INTRODUCTION

One of the major clinical problems with breast cancer treatment today is metastasis, or spread of tumor cells from the breast to secondary sites such as the liver, the brain and the lung [1]. Once breast cancer becomes metastatic, the five-year survival rate drops from 83.9% to 23.8% [2]. The dismal survival rate suggests that new therapies are needed.

Developing such treatments requires examination of recent work on the mechanisms behind metastasis. Many scientists now believe that transitions between epithelial and mesenchymal states drive breast cancer metastasis [3]. Because many breast cancers are derived from epithelial cells, they initially share many properties of the epithelial lineage, growing in sheets and not being very invasive. The theory then states that changes in the tumor environment lead it to undergo a process called the epithelial-to-mesenchymal transition (EMT), which causes cells on the edge of the tumor to change their gene expression patterns to become more invasive and migratory [3]. These cells then invade the bloodstream, are transported through the blood and arrive at the secondary site. Here, the cells convert back to the epithelial state in a process called the mesenchymal-to-epithelial transition (MET), enabling them to grow and form a successful metastasis [3].

This process offers two therapeutic options to inhibit metastasis. By finding a way to block EMT, scientists could prevent tumor cells from escaping the primary tumor, increasing the effectiveness of surgery and targeted radiation. A therapy that blocked MET would prevent cancer cells in the blood from being able to survive and grow at the secondary site reducing the incidence of metastasis.

In our lab, we feel that we may have found a regulator of EMT and MET that has the potential to be used in future therapy: the profilin family. Both profilin-1 and profilin-2 have been reported to have decreased expression in several cancers [4,5]. In our lab, we have recently done experiments to directly examine the role of the profilin family in metastasis. We knocked down expression of profilin-1 in the highly invasive, highly metastatic breast cancer cell line MDA-MB-231, and injected these cells into the bloodstream of mice. We found that cancer cells with profilin-1 knocked down formed fewer metastases in vivo than control cells [6]. These results suggested that altering the expression of the profilin family may regulate metastasis, but does not tell whether these changes arise through alterations in the ability of tumor cells to undergo EMT, MET or some combination of both.

Currently, our approach has been to focus on whether knockdown of the profilin family promotes EMT. EMT is usually assessed by looking in changes in the expression of various molecular markers. One such marker is the cell adhesion protein E-cadherin. In epithelial cancers that aren’t as invasive, such as the MCF-7 breast cancer cell line, E-cadherin is strongly expressed at the cell membrane [3]. However, as the cells progress through EMT, membrane expression of E-cadherin gradually decreases, until it final disappears in the invasive mesenchymal state [3]. Thus, in our study, decreases in membrane expression of E-cadherin are used as a marker for progression of the cancer cell towards EMT.

OBJECTIVE

The objective of this study is to see whether knockdown of profilin-1 and profilin-2 in the MCF-7 breast cancer cell line decreases the membrane expression of E-cadherin, pushing the breast cancer cells towards EMT.

METHODS

There were three overall steps in this study. First, profilin-1 and profilin-2 expression in the MCF-7 cells was knocked down using RNA interference. About 1.67 x 10^5 cells were plated into two 30 mm dishes and allowed to attach overnight. The next day, 100 nM of profilin-1 siRNA and profilin-2 siRNA (Santa Cruz Biotechnology, Santa Cruz, CA) were added to one dish. The other dish had 200 nM non-targeting pool siRNA added to it. Both dishes were then treated with siRNA. Following treatment of the cells with siRNA, the next step was to assess the level of profilin-1 and profilin-2 knockdown via Western blot. About 48 hrs after siRNA was added to the cells, the cells were plated out in 60 mm dishes. They were allowed to grow for two days and were lysed in RIPA buffer with 10% SDS and protease inhibitors. The protein samples were then loaded into a 15% polyacrylamide gel, and separated by molecular weight using gel electrophoresis at 200 V for 45 minutes. The sampled proteins were then transferred to nitrocellulose membrane using wet tank transfer at 100 V for 90 minutes. The membrane was then immunoblottedted using mouse anti-profilin-1 and mouse-anti-profilin-2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), as well as an antibody to B-tubulin (BD Biosciences, San Jose, CA) which acted as Another success criteria in this study was to get complete knockdown of profilin-1 and profilin-2, as observed
by the absence of any bands in the lanes corresponding to cells treated with profilin-1 and profilin-2 siRNA.

Finally, immunofluorescence was performed to assess E-cadherin expression. Cells were plated on glass coverslips coated with rat tail collagen type 1 (BD Biosciences, San Jose, CA) with about 50,000 cells per coverslip, with two coverslips for both the control and for the profilin-1/profilin-2 knockdown group. Cells were allowed to attach overnight and were then fixed in 3% formalin. Cells were then permeabilized with 0.5% Triton-X buffer, and incubated with anti-E-cadherin antibody (Cell Signaling Technologies, Danvers, MA) in 10% goat serum overnight. After washing, cells were incubated using Alexa 594 goat anti-rabbit secondary antibody (Invitrogen, Grand Island, NY). Cells were then mounted on glass cover slips and imaged under a fluorescent microscope. The success criterion for this experiment was observation of strong membrane expression of E-cadherin in control cells. Because control cells should have a high membrane level of E-cadherin, high fluorescence in the membrane area around the control cells would indicate the staining worked.

RESULTS

The two bands corresponding to B-tubulin in the Western blot are equal in intensity, suggesting that the same amount of protein was loaded into each of the wells of the gel (data not shown). This means that the differences observed in the profilin-1 and profilin-2 bands arise from changes in the cells themselves, not from an error during gel loading. We also observe that profilin-1 has a strong band in the control cells but no band in the cells treated with profilin-1 and profilin-2 siRNA (data not shown), suggesting profilin-1 levels were completely knocked down by siRNA treatment. In the profilin-2 bands, we see a strong expression of profilin-2 in the control cells with weak expression of profilin-2 in the cells treated with profilin-1 and profilin-2 siRNA. While profilin-2 was not completely knocked down because the appearance of band in the well treated with profilin-2 siRNA, expression dropped by about 80%, so the knockdown is still decent.

From Figure 2, we can see that the immunofluorescence protocol worked, as the control cells show strong expression of E-cadherin at the cell membrane. The profilin-1/profilin-2 knockdown cells also show a much weaker membrane staining of E-cadherin, suggesting that E-cadherin membrane levels go down after knockdown of profilin-1 and profilin-2.

Figure 2: Knockdown of Profilin-1 and Profilin-2 in MCF-7 Cells Decreases Membrane Expression of E-cadherin

DISCUSSION

Overall, this experiment was successful. Western blotting revealed a complete knockdown of profilin-1 levels following siRNA treatment and a high but not complete knockdown of profilin-2 protein levels. Membrane staining of E-cadherin revealed strong expression of E-cadherin in control cells, and a weaker E-cadherin expression in cells treated with profilin-1 and profilin-2 siRNA, suggesting membrane E-cadherin decreased following knockdown of profilin-1 and profilin-2. Unfortunately, I was able to do only one experiment due to time constraints, failing that success criterion. Overall, this data suggests that decreased expression of profilin-1 and profilin-2 pushes cells toward EMT, because E-cadherin expression dropped following profilin-1 and profilin-2 knockdown.

One limitation of this study is that was performed using in vitro cell culture methods using an established breast cancer cell line, called MCF-7. Concerns have been raised that established cell lines are not “normal” cancer cells, as they have been outside the body for a long time. One possible alternative would be to use emerging methods to culture cancer cells obtained from patient’s tumors. In addition, in vitro cell may not model how cancer cell behaves in vivo very well.

There are several experiments I would do as follow up to this study. I would first examine changes in the expression of markers other than E-cadherin to confirm that profilin-1 and profilin-2 knockdown pushes the cells towards EMT. I would also investigate changes in E-cadherin expression following profilin-1 and profilin-2 knockdown in an in vivo mouse model.

Metastatic breast cancer is a devastating disease that has no curative treatment in medicine today. The results shown here suggest that profilin-1 and profilin-2 may be important regulators of how breast cancer spreads between organs. Further research may unveil more information on these regulators, hopefully leading to more effective treatments to inhibit metastasis in the future.

ACKNOWLEDGMENTS

I would like to thank the Roy lab with technical help with experiments as well as Jingming Chen and Dr. Alejandro Almarza for help with the abstract.

REFERENCES

SUPPLEMENTARY INFORMATION

Figure S1: Profilin-1 and Profilin-2 Expression is Knocked Down by siRNA treatment