Manipulating Mitochondrial Networks for Treating Multi-Drug Resistant Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer is one of the most aggressive forms of cancer and difficult to treat, especially as the cancer becomes drug resistant [6]. When drug resistant, cells show high levels of mitochondrial fusion, blocking the intrinsic apoptotic pathway, a mode of cell death [3]. The goal of this project is to manipulate mitochondrial networks in triple negative breast cancer cells as a therapeutic approach to treatment.

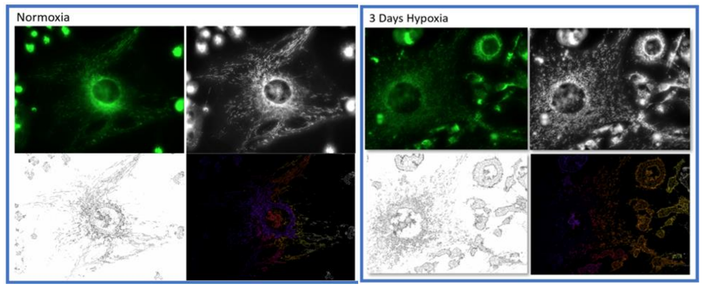

Figure 1. Side by side microscope image of cells stored in normoxic conditions and 3-days hypoxia (left to right), stained with mitotracker green, and processed using MiNa. Cells stored in hypoxia, making them drug resistant, show greater mitochondrial fusion and larger mitochondrial networks.

Background

Mitochondria are dynamic organelles with the ability to fuse and divide, forming complex intracellular networks [3]. There are high levels of fusion with other mitochondria and with the endoplasmic reticulum found in drug resistant cancer cells. This project approached multi-drug resistant (MDR) triple negative breast cancer cells with liposomal drug delivery. When liposomes enter cells, enzymes begin to degrade the vessels, releasing the drugs for absorption [1].

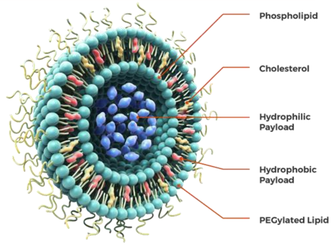

Figure 2. Structure of a liposome, which we are using for drug delivery. https://www.precisionnanosystems.com/areas-of-interest/formulations/liposomes

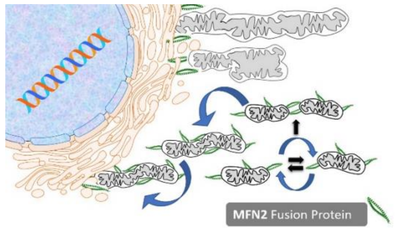

Figure 3. Breaking up of the MFN2 protein causes fission of the mitochondrial network. Copyright Dr. Milane.

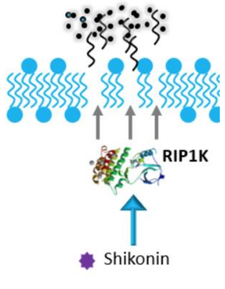

Figure 4. Shikonin activation of necroptosis through RIP1K. Copyright Dr. Milane.

The drug inserted was the mitofusin 2 (MFN2) peptide, which breaks up the mitochondrial network by targeting the MFN2 peptide, a protein that regulates mitochondrial fusion [4]. These cells were then treated with BAM7 and shikonin, which induce a pro-apoptotic factor (Bax) and a necroptosis enzyme (Ripk1) [2][5]. Breaking up the mitochondrial membrane can expose membrane receptors, allowing apoptosis or necroptosis to be induced, killing the cancerous cell.

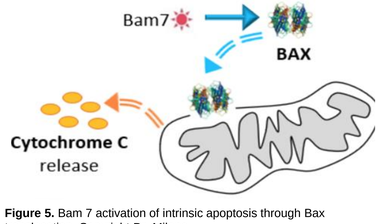

Figure 5. Bam 7 activation of intrinsic apoptosis through Bax translocation. Copyright Dr. Milane.

Experimental Methods

A. Liposome synthesis

1. Combined 587.3 uL DPPC, 558.4uL DOTAP ,185.28 uL cholesterol, and 2668.56 chloroform
2. Used rotary evaporator to form lipid film, vacuum desiccated overnight to remove chloroform
3. Rehydrated using 1 mL PBS (peptide solution)
4. 20 minute freeze thaw cycle (1 minute liquid nitrogen, 1 minute 42ºC water bath)
5. 5 minute probe sonication
6. 15 minute centrifugation at 7.2xg

B. Measuring liposome encapsulation

1. Made serial dilutions of each peptide to test reliability of the NanoDrop 2000c Spectrophotometer
2. Pipetted 1 uL of each supernatant onto the device
3. Recorded concentration of supernatant
4. Subtracted the concentration from 1 mg/mL and multiplied by 100 to find the concentration in the liposome.

C. MTS assay

1. Cells treated with each drug at 0.1, 1, 10, 100 uM concentrations.
2. Combination treatments treated at 10 uM concentration.
3. Absorbance measured using the spectrophotometer
4. Absorbance used to analyze cell viability.

D. Microscopy

1. Added mitotracker green to hypoxic and normoxic cell dishes at 250 nM concentration.
2. Incubated cells with mitotracker green for 45 minutes.
3. Mitochondrial networks photographed under the Keyence All-In-One Fluorescence Microscope
4. MiNA program used to quantify mitochondrial networks.

Figure 6. Rotary evaporator used to evaporate chloroform from the lipid film.
https://www.kisspng.com/png-distillation-rotary-evaporator-laboratory-evaporat-5834303/preview.html

Figure 7. Spectrophotometer (Synergy Microplate Reader) used to measure absorbance in MTS assay.
https://www.biotek.com/products/detection-hybrid-technology-multi-mode-microplate-readers/synergy-h1-hybrid-multi-mode-reader/

Figure 8. Keyence All-In-One Fluorescence Microscope.
https://www.keyence.com/products/microscope/fluorescence-microscope/bz-x700/models/bz-x710/index.jsp

Results

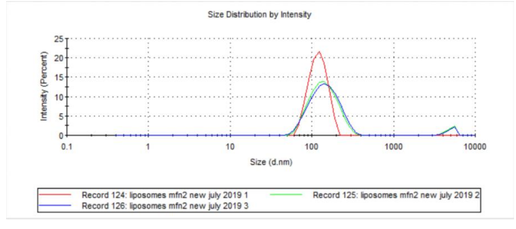

Figure 9. MFN2 loaded liposomes had an average size of 142.2 nm, an encapsulation efficiency of 72.8%, and a zeta potential of 22.2 mV.

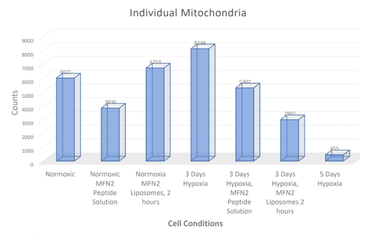

Figure 10. Used MiNa analysis to calculate the individual mitochondria under different conditions. 5 days hypoxia shows a significant decrease in individual mitochondria, demonstrating large amounts of fusion.

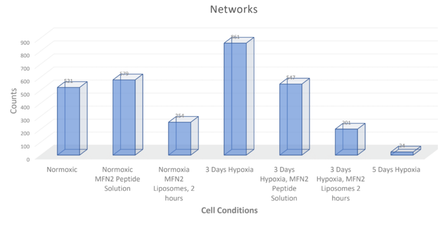

Figure 11. Used MiNa analysis to quantify the mitochondrial networks of cells under different conditions. Again, 5 days hypoxia shows a significant decrease in number of networks as the mitochondria fuses into one large network.

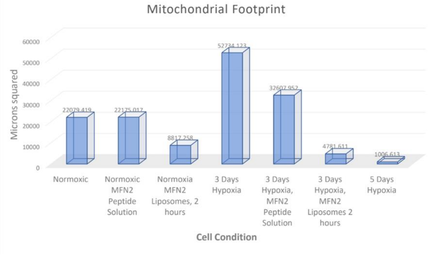

Figure 12. Used MiNa analysis to calculate mitochondrial footprint, which is the area of the cell taken up by mitochondria.

Results Continued

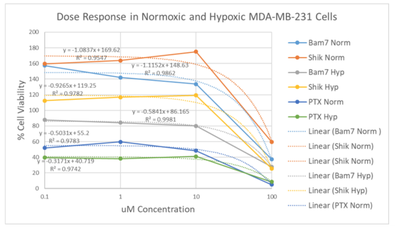

Figure 13. Graph based on single drug treatments. Dose response curve used to measure cell viability and calculate the IC_{50} value.

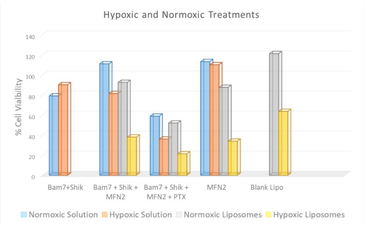

Figure 15. This graph represents the cell viability of each cell condition under different combination drug treatments. There is a significant change in hypoxic cells treated with MFN2 in liposomes. This suggests that drug resistant cells relies on mitochondrial fusion for survival.

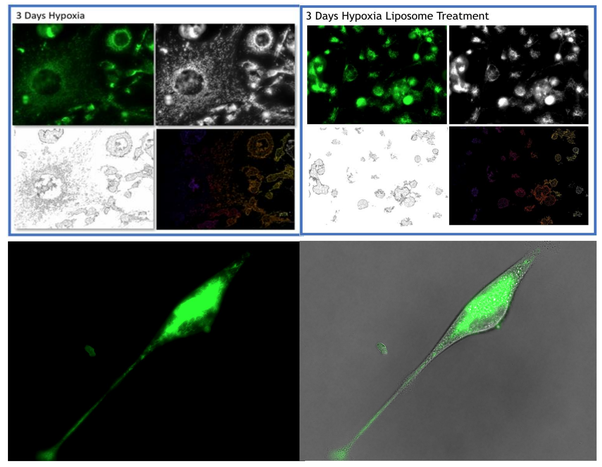

Figure 16. Side by side microscope image of cells stored in 3-days hypoxia, stained with mitotracker green, and processed using MiNa.

Figure 17. Cells stored in 5 days hypoxia and stained with mitotracker green. Significant staining of the endoplasmic reticulum demonstrated increased mitochondrial fusion in MDR cells.

Conclusion and Future Steps

- The MFN2 loaded liposomes was effective in breaking up mitochondrial networks.
- BAM7 and shikonin liposomal delivery will be tested to observe its effectiveness in inducing cell death.
- Research on mitochondrial dynamics will be continued in Alzheimer's Disease in the reverse direction of this project, where mitochondrial fusion will be promoted to preserve neurons

References

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