the low density culture—so that it has a standardized 1 fold expression—it is seen that the medium density culture has a 2.05 fold expression intensity, and the high density culture has a 2.8 fold expression intensity. Figure 1 depicts this expression graphically.

![MCF-7 Pfn1 Levels](image1)

**Figure 1.** A bar graph display of the fold expressions of Pfn-1 based on varying cell density cultures.

Pfn-2 did not show any clear pattern of expression between the low, medium, and high expression. The low culture was again at a normalized 1 fold expression; the medium was at a 1.48 fold expression, and the high was at a 1.35 expression. As can be seen from figure 2, neither the medium nor the high cultures were vastly different then the low density.

It is important to note that the western blots for Pfn-2 were not very good quality. There was a significant amount of background in the blots, and the low culture density Pfn-2 band was most likely not strong enough to compute an accurate expression intensity.

![MCF-7 Pfn2 Levels](image2)

**Figure 2.** A bar graph display of the fold expression of Pfn-2 based on varying cell density cultures.

A statistical analysis was not completed on the data as there were not enough experimental repetitions done to warrant such an analysis. Further trials will be needed to accurately conduct a significance test.

DISCUSSION

Based on the results of the study, the hypothesis was supported for Pfn-1 only. Pfn-2 did not show a clear pattern of change as the culture density changed which suggests that Pfn-2 may not be directly affected by E-cadherin. However, since the western blots were not of good quality, it is likely that the noise interfered with the calculations of the intensities. Given this, more repetitions need to be done in order definitively determine Pfn-2’s potential role in cancer metastasis.

Pfn-1’s increasing expression as density of the culture increases suggests that the more functional E-cadherin there is among the cells, the more Pfn-1 activity there will be. As culture density increases, cells will be in greater contact with their neighbors. Given that MCF-7 cells are epithelial cells, they will be able to form more cell-cell junctions via E-cadherin.

Since both of these proteins show a positive correlation as their levels change, it is likely that they have a synergistic function involving the migration of cells and by extension metastasis. As discussed earlier, since Pfn has a dual role in cancer, it could not be predicted in what direction levels of Pfn will change. Since Pfn-1 levels are changing in the same direction as E-cadherin, it is likely that Pfn-1 is playing more of a tumor suppressive role when levels of E-cadherin are initially changing.

A key limitation in this project was that exact E-cadherin disruption could not be measured in the cell cultures. E-cadherin was not stained for in the western blot, and the method of its functional disruption was rudimentary. Because of this, future trials should check levels of E-cadherin disruption through western blots. Additionally, a more robust method of E-cadherin knockdown, such as siRNA, should be used to ensure proper perturbation of function.

ACKNOWLEDGMENTS

I would like to thank Dr. Partha Roy, the McGowan Institute for Regenerative Medicine, and the Swanson School of Engineering for providing me the opportunity to undertake this project. I would also like to thank Dr. Marion Joy and Dr. Souvik Chakraborty for their technical insight in conducting the experiments. Finally, I would like to thank Dr. Harvey Borovetz and Ms. Casey Hansen for their assistance in the development of this paper.

REFERENCES


