Nanoparticle Engineering for Cancer Imaging and Therapy

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OUTLINE

Introduction:
Nanotechnology and Cancer Imaging/Therapy

Advances in Nanoparticle Engineering:
Novel Engineering/Design of Nanoparticles:
  • Self-destructive nanoparticle

  Novel targeting strategies:
  • Rapid internalization of targeting ligand

Conclusion:
Physical SIZE of Nano

Courtesy of the LBL, DOE
Relative sizes of Bio-entities in nanometer scale

<table>
<thead>
<tr>
<th>Biological Relevant Entities</th>
<th>Size (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen atom</td>
<td>0.1</td>
</tr>
<tr>
<td>Amino acids</td>
<td>0.8</td>
</tr>
<tr>
<td>DNA a-helix (width)</td>
<td>2</td>
</tr>
<tr>
<td>Globular protein</td>
<td>4</td>
</tr>
<tr>
<td>Cell membrane</td>
<td>10</td>
</tr>
<tr>
<td>Liposomes</td>
<td>50-200</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>200-500</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>9000</td>
</tr>
<tr>
<td>Kupffer cells (liver)</td>
<td>15,000-20,000</td>
</tr>
<tr>
<td>Lymphatic microvells</td>
<td>100,000</td>
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- Small size: sub-cellular level
- Function Assembly: multiple function modification
- High surface-volume ratio: high payload
- Stable assembly: carrier/shelter for drug delivery
Clinical Applications Using Nanoparticles

- **Multiple functions**
  - Tissue targeting
    - Tumor-specific binding
  - Sensing or imaging capability
    - Improved sensitivity
    - Multi-modal imaging
  - Non-invasive treatment
    - Therapeutic localized delivery
    - Localized cell kill
    - Lower dose administered
    - Improved side effect profile
NCI Nanotechnology Alliance - Strategies

Major Programs of the Alliance:

1. Centers of Cancer Nanotechnology Excellence
2. Multidisciplinary Research Teams
   - Training
   - Interagency Collaborations
3. Nanotechnology Platforms for Cancer Research
4. Nanotechnology Characterization Laboratory
General construct of nanoparticle for molecular imaging and therapy

Strategies to Tumor Targeting Using NPs

**Passive Targeting**
- Concentrate Drug in tumor Interstitium by EPR (enhanced permeation retention) effect- macromolecules
- Pore cut off size < 400-600nm
- Inability to achieve high level of drug concentration
- Systemic adverse effects

**Active Targeting**
- Administration of Drug delivery vehicle that can recognize unique biophysical characteristics of cancer cell.
- Unique markers – Antigens, receptors overexpressed.
- More efficient, overcomes drug efflux as it gets uptaken by receptor mediated endocytosis.
In vivo Fate of Nanoparticles

In blood (proteins, cells, salts, etc.)

“biofouling”

Reticuloendothelial System (RES)

within several minutes

Clearance by natural protection system (e.g., phagocytosis by macrophages)

<table>
<thead>
<tr>
<th>Particle Size (μm)</th>
<th>Fate</th>
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<tbody>
<tr>
<td>&lt;0.06</td>
<td>liver/several parts of body</td>
</tr>
<tr>
<td>0.06-0.4</td>
<td>liver/spleen</td>
</tr>
<tr>
<td>0.4-8.0</td>
<td>liver/spleen/lung</td>
</tr>
<tr>
<td>&gt;8.0</td>
<td>lung</td>
</tr>
</tbody>
</table>

Surface engineering & Particle size control are key!!
Challenges of Nanoparticles for Cancer Imaging and Therapy

Targeting/Delivery \rightarrow \text{Internalizing targeting}

Toxicity \rightarrow \text{Degradable/biocompatible}
Self-destructive Nanoparticle for in vivo application

Park JH, et al., NATURE MATERIALS. PUBLISHED ONLINE: 22 FEBRUARY 2009
Characterization of LPSiNPs
(Luminescent Porous Silicon Nanoparticle)

structure and \textit{in vivo} degradation process

SEM image

Photoluminescence emission and absorbance spectra

PBS solution at 37 C

Release profile

Cytotoxicity
Biocompatibility and biodegradability of LPSiNPs.

*In vitro* cytotoxicity of LPSiNP towards HeLa cells (48h)  *In vivo* biodistribution and biodegradation of LPSiNPs

Change in body mass of mice injected with LPSiNPs

Histology
In vitro, in vivo and ex vivo fluorescence imaging with LPSiNPs

D-LPSiNP: dextran coated-LPSiNP
Fluorescence images of tumours containing D-LPSiNPs

A strong signal from D-LPSiNPs is observed in the tumor, indicating significant passive accumulation in the tumour by the enhanced permeability and retention (EPR) effect.

(Red and blue indicate D-LPSiNPs and cell nuclei)
Nanoparticle-conjugated tumor-specific ligands/antibodies bind to surface receptors, triggering nanoparticle internalization through an endosome-dependent mechanism.

Detecting cancer early with targeted nano-probes for vascular signatures

NIH Nanotechnology Alliance Platform Grant (Ongoing)

Internalizing Peptide

!!! Confidential: Some of the data are not published yet!
iRGD1 phage extensively internalize into tumor cells in vitro.

A: T7 phage displaying iRGD1 peptides, RGD-4C, or CG7C (control phage) were incubated with various tumor cell lines at 37°C for 30 min.

B: T7 phage displaying iRGD1 peptides (main panel) or CG7C control peptides (right upper window) were incubated with PPC1 cells for 2 hours at 37°C.
A function-blocking antibody against the αV integrin subunit efficiently inhibits both the binding and internalization of the iRGD1 phage.
iRGD1 peptide (FAM-iRGD) internalizes into tumor cells in vitro

Protocol:
- Incubation for 2 hours at 37°C
- Staining with a plasma membrane marker (red) and nuclear stain DAPI (blue)
- Imaging under a confocal microscope

DATA deleted!!
iRGD1 nanoworms labeled with Fluorescein home and spread into pancreatic tumors (confocal microscopy)

PDAC Rip-Tag2

The green signals are detected not only in the blood vessel walls (A and B; asterisks), but also inside the tumor cells (A and B; arrow heads).

The red (A) and blue (A and B) colors represent CD31 and DAPI staining respectively.
iRGD1 micelles home to PDAC tumors.

white light image fluorescent light images

Protocol:
iRGD1 micelles were labeled with fluorescein, 3 hrs post-injection of PDAC mice. Perfusion with 1% BSA/PBS Organs of interest were imaged.

DATA DELETED!!!
Novel internalizing human antibody for cancer imaging and therapy

NIH Nanotechnology R01 Grant (Ongoing)

Note: Some of the data that are not published yet are deleted!
Strategies for internalizing antibody discovery

**In vitro screening**

![Diagram showing the in vitro screening process]

- Naïve phage antibody library
  - Selection on tumor cell lines
    - Sub-library enriched for scFvs binding to internalizing tumor cell surface antigens
  - Selection on tissue by LCM
  - Screen on tumor cell lines to identify candidates

**In vivo evaluation**

- **Radiochemistry:** Site-specific radiolabeling with $^{99mTc}$.
- **Animal model:** Xenograft mouse model with clinical tissue; Xenograft mouse model with tumor cell line
- **Small animal Imaging:** microSPECT/CT.
- **Biodistribution/Ex vivo staining:**

**Imaging**

![Imaging setup with two individuals working]

Tumor cells/Tissues → animals → Imaging
Biodistribution of 99mTc-UA20 in athymic mice with DU-145 subcutaneous tumor xenograft at post-injection of 3 hours.

Tumor uptake in study group was at 4.4%ID/g only lower than that of kidneys (81%ID/g) while liver at 2.7%ID/g. The remaining organ/tissue uptakes were all lower than 1%ID/g.

In control, tumor uptake was only 0.26%ID/g similar to muscle and fat.

Tumor/blood and tumor/muscle ratios were 12:1 and 70:1 as early as 3h post-injection.
Tumor targeting of 99mTc-UA20 at 1h and 3hs post-injection in nude mice with subcutaneous prostate cancer xenograft at lower back.

The remarkable tumor uptake and contrast (tumor/muscle at 70 at 3 hrs) are credited to the rapid internalization and clearance from normal organs.
microSPECT/CT image of nude mice with subcutaneous prostate cancer xenograft at lower back receiving 99mTc-UA20 3hs earlier

Coronal View

Axial view

DATA deleted!!
Internalization of scFv UA20-based liposomes into prostate cancer cells (PC3 and DU145) (microscopy and FACS analysis)

(A) Microscopic examination

(B) DU145

(C) Quantification of FACS
Summary for internalizing antibody

Internalizing antibodies showed accumulation and rapid internalization into tumor cell.

Internalizing antibodies showed rapid homing to tumor and clearance in normal organs, providing remarkable contrast in vivo and great potential for PET cancer imaging with short half-life isotope like $^{18}$F etc.

Nanoparticles decorated with internalizing antibody retain the internalizing functions of scFvs, offering intracellular delivery of imaging reporters as well as chemodrugs and other therapeutic entities such as siRNA and genes for improved imaging and therapy.
# Conclusion

## Challenges

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<th>Technology</th>
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<tr>
<td>Toxicity/Biocompatibility</td>
<td>Novel Chemical Engineering of nanoparticle</td>
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<tr>
<td>Efficacy/Targeting</td>
<td>Internalizing targeting ligands to decorate nanoparticles</td>
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