Nanotechnology in Breast Cancer Therapy

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http://trialx.com/curetalk/wp-content/blogs.dir/7/files/2012/10/Nanotech-drug-delivery.gif
Objectives

• Discuss the use of nanoparticles as a drug delivery system in cancer therapy

• Describe the protocol and assays used to determine the concentration dependence of the combined effects and therapeutic efficacy of paclitaxel and ceramide \textit{in vitro} on the growth of a triple negative breast cancer cell line

• Examine the effects of drugs that are encapsulated in nanoparticles
Introduction

• According to the **CDC** breast cancer is one of the most common cancers among women in the U.S.

In 2009:

• **211,731** women in the United States were diagnosed with breast cancer
• **40,676** women in the United States died from breast cancer
Introduction

• Early diagnosis is essential - most curable stage
• Invasive vs. non-invasive
• Triple negative breast cancer - tends to grow and spread more rapidly then other cancers

Lack the following receptors on their surface:

• Estrogen
• Progesterone
• HER2 protein

• Common treatments e.g. hormone therapy and drugs that target estrogen, progesterone, and HER-2 are ineffective
Introduction

- Conventional treatment - combinations of chemotherapeutic drugs, radiation and/or surgery
- Taxol - natural substance isolated from bark of Pacific Yew tree
- Paclitaxel - synthetic
- Anti-microtubual agent
- Interferes with cell division
- G₂M blockage
Paclitaxel stops the transition from G2 phase to the M (mitosis) phase, among other effects.
Taxol and Ceramide

- Taxol and ceramide demonstrate synergy when used in combination
- Mehta et. al (2000) demonstrated paclitaxel-mediated $G_2$-M phase accumulation decreased significantly with the addition of ceramide in head and neck tumors
- Indicated combined paclitaxel/ceramide treatment resulted in the elimination of Tu138 cells from the S and/or $G_2$-M phases of the cell cycle
Ceramide

- Central molecule of sphingolipid metabolism

Mode of action:
- Cell growth inhibition
- Apoptosis induction
- Senescence modulation
- Endoplasmic reticulum stress responses

http://www.sciforum.net/ecsoc/ecsoc-5/Papers/c0013/c0013_files/fig1-1a-2.jpg
Hypothesis

• Established synergy - combination of paclitaxel and ceramide in head and neck tumors (Mehta et al. 2000)

• Would this synergy work in breast cancer cells?

• Triple negative cell lines?
Research Strategy

• Determine the optimal concentration of paclitaxel and ceramide *in vitro* using triple negative cell line - MDA-MB231

• Single drug and combinations

• Single drug/combination drugs using nanoparticles
Nanotechnology

National Nanotechnology Initiative:

• Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers
  • $1 \times 10^{-9}$ meters
  • $25,400,000$ nanometers in an inch
  • Sheet of paper is about $100,000$ nanometers thick
  • Hemoglobin is $5.5$ nanometers in diameter
Nanoparticles in Drug Delivery

- Increase aggregation of particles and drug at tumor site
- Encapsulation of therapeutic agent into particle – can prevent drug degradation
- Hydrophilic on outside; hydrophobic on outside - can improve delivery of hydrophobic drugs
- Localized therapy
- Formulations to decrease toxicity and increase efficacy
Ideal Nanoparticles:

- Biocompatible and biodegradable
- Small size, high loading capacity
- Prolonged circulation
- Tumor accumulation
- Ability to escape from endosomes
- Ability to penetrate inside cells
- Ability to release cargo
Once nanoparticles are prepared want to make sure homogeneous population achieved at 100 – 150 nm.

A: Particle size analysis by Coulter Counter. B: Scanning electron micrograph of nanoparticles (NP). Original magnification was 17,500.
pH Responsive Triggered Release

A: Fluorescence micrographs of rhodamine containing nanoparticle suspension on a glass slide. B: Nanoparticles after the addition of small amount of pH 5.1 acetate buffer. Rapid dissolution (~5 seconds) of the nanoparticles in low pH is shown by the streaks and an increase in the background rhodamine fluorescence signal.
Drug Loading and Release Studies

A: **Capacity** - Lines parallel each other – different concentrations of paclitaxel picked up by NP.  
B: **Efficiency** - Loading efficiency is ~93%. Control (blue) is paclitaxel alone. (HPLC) Pink - Pac and NP; (NP 10 mg)
Drug release from Nanoparticle Encapsulation

Paclitaxel release from the control and NP at 37°C in 1.0% (w/v) Tween-containing phosphate buffered saline (pH 7.4). (HPLC). Control (pink) - Pac + NP; Blue – Pac + NP + detergent.
## Drug Loading

<table>
<thead>
<tr>
<th>Nanoparticle Formulation</th>
<th>Drug Loading capacity</th>
<th>Loading Efficiency</th>
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<tbody>
<tr>
<td>Paclitaxel</td>
<td>9.32 mg/100mg</td>
<td>&gt; 93%</td>
</tr>
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</table>
Cellular Uptake of NP-Encapsulated Drug
“Passage to Cells”

Differential interference contrast (DIC) and fluorescence confocal images of rhodamine-encapsulated NP internalized in MCF-7 breast cancer cells as a function of time. Original modification was 40x.
**Cellular Uptake of NP-Encapsulated Drug**

“Internalized”

<table>
<thead>
<tr>
<th></th>
<th>DIC</th>
<th>Fluorescence</th>
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<tbody>
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<td>30 min.</td>
<td><img src="image1" alt="DIC" /></td>
<td><img src="image2" alt="Fluorescence" /></td>
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<tr>
<td>1 hour</td>
<td><img src="image3" alt="DIC" /></td>
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<tr>
<td>2 hours</td>
<td><img src="image5" alt="DIC" /></td>
<td><img src="image6" alt="Fluorescence" /></td>
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<tr>
<td>4 hours</td>
<td><img src="image7" alt="DIC" /></td>
<td><img src="image8" alt="Fluorescence" /></td>
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Differential interference contrast (DIC) and fluorescence confocal images of rhodamine-encapsulated NP internalized in BT-20 breast cancer cells as a function of time. Original magnification was 40x.
Confocal Microscopy – MCF-7

Red: Paclitaxel
Green: Ceramide

Cellular uptake and release of drugs
Viability of BT-20 Breast Cancer Cells

% Viability of Breast Cancer Cells treated with PT +/- CE

Synergistic Effect of Paclitaxel and Ceramide at 1nM @ 6 days of treatment
Viability of MCF-7 Breast Cancer Cells

Synergistic Effect of Paclitaxel and Ceramide at 10 μM @ 6 days of treatment
Viability of MB-435 Breast Cancer Cells

Synergistic Effect of Paclitaxel and Ceramide at 1 nM @ 6 days of treatment
ER+/- Breast Cancer Cell Lines

- BT-20 ER-, MCF-7 ER+, MB-435 ER-
- Cell line to cell line variation with drugs
- Different cell lines work better with different concentrations of drugs

- How will drug combinations work on a Triple Negative Cell Line? MB-231
Methods/Procedures

• MDA-MB231 Triple Negative Breast Cancer Cell Line
• Cells passed - weekly basis
• Media (DMEM), Paclitaxel, Ceramide
• Nanoparticles from (Dr Amiji’s Lab) Northeastern Univ., Mass.
• Varied concentrations of drugs alone and in combination (2 fold and 10 fold titrations)
• 30 µl (.5%) MTT Dye Assay – read at 570 nm
• Trypan Blue Assay (1:3 of Trypan Blue and PBS)
Bioassays

- MTT Dye Assay
- Trypan Blue – Cell Count
- Microscopic Analysis
Paclitaxel Mediated Cytotoxicity on Human Breast Cancer Cell Line - MB-231 (MTT Assay)
Paclitaxel + Ceramide Mediated Cytotoxicity on Human Breast Cancer Cell Line - MB-231 (48hr Treatment) (MTT Assay)

Paclitaxel

Paclitaxel + Ceramide at 25ug/mL

+ Ceramide (25ug/mL)

Pac + Ceramide – more cytotoxicity
Paclitaxel + Ceramide Mediated Cytotoxicity on Human Breast Cancer Cell Line - MB-231 (72hr Treatment) (MTT Assay)
Cell Viability Test: Cytotoxic Effect of Paclitaxel + Ceramide (72 hr Treatment) (Trypan Blue)

+ Ceramide (25 ug/mL)

Paclitaxel + Ceramide

Paclitaxel

Concentration (µM)

Live Cell Count

0 100 200 300 400 500 600 700

0 100 200 300 400 500 600

Concentration (µM)
Effect of NP-PT at 24hours (MTT Assay)

Optical Density vs Paclitaxel Concentration
Effect of NP-PT at 48 hours (MTT Assay)

Optical Density vs Concentration (uM)

- Blank Nanoparticles
- No Additions
- NP-PT
- Paclitaxel
Effect of NP-PT at 72hours (MTT Assay)

Optical Density vs. Concentration (μM)

- No Additions
- Blank NanoParticles
- NP-PT
- Paclitaxel

Concentration (μM): 0.1, 1, 10, 100, 1000
Optical Density: 0.1000, 0.3000, 0.5000, 0.7000, 0.9000, 1.1000, 1.3000, 1.5000
Cell Growth Morphology
Comparative Cell Morphology: PT vs. NP-PT

<table>
<thead>
<tr>
<th>24 Hours – Control</th>
<th>PT</th>
<th>NP-PT</th>
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<tbody>
<tr>
<td>![Image](51x249 to 232x378)</td>
<td>![Image](258x249 to 442x378)</td>
<td>![Image](51x84 to 232x212)</td>
</tr>
<tr>
<td>48 Hours - Control</td>
<td>PT</td>
<td>NP-PT</td>
</tr>
<tr>
<td>![Image](259x84 to 442x212)</td>
<td>![Image](480x249 to 654x374)</td>
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Comparative Cell Morphology: PT vs. NP-PT

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<tr>
<td><img src="72Hours_Control.png" alt="Image" /></td>
<td><img src="PT.png" alt="Image" /></td>
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Conclusion

- Paclitaxel concentrations alone appear to kill cells hence we see a steep decline in population at 48 hours
- PT and Ceramide in combination have a gradual decline in cell population after 48–72 hours
- NP alone have no cytotoxic effect on cells
- PT encapsulated in NP has a controlled and gradual decrease in cell population at 48–72 hours
- Microscopic analysis of cell images demonstrated that after 48–72 hours cells treated with PT is cytotoxic
- PT encapsulated in NP after 48 and 72 hours shows controlled cell death
Next Step

• Find the optimal concentrations of Paclitaxel and Ceramide alone and in combination with and without NP in triple negative cell line (600 nm and 60 nm)

• Perform *in vivo* studies in small animals
Acknowledgements

• Colleagues at Northeastern University, Mass. for making nanoparticles
• Dr. Mansoor Amiji and Amit Singh
• Dr. Shashi Mehta
• Kaiwan Mirza – PhD candidate
• Grant - Rutgers University (formerly UMDNJ)