Mitochondrial capacity of chemotherapy resistant and sensitive lung cancer cells.

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Hypothesis: **Chemotherapy resistant lung cancer cells have increased mitochondrial capacity in comparison to their drug sensitive counterparts.**

Abstract:

Lung cancer is a significant cause of mortality in the U.S., the leading among cancer deaths, and more research is needed to improve the clinical picture for patients. Drug resistance remains a barrier to successful treatment of cancer even with the ongoing development of new molecularly targeted therapies. In the diagnosis of cancer, the treatment is determined based on the parameters of the tumor. Non-invasive cancers are often cured by surgery alone, while cancers that have progressed past the lymph node stage are commonly treated with chemotherapy. Chemotherapy drugs are administered systemically and used to kill cancer cells and prevent cancerous cells from producing. There is not a definitive way to distinguish between a chemotherapy resistant or sensitive cancer tumor at the time of diagnosis which is wasting precious time for the patient if the treatment does not work. The main goal of the Skildum lab is to identify biomarkers specific for drug resistant cancer that will allow for earlier and more effective treatment decisions.

We have established a model of cancer resistance, using MCF-7 (sensitive to chemotherapy) and LCC9 (less sensitive to chemotherapy treatment) breast cancer cells. We have shown that LCC9 cells have increased mitochondrial capacity and increased expression of the Tfam gene in comparison to the MCF-7 counterpart. Tfam is a major regulator of mitochondrial DNA replication and transcription. From here curiosity struck to discover if other forms of cancer have increased expression of the Tfam gene in the resistant form of cancer. If we are able to discover a gene that is uniquely expressed in a number of cancers, we may be able to target that gene as a biomarker in the diagnosis of cancer patients.

The goal of the current work was to test whether mitochondrial capacity was elevated in a model of doxorubicin resistant human lung cancer. The techniques used were cell culture, sulforhodamine B assay and hemocytometer to verify doxorubicin treatment in the cells, DNA isolation for mtDNA expression and RNA isolation to express Tfam through qPCR. Preliminary results show that chemotherapy resistant lung cancer cells have increased mitochondrial DNA and Tfam expression in comparison with their sensitive lung cancer counterpart.

Preliminary Data:

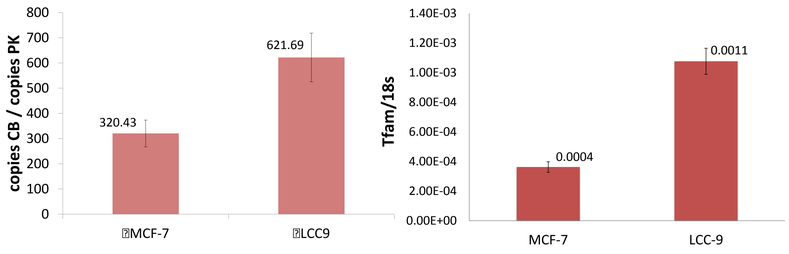

Figure 1. LCC9 cells have twice as much mitochondrial DNA as their parental MCF-7 cells, and Tfam levels in MCF-7 and LCC9 breast cancer cells at the mRNA level. Total DNA and total RNA were extracted from MCF-7 and LCC9 cells cultured in normal growth media. **A.** Portions of the cytochrome B gene (coded on the mitochondrial genome) and the pyruvate kinase gene (coded on the nuclear genome) were amplified using quantitative PCR with a five point standard curve. The average ratio of copies of cytochrome B per copy of pyruvate kinase is shown. **B.** There is a 3-fold increase in Tfam levels in LCC9 cells than MCF-7 cells. 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. The error bars represents standard deviation (n=3).

Plate H69AR cells in 1-24 well plates → (1 day) → Wash plate off, addition of doxorubicin media. → (4 days) → SRB assay (cell abundance measured)

Figure 2: Experimental setup to test doxorubicin resistance in H69 and H69AR cells.

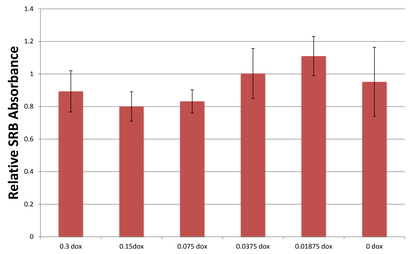

Figure 3. Cellular abundance of H69AR cells treated with doxorubicin. Sulforhodamine B assay done on H69AR cells that were treated with doxorubicin media. Error bars represent standard deviation.

Plate H69 and H69AR cells in 2-24 well plates → (1 day) → Wash plate off, addition of doxorubicin media. → (4 days) → Count total cells

Figure 4: Experimental setup to test doxorubicin resistance in H69 and H69AR cells.

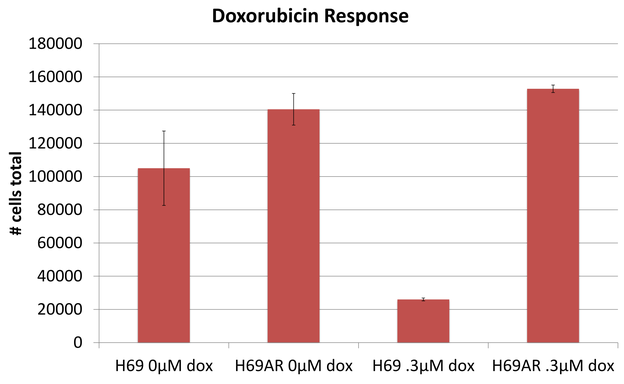

Figure 5. Doxorubicin response of H69 and H69AR cells. Cells were plated in a 24 well plate and treated with 0μM and .3μM doxorubicin and n of 3 per cell line. After 4 days cells were counted via hemocytometer to determine total number of H69 and H69AR cells. Error bars represent standard deviation.

Plate H69 and H69AR cells. → Grow up to get 4 plates/flasks of cells (2 days) → Isolate total RNA and total DNA. Run quantitative PCR to determine mtDNA copy number and Tfam expression.

Figure 6: Experimental setup to determine mitochondrial copy number and Tfam expression.

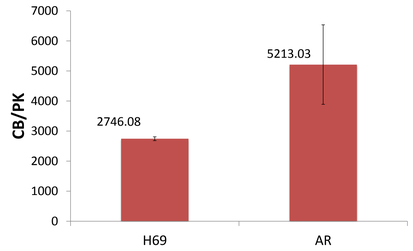

Figure 7. Twice as much mitochondrial DNA in H69AR then in H69 lung cancer cells. Total DNA was extracted from H69 and H69AR cells cultured in RPMI growth media. Portions of the cytochrome B gene and pyruvate kinase gene were amplified using quantitative PCR with a five point standard curve. The average ratio of copies of cytochrome B per copy of pyruvate kinase is shown. The error bars represent standard deviation.

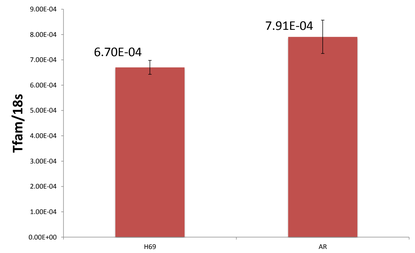

Figure 8. Tfam levels in H69 and H69AR cells at the mRNA level. Total RNA was extracted from H69 and H69AR cells cultured in RPMI growth media. 18s and Tfam were amplified using quantitative PCR with a five point standard curve. The average ratio of copies of Tfam per copy of 18s is shown. The error bars represent standard deviation.

Conclusion:

The preliminary results suggest doxorubicin resistant lung cancer cells have increased mitochondrial capacity. Further research will be done to verify the results obtained.

Future Work:

My next step is to look at four other genes in both breast and lung cancer cells, and determine if there are any significant differences in gene expression between the resistant and sensitive forms of the cancer cells. If there are any significant changes we will be able to investigate further in to whether the altering the gene affects the cancer cells.

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