Overcoming Multidrug Resistance in Triple Negative Breast Cancer via Nanoparticles

Felix Xu, YSP Student, Rana Aishawaibeh, YSP Student, Frankfurt High School
Saket Dolare, Graduate Student, Northeastern University
Rutuja Dige, Graduate Student, Northeastern University

Dr. Lara Milane, Pharmaceutical Sciences, Northeastern University
Dr. Mansoor Amiji, Pharmaceutical Sciences, Northeastern University

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.

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 double-strand breaks 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 and damage can activate caspases like cleaved caspase 3 and induce apoptosis in breast cancer cells [8].

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/beta-catenin, Hedgehog, and Notch pathways [3]. This affects drug efflux in multidrug-resistant cancer cells, cancer cell growth and proliferation, and proliferative signaling [5].

The siRNA targets the mRNA that translates to the gene of interest, which in this case is the DNA-PKcs gene. The DNA-PKcs plays a critical role in DNA repair by allowing cells to repair strands through homologous recombination and non-homologous end joining. [19, 20, 21, 22, 23, 24, 25]. This treatment against DNA-PKcs inhibition resulted in a synergistic effect [26, 27, 28, 29, 30].

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 of the solution
4. Repeat heating and cooling cycle of liquid nitrogen and 4°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

Experimental Methods

Drug Test

A. Size
1. Dynamic Light Scattering with a Zetasizer Nano

B. Drug Encapsulation
1. Centrifuge the liposomes to produce a supernatant
2. Collect the supernatant

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

Bioarkers

- Psycloprotein
- Beta-catenin
- Vimentin
- DNA-PKcs
- Topoisomerase-II

Results

Table 1: Efficacy Testing of Single Agent vs. Combination

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (uM)</th>
<th>CI50</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX</td>
<td>13.91</td>
<td>1.61</td>
</tr>
<tr>
<td>Sal</td>
<td>29.26</td>
<td>1.78</td>
</tr>
<tr>
<td>DOX-Sal</td>
<td>17.28</td>
<td>0.985</td>
</tr>
<tr>
<td>DOX-Sal-NS</td>
<td>86.17</td>
<td>3.23</td>
</tr>
</tbody>
</table>

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


Acknowledgements

Department of Pharmaceutical Sciences
Dr. Mansoor Amiji - Distinguished Professor
Saket Dolare - Graduate Student
Runuja Dige - Graduate Student

Center for STEM Education
Claire Duggan - Director for Programs and Operations
Natasia Zaarour - YSP Coordinator

This work was supported by the National Science Foundation.