In vivo optical and radionuclide imaging for biological application

Hwang do won
Department of Nuclear Medicine, Seoul National University
Molecular imaging provides a visualization of a variety of biological phenomenon at a cellular or molecular level in living subjects.

1. Optical image
   - in vitro cells, small animal
     - (Luciferase, Fluorescence Protein)

2. Nuclear image
   - in vitro cells, animal, human
     - (Sodium Iodide Symporter HSV1-TK, D2R)

3. Magnetic Resonance image
   - large animal, human
The advantages of molecular imaging

**Immunostaining method**
- Invasive manner carrying out tissue biopsy
- Lack of repetitive experimentation
- Complicated technique methods

**Optical and radiotracer methods**
- Noninvasive imaging
- Repetitive imaging in individual animal
- Quantitative information
- Tomographic imaging
- Long-term validation

**Noninvasive molecular imaging**

Invasive immunostaining method
Steps of Molecular Imaging Development

Molecular → In vitro → Clinic → In vivo
영상법 종류

1. Optical image 2. Radionuclide image 3. MR image

Luciferase, Fluorescence protein

I-125, Tc-99m

Iron oxide, Gd-DTPA
microPET microCT microSPECT

Fluorescence imaging

Bioluminescence imaging

Animal MRI
빛의 투과력이 약하기 때문에 작은 실험동물 등에서는 사용

Fluorescence Imaging  Bioluminescence Imaging  MR Imaging
해상도 뛰어남
민감도 약함

Radionuclide imaging
고민감도
대사적 영상 가능
사람 적용
Biological application

therapeutic response to drug
physiologic activity
metabolism
hypoxia
proliferation
apoptosis
angiogenesis
multidrug resistance
cancer migration
developmental process
protein-protein interaction
…………
1. 치료 약물의 효과 판정 (drug response)
1. Monitoring of evaluating therapeutic effect regarding cancer therapy

Visualization of therapeutic effect

<table>
<thead>
<tr>
<th>Days after tumor challenge</th>
<th>pcDNA3.1</th>
<th>pcDNA3-hMUC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>7 days</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>11 days</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>18 days</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>25 days</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

*Jeon et al. Molecular imaging (2007)*
MicroRNA-21 Knockdown Disrupts Glioma Growth \textit{In vivo} and Displays Synergistic Cytotoxicity with Neural Precursor Cell–Delivered S-TRAIL in Human Gliomas

Maarten F. Corsten, Rafael Miranda, Randa Kasmieh, Anna M. Krichevsky, Ralph Weissleder, and Khalid Shah

Corsten, Cancer Res. (2007)
Evaluation of therapeutic effect using radiolabeled probe

hNIS expressing cancer

FDG-PET tumor scans

2. Imaging cell trafficking

- Luciferase
- MRI
- FDG-PET
- Micro-CT
- Flourorescent quantum dots
- Radionuclides (99m Technetium)
Molecular imaging of cell transplantation in living animals

cardiomyoblasts or MSC expressing HSV1-sr39tk reporter gene

=> the locations, magnitude, and survival duration

PET image of MSCs in porcine heart

PET/optical dual imaging in heart
Monitoring of implanted stem cell migration pattern in vivo

Bioluminescence image of stem cell migration

Stem cell migration into tumor region


Cancer metastasis imaging in vivo

Noninvasive Visualization of Retinoblastoma Growth and Metastasis via Bioluminescence Imaging

A Day 0 after intracardiac injection

Metastatic foci at Day 36 after intracardiac injection

B

Brain Submandibular LN Axillary LN Maxilla Femur Skull Paraaortic LN
3. 리포터유전자 기반 신경줄기세포 분화과정 영상화

- Stem cell
- Neuronal differentiation
- Nerve cell

NSE promoter Reporter genes Neuron-specific reporter expression

Transfection

Stem cells Specific factor Neuronal differentiation

Signal-off Signal-on

- Neurons
Neuronal differentiation imaging using reporter gene

4. 마이크로RNA 생체광학영상
Reporter based microRNA imaging for cancer imaging

▸ 光学リポーター基盤マイクロRNA観察

A. CMV luciferase
   - Signal-off
   - miRNA
   - 3 copies of Target Sequence

A. Primary miRNA
   - HT-ori3
   - NPA
   - TPC-1

A. β-actin
   - miR-146b
   - miR-221
   - miR-222

B. Fold intensity
   - miR-146b
   - miR-221
   - miR-222

C. Fold intensity
   - HT-ori3
   - NPA
   - TPC-1

Kim et al, MIB, 2008
In vivo microRNA imaging during neurogenesis

Smirnova et al. (2005)

Krichevsky et al. (2007)

Ko et al, Nat Protoc, 2009
MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling

Chulan Kwon, Zhe Han, Eric N. Olson, and Deepak Srivastava

MicroRNAs as a therapeutic target for cardiovascular diseases

Paras Kumar Mishra, Neetu Tyagi, Munish Kumar, Suresh C. Tyagi*
5. Monitoring transgene expression in embryonic stage
6. Transgenic mouse research for imaging

Transgenic mouse for molecular imaging
Conditional Promoter

NIS-transgenic Mice Under cardiomyocyte-Specific Gene Promoters

Confirmation of specific promoter

Small-animal PET image

- L6
- H9c2

the MHC promoter was confirmed to be active only in cardiomyocytes

Transgenic mouse showed higher uptake of $^{124}$I in heart (H) than in thyroid (T) or stomach (S).

7. In vivo monitoring of **endogenous gene expression using reporter system**
Generation of Cis-p53RE-hNIS/EGFP

p53RE: 15 repeats of TGCCTGGACTTGCCTGG
IRES: Internal Ribosome Entry Site
Scintigraphic Image (Tc-99m)

No treatment

24 hrs after treatment of Adriamycin (2mg/kg i.p.)

5×10^6

1×10^7

NIS
8. 방사성핵종을 이용한 내인성 신경줄기세포의 생체 영상화
Neural stem cell

is potential cell source for recovery of lost nerve tissue
[18F]FLT-PET visualizes endogenous NSCs in vivo.

**F-18-L-thymidine (FLT)**

![Thymidine and FLT structures](image)

**Figure 1.** [18F]FLT labels proliferating NSCs in vitro. A, Fetal rat cortical NSCs grown in monolayer cultures expressed the transcription factor SOX2, verifying their undifferentiated state. B, Rat hippocampal neurons expressed the neuron-specific cytoskeletal protein MAP2. C, Left, Proliferating NSCs incorporated the thymidine analog BrdU, whereas nonproliferating neurons did not. Right, The radiolabeled thymidine [18F]FLT was significantly better incorporated into proliferating NSCs than into nonproliferating neurons (all values displayed as means ± SEM).
Stroke-induced expansion of the NSC niches can be distinguished with PET
Noninvasive real-time imaging of apoptosis
Noninvasive imaging of protein–protein interactions from live cells and living subjects using bioluminescence resonance energy transfer
Enhanced stem cell viability using PLLA scaffold in myocardial infarction rat model

**Biomaterials + Cells**

- CSC only
- Fibrin gel + CSC
- PLLA + CSC

![Image of rodent models comparing CSC only, Fibrin gel + CSC, and PLLA + CSC]
Applications of molecular imaging

- Disease characterization and localization
- Selection & monitoring therapeutic efficacy

- Gene therapy with targeted gene expression
- Monitoring endogeneous gene expression
- Visualizing biological phenomenon (differentiation, development, infection and anti-viral/microbial effect, etc)
- Monitoring disease progression
- Cell trafficking
- Cell therapy
- Drug development
- Transgenic animals evaluation
Molecular Imaging is... to visualize and quantify characterization of biological mechanisms at the cellular and molecular level with non-invasive method.

It is a very powerful technology that allows to study biological phenomenon.

Molecular Imaging is...

Non invasive,
Repeatable,
Quantitative