Ultrasound-guided Delivery of microRNA Loaded Nanoparticles into Cancer

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MicroRNA delivery for Cancer Therapy

• MicroRNA (miR):
  – Small non-coding RNA molecules in cells that regulate expression of genes, including several genes involved in cancer development.
  – Irregular levels of miR are found in several cancers.
  – Restoration of miR can potentially treat cancers.
Challenges in miR delivery into cancer

• miR is rapidly degraded when injected intravenously.
• miR need to be packed in nanocarriers, e.g., PLGA.
• Nanocarriers are usually large (>100nm).
• Delivery of large drug loaded carrier system into tumors via passive accumulation (enhanced permeability and retention effect) is inefficient (<5% of administered drug).
• An active, targeted delivery approach is critical for miR delivery into cancer.
Ultrasound and Microbubble facilitated Drug Delivery

- **Goal**: To facilitate delivery of miR-loaded PLGA into cancers using image-guided ultrasound (US) and microbubble (MB) mediated sonoporation.

- **Sonoporation**: Ultrasound-induced microbubble cavitation \( \Rightarrow \) Permeability change \( \Rightarrow \) Drug delivery
Previously

• Image guided ultrasound drug delivery platform in vitro and in vivo.

• **Pressure** was found to have significant effects on cavitation activities.

• **Today**: *In vivo* nanocarrier delivery results
Ultrasound-guided Nanocarrier Delivery \textit{In Vivo}

- Effects of \textbf{pressure} on delivery outcomes

- Nanocarriers:
  - 120nm semiconducting polymer nanoparticles (SPNs)- super bright, great indicator for spatial distribution of nanocarriers

- Evaluation:
  - Drug delivery profile examined on immunohistochemical staining.
  - Histological/morphological examination
Experimental design

• Effects of **pressure** on delivery outcomes

• Nanocarriers:
  - 115-nm semiconducting polymer nanoparticles (SPNs)
  - 115-nm miR122-loaded FDA approved PLGA nanoparticles.

• Animals euthanized 4 hours post treatments
Evaluation of Treatment Outcomes

• Nanocarrier delivery amounts – *immunohistochemical staining*.

• Nanocarrier penetration depth – *3D confocal microscopy*

• Histological examination – *H&E staining*

• MiR-122 delivery results – *real-time PCR*
Immunohistochemical Staining

Control, no US

Nanocarriers (SPN)
CD31 (Vessel)
F-actin (cells)
US pressure = 5.4 MPa

Nanocarriers (SPN)
CD31 (Vessel)
F-actin (cells)
Immunohistochemical staining – close-up

Control  1.7MPa  2.5MPa
3.9MPa  5.4MPa  6.9MPa

50µm  50µm  50µm
50µm  50µm  50µm
Nanocarrier delivery amount

NP/vessel ratio = area of NPs / area of CD31
NP/Factin ratio = area of NPs / area of Factin
Nanocarrier penetration depth
Nanocarrier penetration depth

3D maximum intensity projection

20 µm

Orthogonal

20 µm
Penetration depth of nanocarriers

N=54 each

Penetration depth (µm) vs. Pressure (MPa)

0.0 1.7 2.5 3.9 5.4 6.9
H&E pathological examination

control  1.2MPa

1.8MPa

5.4MPa

50μm  1mm
H&E pathological examination

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
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<tr>
<td>0</td>
<td>No hemorrhage</td>
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<tr>
<td>1</td>
<td>0 - 10% hemorrhage</td>
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<td>2</td>
<td>11 - 25% hemorrhage</td>
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<tr>
<td>3</td>
<td>26 - 50% hemorrhage</td>
</tr>
<tr>
<td>4</td>
<td>&gt;50% hemorrhage</td>
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</tbody>
</table>

- Slight increase in peritumoral hemorrhage but no significant increase in intratumoral hemorrhage

Pressure →
miR-122 delivery

Fold change in miR122 relative to untreated control animal

Control
- No US
- US

miR-122 loaded
- No US
- US

PLGA

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Conclusion

- 115-nm nanocarriers can be successfully delivered into tumor tissue using US guided MB cavitation.
- Increase of US pressure resulted in increased tumor tissue delivery of nanocarriers along with improved penetration depth.
- Minimal intratumoral damage and slightly increased peritumoral hemorrhage with increasing US pressure.
- Tumor suppressor miR-122 was successfully delivered into tumors.
Ongoing

• Long term therapeutic effect after miR-122 delivery
  – miR122: anti-proliferation, resensitize tumors to chemotherapy
  – Therapeutic effects after delivery of miR-122 alone or miR-122 + chemotherapeutics
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Thank you!
# Experimental series

MB concentration in each treatment cycle $\sim 3.3 \times 10^7$/mL. A total of 5000 pulses were delivered to each treatment location.

<table>
<thead>
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<th>Nanocarriers</th>
<th>Total injected nanocarriers</th>
<th>Peak negative pressure (MPa)</th>
<th>Number of tumors</th>
<th>Number of animals</th>
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<td>1.7</td>
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<td>2.5</td>
<td>5</td>
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<td>3.9</td>
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<td>5.4</td>
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<td>miR122-loaded PLGA</td>
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