PROFILIN FOR THE TREATMENT OF CANCER CELL METASTASIS

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INTRODUCTION
This project focused on cancers originating in the mouth and throat, classified as oral squamous cell carcinomas (OSCC). As reported by the National Institute of Health, there are approximately 30,000 new cases each year in the United States, mostly in patients over 60 years old, and most of which occur in men [1].

Cancer is characterized by the uninhibited growth of epithelial cells, called neoplasia. When left untreated, this growth can form what are called tumors, or abnormal masses of cells. There are two types of tumors; benign and malignant. Benign tumors pose no real threat to the host body, as the mass is more or less homogeneous and bound by a fibrous sheath of connective tissue. Malignant tumors, however, can cause great harm to the host body. They are generally inhomogeneous, have the ability to spread to other parts of the body via either the circulatory system or the lymphatic system, and form secondary growth sites, which are more aggressive than primary growth sites. This spreading is referred to as metastasis [2].

The movement, or motility, of cells is largely spontaneous and is actin based. Actin is a protein found in the cytoskeleton of cells that binds to external substrates in order to produce force, which in turn leads to cell movement. Profilin (Pfn) is a small actin-binding protein also found in the cytoskeleton. Profilin 1 (Pfn1) has previously been shown to be ubiquitously expressed in all cell types except for skeletal muscle. It has also been shown to play a key role in embryonic development and in the regulation of actin dynamics, and to have an anti-migratory effect on breast cancer cells [3]. Expression and function in OSCC cell types, however, remains relatively unknown. Furthermore, there are a growing number of reports on the role of profilin 2 (Pfn2), another isoform of the profilin family, in cell migration [4]. While less is known about Pfn2, the two Pfn isoforms may play a role together in the regulation of cell cytoskeleton.

OBJECTIVE
The goal of this study is to understand the role of Pfn1 and Pfn2 in the regulation of certain processes, such as tumor invasion and dissemination, metastatic colonization, and tumor growth in OSCC. Preliminary data from our lab has shown that Pfn2 is expressed variably across different OSCC, whereas the expression of Pfn1 is consistent. While Pfn1 may not have differential expression, it may still affect the motile dynamics of OSCC. Pfn2, however, may affect the overall survival rate of patients.

SUCCESS CRITERIA
In this study, we will analyze the effect of varying Pfn2 expression in OSCC cell lines that endogenously express high or low levels of Pfn2 on cell migration and proliferation. Furthermore, we will determine if Pfn1 plays a major or minor role in the migration of these cells. Success of the study is based on the completion of these tasks to provide more information on using Pfn as a possible therapeutic target for the treatment of cancer.

METHODS
The study was designed to analyze two key parameters; cell growth and movement. Two OSCC cell lines were used; Cal33, which does not endogenously express Pfn2, and HN5, which does endogenously express Pfn2. Both cell lines were stored under physiologic conditions, at 37°C and 5% CO2. Both cell lines were split into three experimental groups; a control group, in which Pfn expression levels were not altered, an overexpression group, in which Pfn levels were increased, and a knockdown group, in which Pfn levels were decreased. Protein levels were changed using standard plasmid and siRNA transfection protocols.

Cell growth was tracked manually using a hemocytometer. Cells were suspended in media and pipetted into the device. They were then counted manually, using a counter. Cell movement was also tracked manually. Two-hour time-lapse image sequences were taken under a confocal microscope. The sequences were then imported to ImageJ, developed by the NIH, in which the movement of the cells was tracked manually. The data was then exported to Microsoft Excel, where Average speed and net distance travelled was calculated for each experimental group. Data analysis was conducted using a one-way ANOVA. Data was considered statistically significant if calculated p-values were less than 0.05.

Expression levels were analyzed using the Western blot technique, which consists of several steps. First, the proteins were extracted from the cells using the proper lysis buffer. The

Figure 1: Sample well plate experimental setup for Pfn2 experiments.
samples were then loaded into polyacrylamide gels for SDS-PAGE electrophoresis. After electrophoresis, the proteins were transferred from the polyacrylamide gel to a nitrocellulose membrane using the electro-blotting technique. Each membrane was washed with the proper Pfn antibodies and stained with fluorescent dye to allow the samples to be imaged. Imaging was done under the Bio-Rad ChemiDoc MP imaging device.

RESULTS

All values for cell counts were reported as averages between two independent trials. Pfn2 knockdown trials showed that a decrease in Pfn2 expression levels resulted in a statistically significant (p<0.05) increase in growth levels.

Figure 2: Growth rates of Cal33 and HN5 cell lines. ** Indicates p<0.05. Statistical comparison was only made between control and KD within groups using a T-test.

Values for cell movement were also taken between two independent trials. Pfn2 knockdown trials showed that decreased Pfn2 levels led to a statistically significant increase in average speed travelled by HN5 cells. There was no significant change between the control and knockdown groups of the Cal33 cells; this was expected as Cal33 cells do not endogenously express Pfn2.

Figure 3: Random cell migration assay from two independent trials. N = number of cells. ** indicates p<0.05. Ctrl indicates control groups. Pfn2 indicates Pfn2 knockdown groups. Statistical comparison was made using a one-way ANOVA.

Western blots confirmed that Pfn2 expression levels did indeed decrease in both Cal33 and HN5 after knockdown.

DISCUSSION

As of yet, neither of the success criteria have been fully met. We were, however, able to establish the effect of a Pfn2 knockdown on cell growth and motility. We found that if a cell line already expresses Pfn2 endogenously then a Pfn2 knockdown results in a significant increase in both cell growth and cell movement. As expected, there was no significant change when the cells did not endogenously express Pfn2. We can therefore conclude that Pfn2 acts in a suppressing manner towards cell growth and motility. It should be noted that Cal33 cells, which do not express Pfn2, have a naturally higher movement speed than do HN5 cells, which do express Pfn2; this may further support our conclusion. It should also be noted that Pfn2 overexpression data proved to be inconclusive and was thus not included here, and that Pfn1 analysis is still ongoing.

There were a number of limiting factors involved in this study, the biggest of which is human error. Since much of the data was based on manual tracking, there was an increased risk for error while collecting data. Furthermore, this study does not account for confounding variables, in that what is shown by the data may not give the full story. This likely stems from a lack of understanding of the regulation pathways of Pfn.

In the future, we will seek to complete both our Pfn2 overexpression analysis and our Pfn1 analysis. We will also seek to perform similar analyses in varying cell types, and gain an understanding of the regulation pathways involved. We will eventually seek to develop a treatment method by targeting Pfn.

ACKNOWLEDGEMENTS

I would like to acknowledge my PI, Dr. Partha Roy, my mentor, David Gau, and the rest of the team at the Cell Migration Lab. I would also like to thank the NIH for providing funding.

REFERENCES


