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 [9]. 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.

Experimental Methods

A. Liposome synthesis
1. Combined 58.73 uL DPPC, 558.4uL DOTAP, 185.28 uL cholesterol, and 668.56 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 42°C 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 tested 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.

Results

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][9]. Breaking up the mitochondrial membrane can expose membrane receptors, allowing apoptosis or necroptosis to be induced, killing the cancerous cell.

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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