Manipulating Tfam expression and mitochondrial capacity to overcome drug resistance in breast cancer cells.

Cheryl Zehowski[1], Darcy Herman[1], Kendra Nordgren[2], Paulo Oliveira[3], Andrew Skildum[2], Kendall Wallace[2]

1. University of Minnesota-Duluth, Duluth, MN. 2. Department of Biomedical Sciences, University of Minnesota Medical School, Duluth, MN. 3. Coimbra University, Coimbra, Portugal

Abstract:

For a patient diagnosed with breast cancer, the determination of a treatment is based on a biopsy and the markers that are present in the tumor at the time of diagnosis. There are tumors that are difficult to determine whether or not it will respond to chemotherapy treatment at the time of diagnosis. The research that I have been working on supports the main goal of the Skildum lab that discovering predictive biomarkers in tumor samples will help to determine a personalized and more effective treatment path. To model drug resistant breast cancer *in vitro*, I work with MCF-7 (sensitive to chemotherapy treatment) and LCC9 (less sensitive to chemotherapy treatment) breast cancer cells. Tfam is the major regulator of mitochondrial DNA replication and transcription. I discovered that the Tfam gene had an increased expression in the LCC9 cells than in the MCF-7 cells. We then decided to look at the contribution of Tfam expression to drug sensitivity in LCC9 cells. Through siRNA transfection, which interferes with the translation of proteins by binding and promoting degradation of the messenger RNA at specific sequences, we successfully knocked down Tfam mRNA expression. Here we show the consequences of Tfam knockdown are decreased mitochondrial DNA copy number and decreased labeling with a mitochondria specific fluorescent dye. From this finding I have begun to test whether Tfam knockdown in LCC9 cells increases their sensitivity to the chemotherapy drug doxorubicin. If LCC9 cells do have increased drug sensitivity after Tfam knockdown, then the Tfam gene could potentially be used as a biomarker of drug resistance in breast cancer patients.

Preliminary Data:

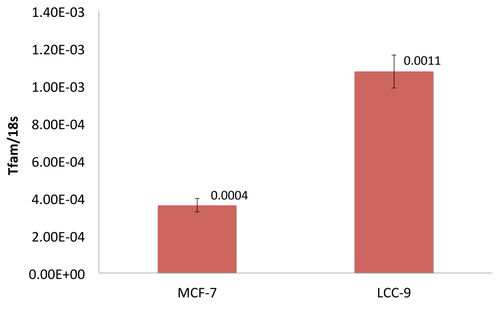

Figure 1. Tfam levels in MCF-7 and LCC9 breast cancer cells at the mRNA level. There is a 3-fold increase in Tfam levels in LCC9 cells than MCF-7 cells. Total RNA was extracted from MCF-7 and LCC9 cells cultured in normal growth media. The Tfam gene (nuclear gene that regulates transcription and translation of the mitochondrial genome) and 18s gene (ribosomal RNA reference gene) were amplified using quantitative PCR with a five point standard curve. The average ratio of copies of Tfam gene per copy of 18s gene is shown, and the error bars represents standard deviation (n=3).

Hypothesis: Tfam knockdown in drug resistant breast cancer cells results in increased sensitivity to doxorubicin.

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Plate LCC9 cells in 2-24 well plates → 1 day → siRNA (+ or – Tfam, negative control). → 2 days → Isolate total RNA and total DNA. Run quantitative PCR to determine Tfam expression and mtDNA copy number. Also measure TMRM labeling of the mitochondria.

Figure 2: Experimental setup to verify knocking down the Tfam gene in LCC9 cells

Results:

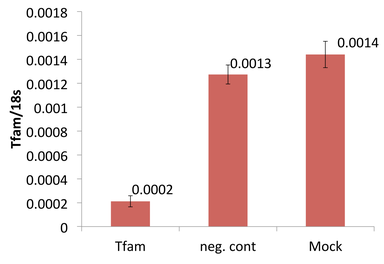


Figure 3. Tfam mRNA can be depleted in LCC9 cells by siRNA transfection. LCC9 cells transfected with Tfam siRNA and control siRNA. Tfam gene was normalized to 18s gene to get the copies of Tfam per cell. Error bars represent standard deviation.

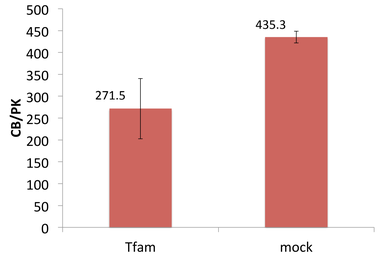
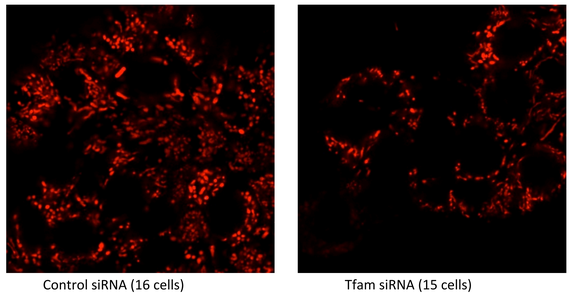


Figure 4. Mitochondrial copy number is decreased in LCC9 cells transfected with siRNA. Portions of the cytochrome B gene and the pyruvate kinase were amplified. The average ratio of copies is shown. Error bars represent standard deviation.



Control siRNA (16 cells) Tfam siRNA (15 cells)

	Control siRNA	Tfam siRNA
Average TMRM intensity:	12.48	6.45
Average TMRM intensity/nuclei:	0.81	0.36

Figure 5. TMRM labeling of the mitochondria is decreased in Tfam siRNA transfected LCC9 cells. These are confocal images of cells labeled with a cationic fluorescent dye, tetramethylrhodamine methyl ester, that labels mitochondria in proportion to their charge and abundance. Average intensity of the image was taken from 0-255 pixels.

Plate LCC9 cells in 1-24 well plates → 1 day → siRNA (+ or – Tfam, negative control). → 6 hours → Reagents washed off, addition of doxorubicin media. → 4 days → SRB assay (cell abundance measured)

Figure 6: Experimental setup to test doxorubicin sensitivity in Tfam siRNA transfected LCC9 cells.

Preliminary Results:

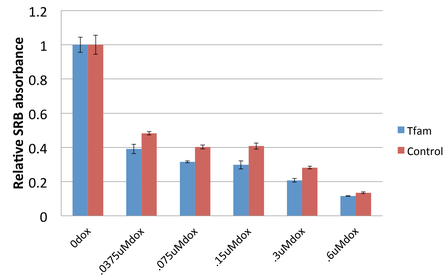


Figure 7. Cellular abundance of Tfam siRNA transfected LCC9 cells treated with doxorubicin. Sulforhodamine B assay done on transfected LCC9 cells treated with doxorubicin media. Error bars represent standard deviation.

Conclusions:

The main conclusions from experiment 1 are:

- Tfam is successfully knocked down at the mRNA level.
- Tfam knockdown decreases mtDNA in the LCC9 cells.
- Tfam knockdown decreases TMRM labeling in LCC9 cells.

The main conclusion from experiment 2 is that there may be increased sensitivity to doxorubicin after Tfam has been knocked down in the LCC9 cells.

Future Work:

My next step is to look at the effect of Tfam in LCC9 cells and treating with anti-estrogens.

I will then look at other cancer models that have doxorubicin resistant cell derivatives and discover any similarities to the breast cancer model created in the Skildum lab.

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