Converging physical and biological strategies for radiation sensitization of tumors using nanoparticles

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Disclosure Information
Sunil Krishnan

I have the following financial relationships to disclose:

Grant or research support from:
Genentech, Merck, Hitachi

Honoraria from:
Carestream Molecular Imaging

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

Nanoparticles

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 5 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 x 10^{-14} m^2 vs. 1.66 x 10^{-20} 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

Survival of Nanoshell-Treated Mice vs. Controls
Nanoshell Treated (n=11)
Sham Treatment (n=6)
Untreated Control (n=17)


**Gold nanoshell mediated hyperthermia**

- **Laser:** Diomed – 15 plus
- **\( \lambda_{max} \):** 808 nm
- **\( P_{max} \):** 15 W
- **Delivery:** Fiber optic cable, collimating lens
- **Beam Dia:** 1 cm
- **Exp time:** 15 to 20 minutes
- **Aiming beam:** 632 HeNe laser
- **Class:** 3b or 4

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**Is thermoradiotherapy underutilized?**

- **Thermoradiotherapy is underutilized for the treatment of cancer**

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**Temperature measurements**

- **Invasive method:** Needle thermocouple
- **Non-invasive method:** Magnetic Resonance Thermal imaging (MRTI)
Thermocouple measurements

- Laser power:
  - 0.8 W/cm²
  - 0.6 W/cm²
  - 0.4 W/cm²

- ΔT (in tumor center):
  - 0.8 W/cm²: ~13 to 15 °C (n=2)
  - 0.6 W/cm²: ~10 ± 1.5 °C (n=4)
  - 0.4 W/cm²: ~4 to 5 °C (n=2)

MRTI

- ΔT (~11 °C) from a baseline of ~30 °C
- Irradiation with laser alone (no nanoshells) demonstrated a ΔT ~2 to 3 °C

Real time MRTI

- Temperature profiles
  - ΔT (°C)
  - Time (sec)
  - Tumor-bottom
  - Tumor-core

- Laser parameters:
  - λ = 808 nm
  - Power = 0.6 W (75% duty cycle)
  - Power density = 350 mW/cm²

- Beam Dia = 1 cm

Temperature profiles

- Therocouple
  - Time (sec)

- MRTI
  - Exp time = 20 min
  - Beam Dia = 1 cm
Dynamic contrast enhanced MRI
Pre-Hyperthermia Post-Hyperthermia

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

**Contrast uptake**

**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
- Radiation
- Hyperthermia
- Thermoradiotherapy

**Tumor doubling time**

- Control
- Hyperthermia
- Radiation
- Thermoradiotherapy

*P < 0.005*

**H&E**

- Periphery
- Core

**Hypoxia, cell proliferation, perfusion**

- Hypothermia
- Radiation
- Hypothermia + Radiation
Scanning Electron Microscopy

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:
  1. an initial increase in vascular perfusion of the hypoxic core of the tumor resulting in tumor cell radiosensitization, and
  2. 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>
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<tbody>
<tr>
<td>Anti-hypoxic effect</td>
<td>Vascular disrupting effect?</td>
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</tbody>
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Enhancing physical dose enhancement

- Anti-hypoxic effect
- Vascular disrupting effect?
- Nanoparticles
- Passively targeting
- Emission spectrum of blue 12nm nanocrystal excited at 360 nm
- Active targeting
- Nanoparticles + peptides

Enhancing physical dose enhancement on the order of 10 μm
Nanoparticles

Emission spectra of nanocrystals of varying sizes all being excited at the same wavelength (360nm)

1. Flexible excitation but very discrete emission
   Comparison to other fluorescent dyes:
   - Black – Alexa488
   - Blue – Cy3
   - Red – Alexa568
   - Green – Nanocrystal
   Quantitative, can be multiplexed

2. Lower limits of detection (dye curves were blown up 10-1000 times to be seen on this graph)
   Nano- or pico-molar concentrations enough

3. Less photobleaching
   Photostable, permits repetitive imaging

Peptide-nanoparticle bioconjugates


In vivo imaging and spectral unmixing
In vivo testing

After Cetuximab:

EGF-QD-800: 10 pmol
HCT 116 colorectal xenografts

In vivo quantification

(m) uptake
EGF-QD-800 QD-800
accumulation

Ex vivo characterization

Ex vivo characterization

EGFR CD31
A unique particle

Conjugated gold nanorod

Gold nanorod

Tumor regrowth delay

Krishnan lab, unpublished data
Biodistribution

Clonogenic survival

DNA damage

DNA damage
**Total oxidative stress**

**Protein carbonyl assay**

- Control
- Immediate
- 1 hr
- 4 hrs

**Time after 4 Gy radiation**

- Intensity of normalized protein carbonyl content
- Rad
- GNR
- CGNR

**Normalized protein carbonyl content**

**Tissue effects**

- Post irradiation time
  - 4 hrs
  - 4 days

- Radiation
- PEG-GNR
- C225-GNR

**Radiation (10 Gy)**

- GNR + Rad (10 Gy)
- C225-GNR + Rad (10 Gy)

**Average microvessel density per field of view with 10X objective**

- Post irradiation period

**Intracellular distribution**

**Time**

- 4 hrs
- 4 days

**Intracellular distribution**

- Distribution of markers

**Intracellular markers**

- Reduced
- Increased

**Cellular effects**

- Immediate
- 1 hr
- 4 hrs

**Control**

- Immediate
- 1 hr
- 4 hrs
Modeling dose

Tissue distribution

Summary

• 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
Other nanoparticles

Krishnan et al. Intl. J Hyperthermia. 2010

Translational issues

- Biocompatibility – toxicity, stability
- Biodistribution/kinetics
  - renal filtration
  - RES capture
  - internalization
  - modeling
- Quantify, Visualize, Predictive Dosimetry
- Clinical applicability
  - Light: depth of penetration - ?intraop
  - Magnetic: focusing, AMF field strength
Renal filtration


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RES capture

Diagaradjane et al. ACS Nano, 2010

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Quantifying gold nanoparticles in tumor


Imaging gold nanoparticles in tumors

Imaging gold nanoparticles in tumors

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**Thermal dosimetry**

Before Surgery
Immediately after surgery
3 weeks after surgery


Larger particles for vascular-targeted applications (thermolysis, hyperthermia, vascular imaging)

• Smaller particles for parenchymal applications (imaging, targeted payload delivery)

• Radiation dose enhancement

• Combinations of above

• Unresolved issues related to clinical translation

Summary

Acknowledgements

Krishnan lab
Parmesh Diagaradjane
Amit Deorukhkar
Dev Chatterjee
Nga Ta
Krystina Sang
Jacobo Orenstein Cardona
Norman Colon
Hee Chul Park
Brook Walter

Imaging Physics
John Hazle
Jason Stafford
Anil Shetty
Andrew Elliott

Exp Diag Imaging
Juri Gelovani

UT Austin
James Tunnell
Rayan Zaman
Priya Puranakrishnan
Jaesook Park

Georgia Tech
Sang Cho
Seong-Kyun Cheong
Bernard Jones

Baylor
Jeffrey Rosen
Rachel Atkinson

Nanospectra
Don Payne
Joe Schwartz
Glenn Goodrich
James Wang

Funding
NIH - KL2, R21, R01, U01
DOD – pre-center grant
UT Ctr Biomed Engg. Hitachi, MDACC