# The Effects of Transposing Mitochondria From Drug Resistant to Drug Sensitive Breast Cancer Cells

By: Shahzaad Jahangier
Department of Chemistry and Biochemistry
University of Minnesota Duluth

# Breast Cancer

- Increase in recent years
- Cancer is the highest leading cause of death
- Breast cancer has the second highest mortality rate in cancer patients
- 12% of American women will contract breast cancer
- One in six will die from it
- Even higher if it runs in the family

# Goals

- To detect drug resistance in early stages of cancer development
  - Improve patient care by avoiding antiestrogen therapy and the risks associated with it
- To better understand cancer cell metabolism
  - Potential to synthesize new drugs

# Model System

- Drug sensitive breast cancer cells: MCC-MCF7
- Drug resistant breast cancer cells: MCF7-TamR. Selected for tamoxifen resistance.

# Observation: Higher Mitochondrial Mass and mtDNA in TamR

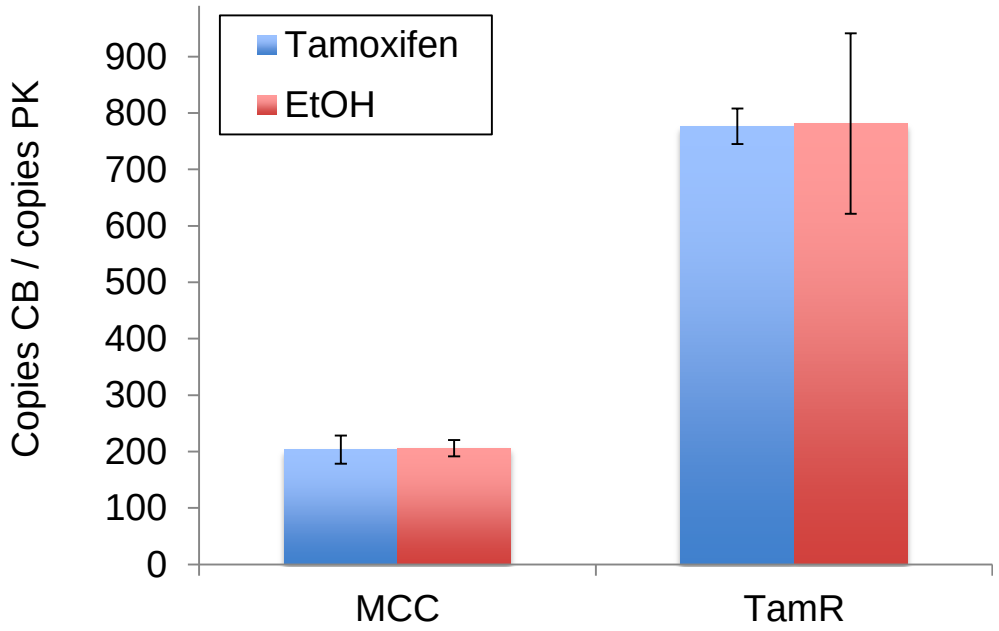


*Figure 1: mtDNA copy number for MCC and TamR cells. On the y-axis we have the ratio of the mitochondrial gene cytochrome B and the nuclear gene pyruvate kinase. Treatments were two days in 1nm tamoxifen or ethanol vehicle.*

# Setting the Stage

- Investigate the mitochondrial phenotype (and dynamics) further and correlate it to drug resistance
- Hypothesis: Transposing mitochondria from resistant to sensitive cells would infer resistance. Similarly we expect no change to the recipient cell if the donor cell was already sensitive

# Our (Theoretical) Approach

- First step: visualization
- Confocal microscopy to observe transposition
- Optimize visualization conditions
  - JC-1 and TMRM at various concentrations: 0.5 – 5 μM for JC-1 and 20-200 nM for TMRM
    - mtGFP as an alternative to JC-1
      - Baculovirus vector used to propagate a GFP tag on a mitochondrial structural protein that is synthesized in the nucleus of the cell

# Our (Theoretical) Approach

- Second step: transposition
- Isolate labeled mitochondria using differential centrifugation (Pierce mitochondrial isolation kit)
- Confirm that mitochondria are structurally intact with TMRM
- Introduce mitochondria to recipient cells (mitochondrial uptake through pinocytosis)

# Our (Theoretical) Approach

- Third and final step: assay for drug resistance
- Sulforhodamine B assay
  - Tamoxifen concentration range 1 nM to 1 μM
  - Ethanol as a vehicle
  - Original MCC and TamR cell lines as a positive and negative control respectively

# Visualization

- JC-1: less than ideal
  - Solubility problems
  - Diffuse staining
  - Low resolution

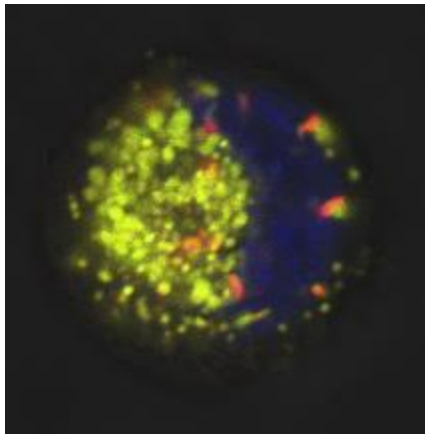

*Figure 2: JC-1 (mitochondrial marker) and DAPI (nuclear marker) staining in TamR*

# Visualization

- mtGFP: not perfect, but better
  - No solubility problems
  - No diffuse staining
  - Higher resolution
  - Major problem: dilution through cell division
    - Easier to work with than JC-1

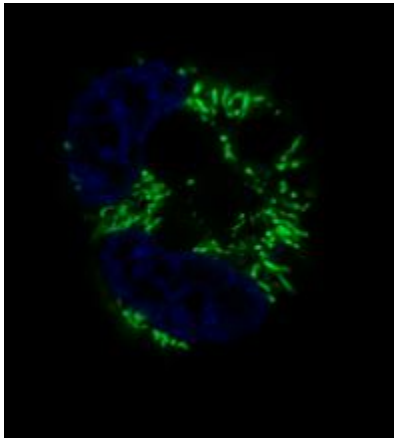

*Figure 3: mtGFP (mitochondrial marker) and DAPI (nuclear marker) staining in TamR*

# Transposition

- Able to successfully get a mitochondrial enriched pellet

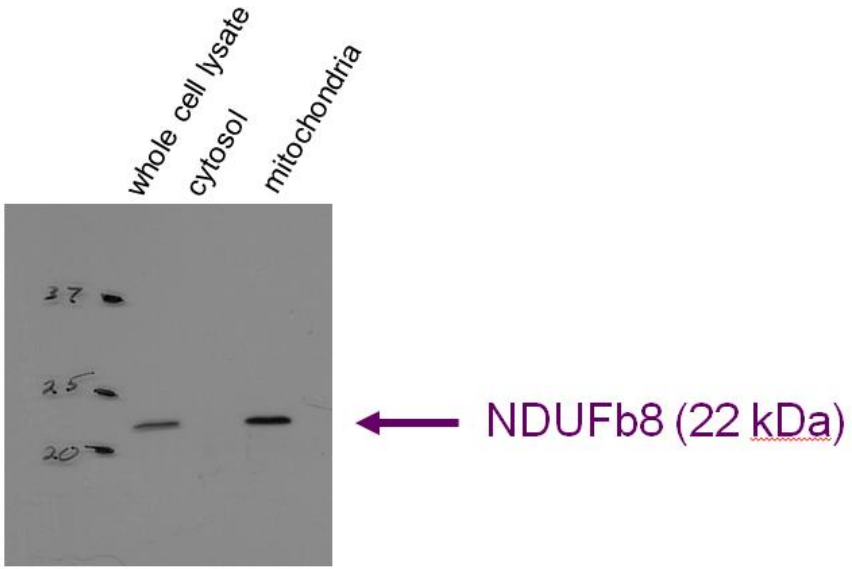


*Figure 4: Western blot that shows an enriched NDUFb8 (Complex I) band for the mitochondrial pellet.*

# Transposition

- However, we were unable to get healthy polarized mitochondria

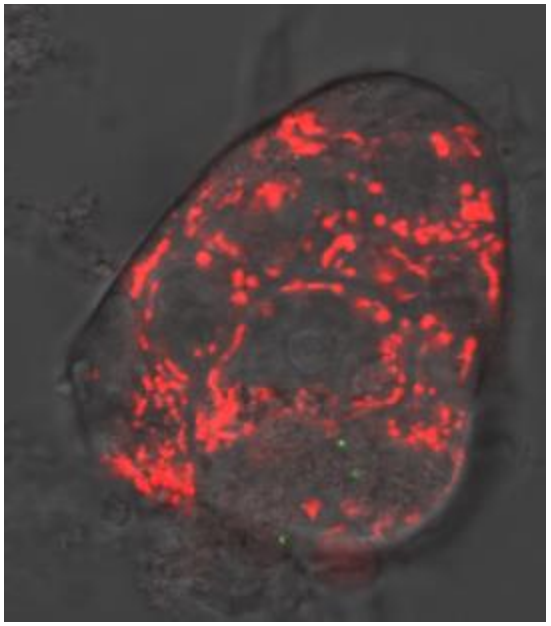

*Figure 5: Cluster of MCC cells that show no colocalization of TMRM and mtGFP*

# Possible Causes

- Dounce homogenization too rough on the cells
- Non-optimal buffers

# What's Next?

- Try different methods to isolate mitochondria
  - e.g. teflon instead of glass pestle to reduce stress on the cells
  - Use chemicals to lyse cells
- Currently working on optimizing conditions for mitochondrial isolation

# Credits


- Dr. Andrew Skildum, department of biomedical sciences, University of Minnesota Duluth
  - For working closely with me on the project and overcoming some of the bumps we had along the road
- UROP
  - Funding
  - Opportunity to make this project possible