Combining nanoparticles with radiation rationally – are we there yet?

Sunil Krishnan, MD
Director, Center for Radiation Oncology Research
MD Anderson Cancer Center

Disclosure Information
Sunil Krishnan

I have the following financial relationships to disclose:

Grant or research support from:
- Genentech, Merck, Hitachi, Shell, MPOB, FUSF

Honoraria from:
- Carestream Molecular Imaging

I WILL include discussion of investigational or off-label use of a product in my presentation.

Gold nanoshells

- Dielectric silica core
- Thin gold coating
- Light absorbed by the free electrons on the gold is converted to heat
- Core-shell ratio determines the optical characteristics
**Electromagnetic spectrum**

Light – non-ionizing, safe, affordable, non-invasive

Penetration depth in tissues depends on the wavelength and tissue type

Near infrared region

Clinical optical window

Tissue penetration up to 2-3 cm

---

**Why gold nanoshells?**

Robust structure
- less susceptible to chemical/thermal denaturation

Biocompatibility (silica, noble metal surface)
- acceptable toxicity at high concentrations (up to 3% of body weight) of gold in the body

Very high absorption cross section
- \(3.8 \times 10^{-14} \text{ m}^2\) vs. \(1.66 \times 10^{-20} \text{ m}^2\) for ICG
L. R. Hirsch et al., PNAS, 100 (23), 13549-13554.

Ease of surface modification for bioconjugation and PEGylation
- less uptake in liver
- longer biological half-life in blood due to slower clearance from the body

---

**Accumulation in tumors**

Enhanced Permeability and Retention (EPR) effect through leaky vasculature and inefficient lymphatic drainage of tumors
(size : 60 to 400 nm size)

Wide interendothelial junctions, incomplete or absent basement membrane, a dysfunctional lymphatic system and large number of transendothelial channels.

Gold nanoshells

Gold nanoshell mediated hyperthermia

Is thermoradiotherapy underutilized?

Thermoradiotherapy is underutilized for the treatment of cancer

Invasive techniques
No real-time temperature monitoring or dosimetry
No uniform description of dose, time attributes

Laser
Diomed – 15 plus

\( \lambda_{\text{use}} \)
808 nm

\( P_{\text{max}} \)
15 W

Delivery
Fiber optic cable
collimating lens

Beam DIa
1 cm

Exp time
15 to 20 minutes

Aiming beam
632 nm HeNe laser

Class 3b or 4

Temperature measurements

**Invasive method**
- Needle thermocouple

**Non-invasive method**
- Magnetic Resonance Thermal imaging (MRTI)

Thermocouple measurements

![Graph showing temperature changes over time](image)

- Laser power
  - 0.8 W/cm²
  - Temperature range: 10 ± 1°C (n=4)
- 0.6 W/cm²
  - Temperature range: 10 ± 1.5°C
- 0.4 W/cm²
  - Temperature range: 4 to 5°C

MRTI

- MRTI & thermocouple measurements demonstrated a ΔT ~ 11°C (from a baseline of ~30°C)
- Irradiation with laser alone (no nanoshells) demonstrates a ΔT ~ 2 to 3°C
Real time MRTI

(0.6 W/cm² for 20 min at 808-nm)

Temperature profiles

Thermocouple

MRTI

Power = 0.6 W (75% duty cycle);
Power density = 350 mW/cm²

Dynamic contrast enhanced MRI

Pre-Hyperthermia

Post-Hyperthermia
Increased perfusion with a 2-fold increase in the contrast enhancement was observed immediately (3 to 5 min) after gold nanoshell mediated hyperthermia. 

**Contrast uptake**

- **Tumor Center**
- **Whole Tumor**

**Experimental groups**

- Control (n=7)
- Hyperthermia (n=7)
- Radiation (n=7)
- Hyp + Rad (n=7)

**Radiation Dose**

- Phillips RT-250 Orthovoltage X-ray Unit
- 125 Kv: 20 mA; 2 mm Al filter
- Skin cone = 1.5 cm diameter
- Total delivered dose = 10 Gy
**Normalized tumor volume**

- Control
- Rad
- Hyp
- Rad+Hyp

Tumor doubling time

- Control
- Hyperthermia
- Radiation
- Hyp+Rad

* P < 0.005

**H&E**

- Control
- Hyperthermia
- Radiation
- Thermoradiotherapy
Conclusions

- Optically activated gold nanoshells serve as a novel means to non-invasively generate hyperthermia.
- Temperature profiles can be monitored regionally and globally within tumors using MRTI.
- Combining low-dose hyperthermia with radiation therapy leads to potent radiosensitization that is characterized by the dual effect of:
  - an initial increase in vascular perfusion of the hypoxic core of the tumor resulting in tumor cell radiosensitization, and
  - a subsequent disruption of vasculature that results in a profound increase in the size of the necrotic core of the tumor.

Conclusions

<table>
<thead>
<tr>
<th>Early effects</th>
<th>Late effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypoxic effect</td>
<td>Vascular disrupting effect?</td>
</tr>
</tbody>
</table>
**Physical dose enhancement**


<table>
<thead>
<tr>
<th>Treatment (Tumor T7)</th>
<th>1060</th>
<th>1000</th>
<th>108</th>
<th>3N</th>
<th>Tumor Initiation Cell Frequency (TIC) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mek</td>
<td>0/6</td>
<td>0/6</td>
<td>1/6</td>
<td>0/6</td>
<td>1.12 (1.1-1.14)</td>
</tr>
<tr>
<td>5-OY</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
<td>1/6</td>
<td>1.1 (0.1-1.16)</td>
</tr>
<tr>
<td>5-OH -4°C</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>1.02 (0.02-4.66)</td>
</tr>
</tbody>
</table>


**Physical dose enhancement**

Physical dose enhancement


Physical dose enhancement

Cho, Krishnan Med Phys 2010
Physical dose enhancement

Enhancing physical dose enhancement

Passive targeting
Active targeting

Peptide-nanoparticle bioconjugates

In vivo quantification

Conjugated gold nanorod

Gold nanorod

Krishnan lab, unpublished data
Tumor regrowth delay

Control
PEG-GNR
C225-GNR
Cetuximab
Rad
Cetuximab + Rad
PEG-GNR + Rad
C225-GNR + Rad

Biodistribution

Clonogenic survival

DEF 10%
DEF 15%
DNA damage

Average Number of Foci per cell
Time after irradiation (hrs)
No Radiation
Radiation (4 Gy)
GNR + Rad (4 Gy)
C225-GNR + Rad (4 Gy)

Apoptotic markers
Caspase-mediated apoptotic markers
Mitochondrial-mediated apoptotic markers

Caspase 3, 8, 9
Procaspase 3, 8, 9
Actin
Bax, Bcl-2, PUMA
Caspase 3, 8, 9
Procaspase 3, 8, 9
Actin

NTP ratio
Rad 1 hr
Rad 24 hr

G
pGNR + Rad
cGNR + Rad
Rad (4 Gy)
C     0     1    4   24
C     0     1    4   24
C     0     1    4   24

Rad (4 Gy)
C     0     1    4   24
C     0     1    4   24
C     0     1    4   24

Procaspase 3
Procaspase 8
Procaspase 9
Actin
Intracellular distribution

Time

Tissue distribution

Tissue distribution
Modeling dose

• Targeted payload delivery feasible with smaller nanoparticles bioconjugated to peptides/antibodies

• While the tumor accumulation does not increase dramatically, the distribution is altered at the cellular (internalized) and tissue (more perivascular) levels

• Both the intracellular localization and the perivascular sequestration result in greater radiosensitization at a biological level, mediated primarily by:
  • Increased DNA damage and downstream signaling
  • Increased oxidative stress
  • Increased vascular disruption

Summary

Another approach
Radiosensitization

Deep penetration of tumors

Summary

- Delivery of nanoparticles using thermosensitive liposomes enhances deep penetration of nanoparticles when triggered by hyperthermia.

- Deep penetration of gold nanoparticles improves radiosensitization independent of the effect of hyperthermic radiosensitization.

- In principle, this could be a class solution for a variety of tumors accessible by ultrasound.
RES capture

Diagaradjane et al. ACS Nano, 2010

Thermal dosimetry


Quantifying gold nanoparticles in tumor

Photoacoustic imaging

• Larger particles for vascular-targeted applications (thermoablation, hyperthermia, vascular imaging)
• Smaller particles for parenchymal applications (imaging, targeted payload delivery)
• Combinations of above
• Unresolved issues related to clinical translation

Summary

• Overcome radioresistance via
  • increased perfusion, reduced hypoxia
  • stem cell sensitization
  • vascular disruption
  • physical radiation dose enhancement
    • oxidative stress
    • DNA damage
  • triggering these effects deep within tumor core
Vascular targeted nanoshell

Days post surgery

NIH - KL2, R21, R01 x 2, U01
DOD PCRP, ANH pre-center grant, Shell
UT Ctrl Biomed Engg, Hitachi, FUSF, MDACC

Thank you
X 5K  X 25K  X 50K  X 100K

cGNR

Chlorpromazine  
+ cGNR 

Bafilomycin 
+ cGNR 

Lactacystin  
+ cGNR 

Chloroquine 
+ cGNR
Delivery of Proton Radiation to Prostate Tumors in Mice (xenograft subcutaneous)

Cells implantation → Select tumors with 10^6 PC3 cell, longer axis 9-11 mm → gAuNP injection (24h prior irradiation) → Irradiation

Request mice to be transported and housed over the PTC.

Proton Irradiation

Request mice to be transported back to BSRB for follow up.

Experimental Arms

No radiation

No NPs

gAuNR

pAuNR

Proton radiation

No NPs

gAuNR

pAuNR

No NPs

gAuNR

pAuNR

Beam Entrance