Tumor-Homing Chitosan-Based Nanoparticles for Cancer Theragnosis

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Biomedical Research Center
Korea Institute of Sci. & Tech.
2nd Annual KIST/PU Symposium
Molecular Imaging and Theragnosis
Angiogenesis in Cancer

Pathological angiogenesis is a hallmark of cancer.

Without blood vessels, tumors cannot grow beyond a critical size (~5 mm in diameter) due to the lack of oxygen and nutrients for their survival.

Antiangiogenic Cancer Therapy

1971 – Folkman J.
Tumor angiogenesis: therapeutic implications.
New England Journal of Medicine
1971; 285: 1182-86.

2004 – Hurwitz H et al
Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer.
New England Journal of Medicine
2004; 350:2335-42
Fig. 2. Chemical structure of SMANCS. SMA, poly(styrene-co-maleic acid-half-\(n\)-butylate); NCS, neocarzinostatin. There is a chromophore in the center, an edenyn compound, which generates superoxide radical extensively in cells by NADPH/cytochrome reductase and molecular oxygen in the presence of NADPH [48].
Macromolecules passively accumulate in solid tumor more than the low molecular weight anticancer agents do.

Unique biological properties of tumor
- High level angiogenesis / hypervascuclature
- Defective vascular architecture
- Deficient lymphatic drainage from tumor tissue
HGC nanoparticles labeled with Cy5.5

glycol chitosan shell

Cy 5.5

hydrophobic core

Fluorescent spectrum of HGC-Cy5.5 in PBS (10μg/mL) (ex=675nm, and em=694nm)
Structural Characteristics

PBS (pH 7.4)

Chloroform

Sonication

Decrease in polymer concentration

After 5 days of the SCC injection, HGC-Cy5.5 (5mg/Kg) was injected into tail vein.
Biodistribution of HGC-Cy5.5 nanoparticles
Angiogenesis in Cancer

Table 3. Pore cutoff size vs. effective permeability to BSA

<table>
<thead>
<tr>
<th>Tumor cell line (n)</th>
<th>Pore cutoff size, nm</th>
<th>Permeability (×10^7 cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCa-1 (5)*</td>
<td>380–550</td>
<td>2.06 ± 1.44 (1.60–3.99)</td>
</tr>
<tr>
<td>LS174T (6)*†</td>
<td>400–600</td>
<td>1.24 ± 0.45 (0.56–1.67)</td>
</tr>
<tr>
<td>ST-8 (5)*</td>
<td>550–780</td>
<td>3.73 ± 3.34 (1.67–9.28)</td>
</tr>
<tr>
<td>MCa IV (8)*</td>
<td>1,200–2,000</td>
<td>2.5 ± 1.5 (1.2–5.1)</td>
</tr>
<tr>
<td>MCa IV (6)§</td>
<td>380–550</td>
<td>1.9 ± 0.5 (1.3–2.5)</td>
</tr>
<tr>
<td>U87 (6)‡</td>
<td>7–100</td>
<td>3.8 ± 1.2 (2.4–5.0)</td>
</tr>
</tbody>
</table>

n, number of animals.

*Grown in dorsal chamber.
†Yuan et al. (16).
‡Grown in cranial window.
§Yuan et al. (12).
Bodydistribution Test

- SCC-7cell (3x10^6 cell)
- tail vein injection of HGC-Cy5.5 (5mg/kg)
Preparation of Nanoparticles for tumor targeting

HGC nanoparticles (hydrophobically modified glycol chitosan nanoparticles)

Glycol chitosan (Mw = 20, 100, 250kDa)

In aqueous condition

glycol chitosan shell

cholanic acid cores

Characterization of HGC nanoparticles

<table>
<thead>
<tr>
<th>Samples</th>
<th>(M_n^b)</th>
<th>DS(^c)</th>
<th>(\frac{\mu_0}{r^2})</th>
<th>Size(^d)(nm)</th>
<th>(\xi)(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGC20kDa</td>
<td>21,180</td>
<td>4.7</td>
<td>0.003</td>
<td>231</td>
<td>10.1</td>
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<tr>
<td>HGC100kDa</td>
<td>109,070</td>
<td>4.7</td>
<td>0.011</td>
<td>271</td>
<td>11.4</td>
</tr>
<tr>
<td>HGC250kDa</td>
<td>271,420</td>
<td>4.8</td>
<td>0.015</td>
<td>310</td>
<td>10.8</td>
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</tbody>
</table>

Average diameter of glycol chitosan nanoparticles
Delivery Carriers: Tumor Targeting

Tumor uptake of HGC nanoparticles

Fluorescence intensity in tumor

Contrast (Tumor/Normal)
Delivery Carriers: Tumor Targeting

In vitro stability of HGC nanoparticles

Ex vivo tumor targeting ability of HGC nanoparticles

Optical NIR

Blood

Tumor
Effect of Deg. Substitution

Filter Condition:
- GC
- 7.5 % HGC
- 13 %
- 23 %
- 35 %

Fluorescence Intensity (%):
- 7%
- 13%
- 23%
- 34%

St 800nm 450nm 200nm

Intensity(UC)
- 3.59e+004
- 2.72e+004
- 1.84e+004
- 9.72e+003
- 1e+003

After 6 hr
Brain Tumor model

Animal: Athymic Nude mice
Tumor: U87 Malignant Glioma
Injection sites: Brain and Flank \((5 \times 10^5 \text{ cells/} 5 \mu l)\)
Probe: HGC-Cy5.5 (250kDa), 5 mg/ kg, i.v.

Drilling → Tumor Xenograft → Probe Injection → In vivo Imaging (at 24h)

U87 Malignant Glioma \((5 \times 10^5 \text{ cells/} 5\mu l)\)

Kodak Optix Explore
Ex Vivo Imaging

1~4: U87 tumor, HGC-Cy5.5
5: U87 tumor, no treat
6: Normal, no treat
1: U87 tumor, HGC-Cy5.5
2: U87 tumor, HGC-Cy5.5
3: U87 tumor, no treat
Imaging of Early Metastases of Lung Cancer

Z-axis

15-16 mm  16-17 mm  17-18 mm  18-19 mm  19-20 mm

20-21 mm  21-22 mm  22-23 mm  23-24 mm  24-25 mm
The graph shows the tumor volume (mm³) over time (days) for different treatments. The treatments include Saline, HGC, Tax-5, Tax-10, Tax-20, PTX-5, PTX-10, and PTX-20. The tumor volume increases over time for all treatments, but the graph indicates that the treatments with higher doses (Tax-10 and Tax-20, PTX-10 and PTX-20) show a slower rate of increase compared to the lower doses (Tax-5, PTX-5). The images on the right show the tumor weight (g) for each treatment group at the end of the experiment. The images indicate that the tumors treated with higher doses of Taxol and PTX-HGC are smaller than those treated with lower doses.
Molecular Imaging
Molecular Imaging

Visualization of biological process
Molecular events at molecular and cellular level
In living systems
Using remote imaging detectors

“The characterization and measurement of biological processes in living animals, model systems, and humans at the cellular and molecular level by using remote imaging detectors”

Lucker GD and Piwnica-Worms D Acad. Radiol. 2001;8;4
When Tumoral Angiogenesis Starts

Beyond the critical volume of 2 cubic millimeters, oxygen and nutrients have difficulty diffusing to the cells in the center of the tumor, causing a state of cellular hypoxia that marks the onset of tumoral angiogenesis.
VEGF and bFGF are first synthesized inside tumor cells and then secreted into the surrounding tissue. When they encounter endothelial cells, they bind to specific proteins, called receptors, sitting on the outer surface of the cells. The binding of either VEGF or bFGF to its appropriate receptor activates a series of relay proteins that transmits a signal into the nucleus of the endothelial cells. The nuclear signal ultimately prompts a group of genes to make products needed for new endothelial cell growth.
The activation of endothelial cells by VEGF or bFGF sets in motion a series of steps toward the creation of new blood vessels.

First, the activated endothelial cells produce matrix metalloproteinases (MMPs). The MMPs break down the extracellular matrix—support material that fills the spaces between cells and is made of proteins and polysaccharides.

Breakdown of this matrix permits the migration of endothelial cells. As they migrate into the surrounding tissues, activated endothelial cells begin to divide.
The Angiogenic Sequence

- A cell activated by a **lack of oxygen** releases angiogenic molecules that **attract inflammatory and endothelial cells** and promote their proliferation.
- During their migration, inflammatory cells also secrete molecules that intensify the angiogenic stimuli.

- The endothelial cells that form the blood vessels respond to the angiogenic call by differentiating and by secreting matrix **metalloproteases** (MMP), which digest the blood-vessel walls to enable them to escape and migrate toward the site of the angiogenic stimuli.

http://www.angioworld.com
In vivo molecular target assessment of matrix metalloproteinase inhibition

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Fluorochrome Peptide substrate

Gly-Pro-Leu-Gly-Val-Arg-Gly-Lys-

FITC

Gly-Pro-Leu-Gly-Val-Arg-Gly-Lys-

FITC

MPEG

MPEG
Time dependence of p-nitroaniline release from the polymeric substrate P-Gly-Phe-Leu-Gly-NAp (P…. polymer) catalyzed by rat liver tritosomes, cathepsin B, cathepsin L and cathepsin H.
Chemically Associated MMP probes

- Stable chemical conjugates
- Cell-permeable
- Use for in vitro and in vivo
- Specified chemical entities

**Glycol chitosan**

**Hydrophobic moiety**

**MMP substrates**

**Cy 5.5**

**Blackhole quencher**
In vivo image (eXplore Optix)

1: Tumor: SCC7
2: MMP-2 probe: 5 mg/kg
3: MMP-2 inh.: 100 $\mu$g/kg

<table>
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<th>HT1080</th>
<th>LLC</th>
<th>B16F10</th>
<th>SCC7</th>
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<tbody>
<tr>
<td>MMP-9</td>
<td>MMP-2</td>
<td></td>
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Normalized photon count in tumor (50 mm²)

- No treat
- MMP-2 probe
- MMP-2 probe + Inh.
In vivo image (eXplore Optix)

- No treat
- MMP-2 Probe
- MMP-2 Probe + inhibitor

Tumor uptake of HGC nanoparticles
Z-axial slice images

1: MMP-2 probe in SCC7, 2: MMP-2 probe + inhibitor in SCC7, 3: No treat in SCC7
Ex vivo image (Kodak)

1: MMP-2 probe in SCC7
2: MMP-2 probe + inhibitor in SCC7
3: No treat in SCC7

White light  NIR
Histology

MMP-2 probe  MMP-2 probe + inhibitor  No treat

MMP-2  H&E
Fig. 5. (A) Chest radiographs from a patient with adenocarcinoma of the lung treated with SMANCS/Lpd via the bronchial artery three times and with aqueous intravenous SMANCS ×3/week during several weeks. A remarkable reduction in tumor size is seen. (B,C) CT scans and (D) chest X-ray images from different lung cancer patients. Please note tumor size reduction between the date given.
Tumor Biology with PET

- VX-2 Tumor bearing Rabbit
- Three different radiopharmaceuticals in the same animal

\[
\begin{align*}
\text{[}^{18}\text{F}]\text{FDG} & \quad \text{[}^{18}\text{F}]\text{FLT} & \quad \text{[}^{18}\text{F}]\text{FMISO}
\end{align*}
\]
Research Progress and Output

Rheumatoid Arthritis Imaging
Passive Tumor Targeting Nanoparticle

RA diagnosis

Normal  Adv. RA  Late RA  Normal  Adv. RA  Late RA

Imaging of RA Progress

Monitoring of Drug Response

MTX 0 mg/ kg  MTX 5 mg/ kg  MTX 10 mg/ kg
Theragnosis

Therapy + Diagnosis at the same time

Real time, Non-invasive
In vivo images on Therapy
Drug screening and mechanistic studies
Research Progress and Output

Imaging MMP-13 Expression
Osteoarthritis: MMP-13 activatable nanoparticle

In vitro MMP-13 assay

<table>
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<tr>
<th>Enzyme Conc.</th>
<th>+ Enz</th>
<th>- Enz (Control)</th>
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</thead>
<tbody>
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<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>0 (μg/ml)</td>
</tr>
</tbody>
</table>

| Probe conc. | 1 | 5 | 10 | 50 | 100 | 0 (μg/ml) |

In vivo Optical Imaging

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(a) OA + Probe, (b) Normal + Probe, (c) OA + Probe + Inh.

Intensity (NC)

- 2.25e+005
- 1.7e+005
- 1.14e+005
- 5.85e+004
- 3e+003

White light  NIRF  Merged
Imaging Kinase Activity
### Protein kinase inhibitor in pharmaceutics

- **Herceptin**
  - metastatic breast cancer
- **Gleevec**
  - chronic myeloid leukaemia
  - gastrointestinal stromal tumor
- **Iressa**
  - non-small cell lung cancer
- **Erbitux**
  - metastatic colorectal cancer
Protein kinase is an enzyme that can transfer a phosphate group from a donor molecule (usually ATP) to an amino acid residue of a protein. The protein kinase mechanism is used in signal transduction for the regulation of enzymes: phosphorylation can activate (or inhibit) the activity of an enzyme.
Scheme for cellular imaging

- **Cy5.5 LRRASLG** PKA-specific substrate (kemptide)
- Positively charged polymer
- Polymer mixture in aqueous condition
- Negatively charged polymer
- Polyion-induced (PIC) nanoparticles
- PIC nanoparticles
- NIR fluorescence amplification

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Published in *Chem Comm* 13 (2007) 1346
Characteristics of PIC nanoparticle

(a) Effect of Cy5.53-PEI-kemptide25/PAA molar ratio on the scattering intensity (○) and polydispersity index (●) of PIC nanoparticles in PKA reaction buffer at 25°C.

(b) A TEM image of PIC nanoparticles formed when the molar ratio was 0.8, in distilled water.

(c) A photograph of Cy5.53-PEI-kemptide25 and PIC nanoparticle solutions in PKA reaction buffer; 1: Cy5.53-PEI-kemptide25 mixture, 2: PIC nanoparticles formed when the Cy5.53-PEI-kemptide25/PAA components were in a molar ratio of 0.8, 3: PIC nanoparticles precipitated after centrifugation at 10,000 g.

(d) Quenching efficiency of PIC nanoparticles as a function of the molar ratio of the polymer mixture. Inset: NIR fluorescence from wells containing polymer mixtures.
PKA specificity of PIC nanoparticle

(a) The fluorescence spectra (excitation frequency of 675 nm) of PIC nanoparticles with a Cy5.53-PEI-kemptide 25/PAA molar ratio of 0.8 in PKA reaction buffer with 1 mM dithiothreitol at 37°C for 30 min; □ PIC, ○ PIC/PKA/ATP, △ PIC/PKA, ▽ PIC/PKA/ATP/PKA inhibitor, ▼ PIC-scrambled peptide/PKA/ATP.

(b) NIR fluorescence image from wells containing PIC nanoparticles incubated with PKA stimuli or inhibitors.

(c) Photograph of PIC nanoparticles in the PKA reaction; 1: PIC nanoparticles, 2: PIC nanoparticles/PKA/ATP, 3: PIC nanoparticles with scrambled peptide/PKA/ATP.

(d) The relationship between fluorescence intensity and relative scattering intensity of PIC nanoparticles in the presence of PKA and ATP at 37°C.

<table>
<thead>
<tr>
<th>optic</th>
<th>FITC</th>
<th>Cy 5.5</th>
<th>merge</th>
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<tr>
<td>100 μg</td>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>50 μg</td>
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
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1 × 10^5 HeLa cells, 1 hr incubation
<table>
<thead>
<tr>
<th>Time</th>
<th>optic</th>
<th>FITC</th>
<th>Cy 5.5</th>
<th>merge</th>
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<td><img src="image2" alt="5 min FITC" /></td>
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<tr>
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<td><img src="image3" alt="30 min Cy 5.5" /></td>
<td><img src="image4" alt="30 min merge" /></td>
</tr>
</tbody>
</table>

1 × 10^5 HeLa cells, 250 μg NP
1 $\times$ 10^5 HeLa cells, 250 $\mu$g NP
PKA imaging in single living cell

- Transfection time: 48 h
- 10 μg/ml PIC nanoparticle
- Delta VisonRT
- SoftWoRx Explorer Suite

<table>
<thead>
<tr>
<th>Transfection time (hour)</th>
<th>PKA C-beta</th>
<th>beta actin</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Images:
- PIC 5 min
- PIC 10 min
- PIC 30 min
- PIC 1h
- PIC/inhibitor 1h
- PIC/scramble 1h
Future
Cancer Theragnosis
Enzyme (e.g. MMP)
cancer NANOTECHNOLOGY

Going Small for Big Advances
Using Nanotechnology to Advance Cancer Diagnosis, Prevention and Treatment

Investigators
Access detailed information on open and closed funding opportunities, grant mechanisms, and application guidelines. more >

Patients & Providers
View resources on the basics of cancer imaging and locate cancer imaging trials. more >

News & Announcements

R01 Grant Application Alert - new process and forms required for Feb 2007 and beyond

National Cancer Imaging Archive

NIH Fiscal Policy for Grant Awards - FY 2007

Change in Standing Receipt Dates

New Limits for Appendix Materials

Multiple PI Awards for Support of Team Science Projects

View All News

The mission of the Cancer Imaging Program, National Cancer Institute, is to promote and support Cancer-related basic, translational and clinical research in imaging sciences and technology, and integration and application of these imaging discoveries and developments to the understanding of cancer biology and to the clinical management of cancer and cancer risk. more >

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Inflammation
Cancer
Autoimmunity
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