Overcoming Multidrug Resistance in Triple Negative Breast Cancer via Nanoparticles

Felix Xu, YSP Student, *Belmont High School*
Rania Alshawabkeh, YSP Student, *Franklin High School*
Saket Dolare, Graduate Student, *Northeastern University*
Rutuja Dighe, Graduate Student, *Northeastern University*
Dr. Lara Milane, Pharmaceutical Sciences, *Northeastern University*
Dr. Mansoor Amiji, Pharmaceutical Sciences, *Northeastern University*

Northeastern University
Center for STEM Education

Abstract

Triple-negative breast cancer (TNBC) accounts for 10-20% of breast cancer cases and has proven to be extremely hard to treat due to its multi-drug resistant (MDR) nature and aggressive growth. TNBC cannot be treated with hormone regimens due to its lack of progesterone and estrogen receptors along with its low amount of HER2 proteins. The presence of breast cancer stem cells (BCSC), which are highly proliferative and tumor-initiating, contribute to TNBC's metastatic nature. [1, 10, 11]. To combat TNBC, a new drug therapy utilizing doxorubicin (DOX), salinomycin (Sal), and inhibition of the non-homologous end joining (NHEJ) DNA repair pathway by siRNA interference that will be encapsulated in liposomes and injected intravenously into patients has been proposed. This lab seeks to develop a new translational liposomal nanomedicine to overcome multidrug resistance in doxorubicin-treated cells.

Introduction

Nanoparticles have several benefits over conventional treatments, such as having a large surface area to volume ratio, allowing smaller doses of drug to have greater effect. Tumors also exhibit enhanced permeability and retention due to their leaky vasculature and reduced lymphatic drainage, which nanoparticles take advantage of. Liposomes will be used as the nanoparticle, as their hydrophobic membrane and hydrophilic core allow us to load both hydrophobic and hydrophilic drugs.

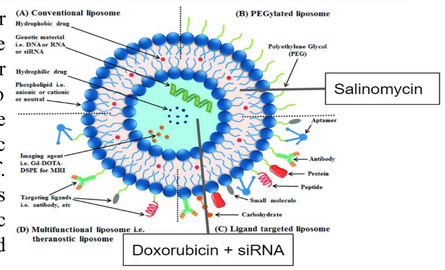

Figure 1. Diagram of liposome, and where drugs are loaded. https://www.researchgate.net/figure/Liposomes-Conventional-liposomes-are-made-of-phospholipids-A-PEGylated-stealth_fig1_322351308

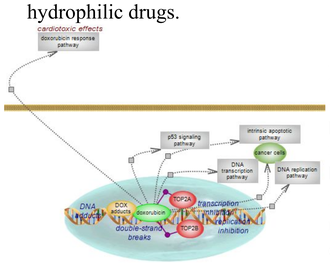

Figure 2. Doxorubicin mechanism of action. rgd.mcw.edu

Doxorubicin is an anthracycline that acts by intercalating into DNA, stabilizing the topoisomerase II-DNA complex, and producing free radicals [9]. Intercalating into DNA can result in damage to DNA, such as double-strand and single-strand breaks [6]. By stabilizing the topoisomerase II-DNA complex, DNA ligation and re-ligation is prevented, preventing replication. This damage can activate caspases like cleaved caspase 3 and induce apoptosis in breast cancer cells [8].

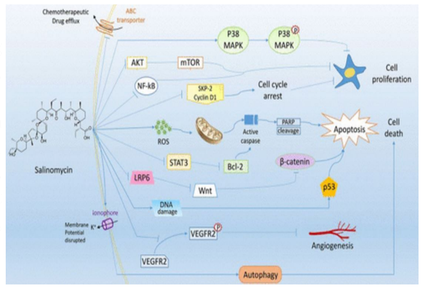

Figure 3. Salinomycin mechanism of action. www.cancertreatmentsresearch.

Salinomycin is an ionophore antibiotic affecting multiple pathways. It can target the EMT transition, inhibit the binding of ATP to ATP-binding cassette transporters like p-glycoprotein, target cancer stem cells, and inhibit the Akt signaling pathway, Wnt/β-catenin, Hedgehog, and Notch pathways [3]. This affects drug efflux in multidrug-resistant cancer cells, cancer cell growth and proliferation, and proliferative signaling [5].

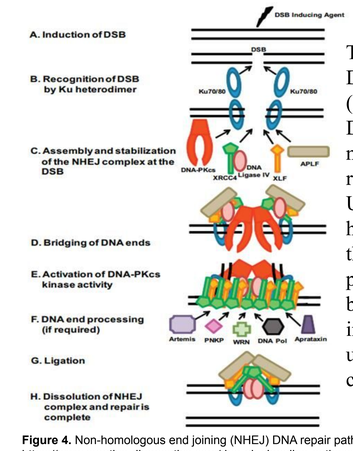

Figure 4. Non-homologous end joining (NHEJ) DNA repair pathway. https://www.creative-diagnostics.com/dna-pk-signaling-pathway.htm

The siRNA targets the mRNA that translates to the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) that binds to the Ku heterodimer, forming the DNA-PK complex. DNA-PK is crucial to the non-homologous end joining (NHEJ) DNA repair pathway, repairing double-stranded breaks in mammalian cells [2]. Unlike homologous recombination, which requires a homologous template to restore the DNA strand and therefore occurs in the G1-S phase, NHEJ can occur at any point in the cell cycle because it directly re-ligates the two broken DNA strands [7]. The NHEJ pathway is more active in breast cancer cells, and DNA-PK has been shown to be upregulated in human glioblastoma and murine breast cancer cells treated with and resistant to doxorubicin [4, 8].

Experimental Methods

Liposome Formulation

1. Dissolve phospholipids in a chloroform solution and dry with rotary evaporator
2. Prepare a PBS buffer with salinomycin, doxorubicin, and siRNA
3. Hydrate the lipid with a film with 1mL of the solution
4. Repeat heating and cooling cycle of liquid nitrogen and 40°C water bath 6 times
5. Sonicate the film to break the MLVs into liposomes
6. Centrifuge to separate the liposomes

Cell Culture

A. Cell Lines: MDA-MB-231 and BT549

Efficacy Testing

A. MTS Assay

1. Treat cells with drugs at different concentrations in 96 well plates
2. Analyze formazan absorbance with Synergy H1 Microplate Reader
3. Determine the IC50 of single agents based on cell viabilities calculated relative to absorbance of cell growth in medium only.
4. Determine combinations

Drug Tests

A. Size

1. Dynamic Light Scattering with a Zetasizer Nano

B. Drug Encapsulation

1. Centrifuge the liposomes to produce a supernatant
2. Separate out the vesicles
3. Determine concentration of drug in supernatant with spectrophotometer
4. Quantify the amount of drug in liposome

C. Drug Release

1. Place cells in a solution containing PBS and PVA
2. Collect absorption spectra through spectrophotometer

Protein Analysis

A. Western Blotting

1. Prepare samples through protein extraction with lysis solution
2. Perform BCA assay to determine total protein concentration
3. Protein samples undergo electrophoresis to determine the size
4. Transfer proteins to nitrocellulose, use dried milk on non-specific binding sites
5. Incubate with primary antibodies
6. Incubate with secondary antibodies
7. Visible bands generated can be analyzed with spectroscopy

Biomarkers

- P-glycoprotein
- Beta-catenin
- Vimentin
- DNA-PKcs
- Topoisomerase-II

Figure 5. Synergy H1 Microplate Reader. https://niom.no/materials-testing/testing-facilities/multi-mode-reader/

Figure 7. Zetasizer Nano. https://www.malvernpanalytical.com/

Figure 8. NanoDrop 2000 spectrophotometer. https://www.midlandsci.com/default.aspx?page=item+detail&itemcode=THERMO%20ND-2000

Results

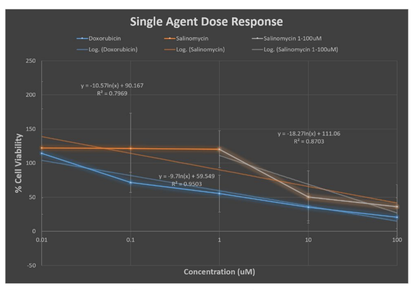

Figure 9. Theoretical efficacy evaluation data for single agent doxorubicin and salinomycin based on MTS assay in 96 well plate. IC50 for DOX = 2.66 uM, IC50 for Sal = 44.36 uM. (Error bars +/- SD)

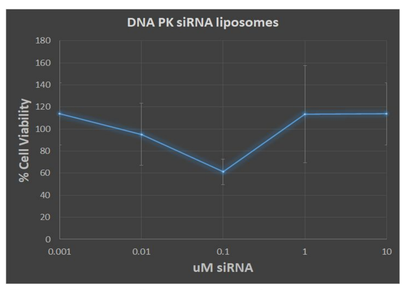

Figure 10. Theoretical efficacy evaluation data for single agent DNA-PKcs siRNA based on MTS assay in 96 well plate. We expect that single agent DNA-PKcs siRNA treatment will not induce cell death, so no IC50 was calculated. (Error bars +/- SD)

Results (cont.)

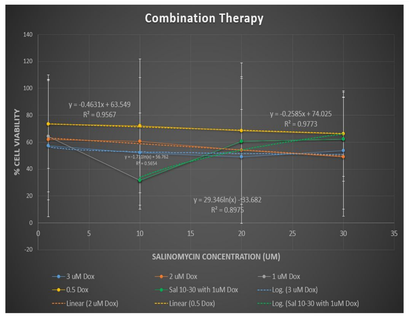

Figure 11. Theoretical efficacy evaluation data for combination treatment of DOX and Sal based on MTS assay. Our optimal concentration (lowest CI50) was determined to be 1 uM DOX with a Sal IC50 of 10 uM.

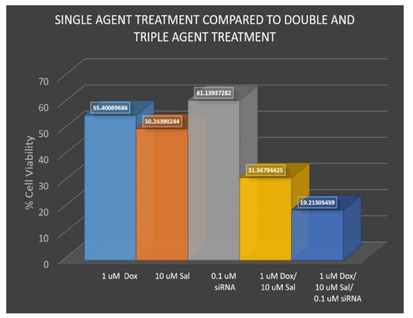

Figure 12. Theoretical efficacy evaluation data for all drug combinations at optimum amounts. The 1 uM Dox, 10 uM Sal, and .1 uM siRNA proved to be most effective, leaving < 25% of cells viable after treatment.

	Sal IC50	CI50
3 uM DOX	13.81	1.61
2 uM DOX	29.26	1.78
1 uM DOX	17.28	0.985
0.5 uM DOX	86.17	3.23

CI50 = 0.985 < 1 → synergistic effect

Conclusion and Future Steps

- The nanomedicine has shown its effect on cell pathways associated with stem cell conversion, specifically the Wnt and Hedgehog pathways. It would also affect drug efflux, like p-gp.
- The liposomal nanomedicine combination therapy consisting of 1 uM Dox, 10 uM Sal, and .1 uM siRNA has shown its potential to decrease cancer cell viability to <20% while maintaining a CI50 of 0.985 < 1, producing a synergistic effect.
- In the future, specific ligands could be explored to improve the specificity of the nanomedicine. Different proteins could also be targeted for silencing, such as proteins associated with stemness pathways like SOX2 or beta-catenin.

References

1. Al-Mahmood, S., Sapiezynski, J., Garbuzenko, O. B., & Minko, T. (2018). Metastatic and triple-negative breast cancer: Challenges and treatment options. *Drug Delivery and Translational Research*, *8*(5), 1483–1507. https://doi.org/10.1007/s13346-018-0551-3
2. Davis, A. J., & Chen, D. J. (2013). DNA double strand break repair via non-homologous end-joining. *Translational Cancer Research*, *2*(3), 130–143. https://doi.org/10.3978/j.issn.2218-676X.2013.04.02
3. Dewangan, J., Srivastava, S., & Rath, S. K. (2017). Salinomycin: A new paradigm in cancer therapy. *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *39*(3), 1010428317695035. https://doi.org/10.1177/1010428317695035
4. Friesen, C., Uhl, M., Pannicke, U., Schwarz, K., Miltner, E., & Debatin, K.-M. (2008). DNA-Ligase IV and DNA-Protein Kinase Play a Critical Role in Deficient Caspases Activation in Apoptosis-resistant Cancer Cells by Using Doxorubicin. *Molecular Biology of the Cell*, *19*(8), 3283–3289. https://doi.org/10.1091/mbc.E08-03-0306
5. Hermawan, A., Wagner, E., & Roidl, A. (2016). Consecutive salinomycin treatment reduces doxorubicin resistance of breast tumor cells by diminishing drug efflux pump expression and activity. *Oncology Reports*, *35*(3), 1732–1740. https://doi.org/10.3892/or.2015.4509
6. Ross, W. E., & Bradley, M. O. (1981). DNA double-stranded breaks in mammalian cells after exposure to intercalating agents. *Biochimica Et Biophysica Acta*, *654*(1), 129–134. https://doi.org/10.1016/0005-2787(81)90145-3
7. Sishc, B. J., & Davis, A. J. (2017). The Role of the Core Non-Homologous End Joining Factors in Carcinogenesis and Cancer. *Cancers*, *9*(7). https://doi.org/10.3390/cancers9070081
8. Stefanski, C. D., Keffler, K., McClintock, S., Milac, L., & Prosperi, J. R. (2019). APC loss affects DNA damage repair causing doxorubicin resistance in breast cancer cells. *Neoplasia*, *21*(12), 1143–1150. https://doi.org/10.1016/j.neo.2019.09.002
9. Thorn, C. F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T. E., & Altman, R. B. (2011). Doxorubicin pathways: Pharmacodynamics and adverse effects. *Pharmacogenetics and Genomics*, *21*(7), 440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56
10. *Triple-negative Breast Cancer | Details, Diagnosis, and Signs*. (n.d.). Retrieved July 12, 2020, from https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/types-of-breast-cancer/triple-negative.html
11. *Triple-negative breast cancer recurrence: Risks and survival*. (2019, January 24). https://www.medicalnewstoday.com/articles/324272

Acknowledgements

Department of Pharmaceutical Sciences
Dr. Mansoor Amiji - Distinguished Professor
Dr. Lara Milane - Assistant Professor
Saket Dolare - Graduate Student
Rutuja Dighe - Graduate Student

Center for STEM Education
Claire Duggan - Director for Programs and Operations
Natasha Zaarour - YSP Coordinator
Salima Amiji - YSP Coordinator

Young Scholars' Program at Northeastern University
Claire Duggan, Program Director

This work was supported by the National Science Foundation