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Molecular Imaging: An Old and New Field Connecting Basic Science and Clinical Medicine

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Abstract Combination of recent progress in imaging technology and molecular biology/gene technology has evolved a new field named ''molecular imaging''. It includes wide range of imaging technique from basic research to clinical practice. For basic researchers, we focused on the part of in vivo imaging in human, and introduce the recent progress of modern biomedical imaging, as a crossing point of basic science and clinical medicine. J. Cell. Biochem. Suppl. 39: 85–89, 2002.

Key words: antisense; gene expression; PET; nuclear medicine

Recent progress in biomedical imaging has realized non-invasive imaging of human body with sub-millimeter resolution, multiple information intensity as well as reasonably quantitative data. Traditional radiological imaging techniques using X-ray (conventional X-ray and X-CT) are based on the interaction between the electro-magnetic wave (X-ray) and atoms constructing human body. Thus, in principle, it does not bring any information concerning biological molecules. On the other hand, nuclear medicine (NM) imaging and magnetic resonance (MR) imaging are based on the molecule–molecule interaction and molecule–magnetic field (electro-magnetic field) interaction respectively, and thus they can provide molecular information, by means of interaction between an appropriate molecular probe and living organisms in vivo. Fortunately, great progress in molecular biology clarified enormous amount of knowledge about cascade of gene expression and interaction between gene and gene products including DNA, RNA, protein, and biologically active small molecules. Using the knowledge, NM as well as MR imaging has been moved to a new discipline "molecular imaging" [Sharma et al., 2002]. "Molecular imaging" is broadly defined as in vivo characterization and measurement of biologic processes at the cellular and molecular level [Weissleder and Mahmood, 2001], and it includes not only biomedical imaging but also basic imaging technologies like in situ hybridization. However, this article is not to remind the basic scientists a history of the latter, but to review the recent approach of "biomedical imaging" to connect molecular and cellular biology and clinical medicine. For this purpose, up-to-date modalities for biomedical imaging will be introduced, then examples of clinical "molecular imaging" approach will be shown.

MODALITIES FOR BIOMEDICAL IMAGING

In nuclear medicine, molecular probe labeled with short-lived positron- or gamma (shingle photon)-emitting radionuclide is injected into human body, and its distribution was analyzed with positron emission computed tomography (PET, PECT) or single-photon emission computed tomography (SPECT, SPET) to understand the interaction between the radiolabeled molecular probe and its target molecules in vivo. Figure 1 shows a PET image of [F-18]-2-fluoro-2-deoxy-D-glucose (FDG) visualizing glucose

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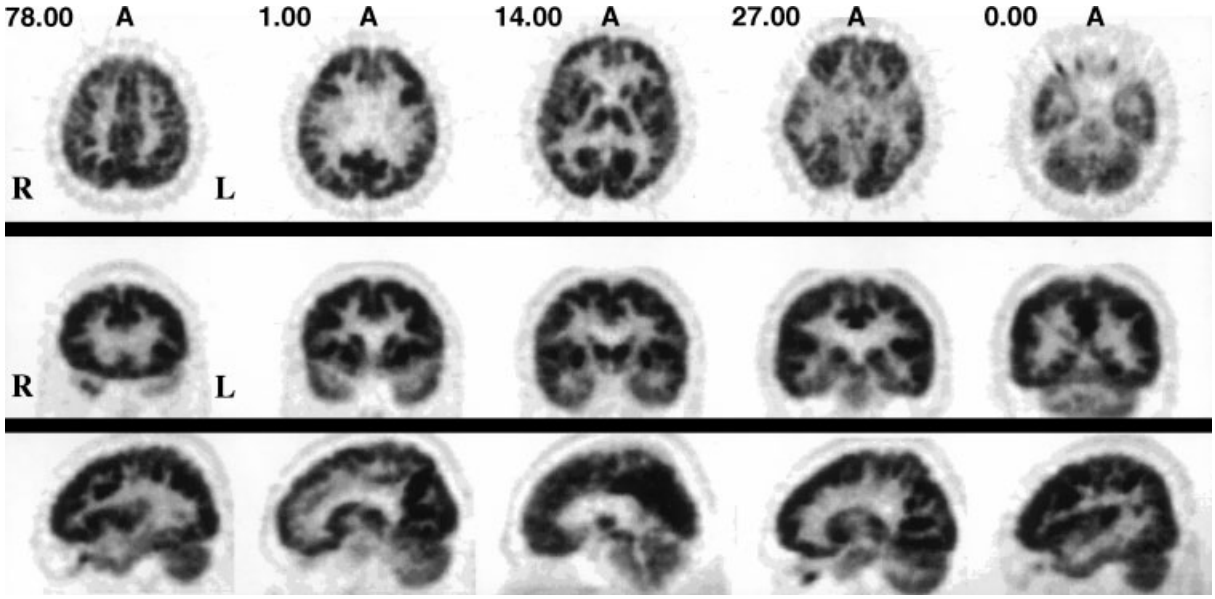

Fig. 1. FDG–PET images in normal human brain.

utilization in human. This PET image can be considered as an indication of interaction between glucose analogue and hexokinase molecule. At present, special resolution is around few millimeters for clinical PET and nearly one millimeter for small animal PET. FDG–PET diagnostic imaging is now routinely used in clinical practice approved by health insurance system. In general, PET facilities must have small cyclotron(s) to make ultra-short half-lived radionuclides (F-18: 108 min, C-11:20 min, N-13: 10 min, O-15: 2 min), but it allows us to make various kinds of originally designed molecular probes. On the other hand, SPECT-probes can be obtained as commercially available radiopharmaceuticals, but radionuclides used for labeling are biologically heterogenous, and variety of probes available is still limited.

Another modality is MR imaging (MRI). Principally, regional signal strength, relaxation time, and chemical shift of proton in water molecule is measured to visualize the concentration as well as surrounding atmosphere of water molecule. Paramagnetic metals like Gd, Mn, Fe, etc., can modify these signals, so that relative amount of these metals can be measured using MRI, and molecular probes labeled with these metals are used for the detection of molecular reaction. Although the sensitivity is very low when compared with PET or SPECT, very high resolution of MRI (sub-millimeter level) allows us to visualize small organs even in rats or mice.

Based on these modalities and the development of suitably labeled molecular probes, clinical molecular imaging has become realized.

MESSENGER RNA

Just as Northern blot analysis or in situ hybridization, detection of messenger RNA as gene transcription product is considered to be a direct indication of gene expression. For this purpose, stable antisense probes with very high specific radioactivity have been designed, using the knowledge of basic biotechnology. Figure 2 shows our approach for the probe design as an example [Fujibayashi et al., 1999]. This probe design allowed us to get a clinically applicable gamma-emitting probe with high specific radioactivity and purity, by simple mixing of the precursor and commercially available In-111. To realize the imaging of specific probe-mRNA binding, non-specific delivery of antisense oligo-probe into the cells (hybridize phase) and washout from non-target cells (washing phase) is essential, and rather big molecular size of oligo-probe might be a problem. However, Sato et al. successfully enhanced the delivery of this probe into cells using dendrimer as a counter cation [Sato et al., 2001]. Lee et al. reported a carrier mediated approach to improve the delivery/washout of antisense probe to/from brain tissue through the blood brain barrier (BBB), an essential point to realize "hybridize and wash" in the brain [Lee et al., 2002]. These

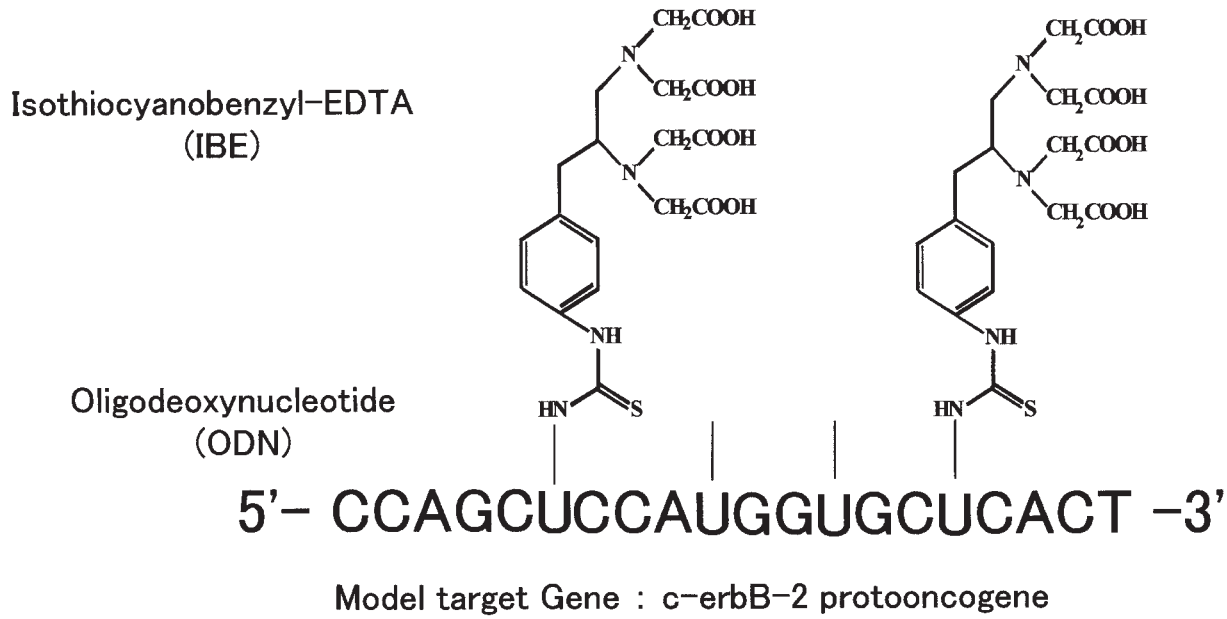

Fig. 2. Schematic structure of In-111-labeled antisense probe with multi-chelating sites.

approaches will accelerate the progress and realize the "in vivo hybridization" imaging in living animal near future, although there still be some problems to be solved.

PROTEIN

Western blot analysis is a direct detection method of gene-translation product, namely protein, using antigen–antibody reaction. In biomedical imaging, radiolabeled antibodies against cell-membrane antigens have been used for tumor imaging for many years, and several antibody-based radiopharmaceuticals for tumor imaging are commercially available now. Using this technique, vascular endothelial growth factor (VEGF) was visualized for the prediction of tumor immunotherapy using anti-VEGF monoclonal antibody as molecular probe as well as therapeutic agent [Jayson et al., 2002].

Detection of protein, based on its biological function, is also widely applied to biomedical imaging of transporters, enzymes, receptors, and so on [Halldin et al., 2001; Kwekkeboom and Krenning, 2002; Warner and M O'Dorisio, 2002]. FDG, mentioned above, is a typical example of enzyme-seeking probe [Phelps, 1991; Silverman et al., 1998; Smith, 2000]. Recent progress of molecular biology and combinatorial chemistry/peptide library technique realized enormous number of ligand–protein combinations, and it will bring ideal sets of molecular probe—target protein for biomedical imaging.

PHYSIOLOGY

Gene expression finally reflects physiological changes. For example, injection of vascular endothelial growth factor cDNA (VEGF-cDNA) induces angiogenesis, and regional perfusion is a good indication of the gene therapy of ischemic myocardium. To evaluate the efficacy of gene therapy, SPECT imaging, PET imaging, echocardiography as well as MRI are used [Huwer et al., 2001; Sarkar et al., 2001]. This kind of trials has been widely performed not only in heart but brain [Yoshimura et al., 2002].

REPORTER GENE

Now, gene transfection technique has gradually moved from basic research technique to clinical gene therapy. Use of VEGF, hepatocyte growth factor (HGF) [Aoki et al., 2000; Taniyama et al., 2002], fibroblast growth factor (FGF) [Horvath et al., 2002] for angiogenesis gene therapy has been reported, and some of them have been evaluated using biomedical imaging technique for the assessment of therapeutic effect. Although this approach is useful for clinical practice, these are still indirect evaluation of gene expression and interpretation is not simple.

For the direct assessment of transfected gene expression, use of co-expressing reporter gene is useful. In cell or small animal studies, beta-galactosidase (lac-Z), green fluorescence protein (GFP), luciferase and so on, are widely used as reporters for specific gene expression. However, the signals from these reporters are enzymatic coloring or visible photon emission, so that non-invasive detection in living animal is only limited to surface region of the body, and quantitative evaluation of gene expression is difficult. For clinical practice, combination of biomedical imaging technique and gene technology is essential. As listed above, imaging principles of molecular reactions using labeled probes are in mature stage, and new reporter system specially designed for the detection of gene expression can be developed based on them.

Selection of labeled probe and reporter protein is of important issue. As a reporter protein, it should be expressed only in the region of high target gene expression. From this point, heterogenous protein originated from other species is better, but it must have immunogenicity and the cells expressing such protein are going to be rejected from the body soon. It is acceptable in case of suicide gene therapy of tumors. Transfection of HSV1-Tk into tumor cells is originally developed for gene therapy in combination with antiviral drug acycloguanosine, and PET/SPECT monitoring of gene expression can be done using radiolabeled acycloguanosine (e.g., F-18-fluoro-ganciclovir) [Herschman et al., 2000; MacLaren et al., 2000]. This is the case that therapeutic (suicide) gene and reporter gene is exactly the same. However, if HSV1-Tk were used merely as a reporter for monitoring another functional gene, antiviral drug (e.g., acycloguanocine) treatment become unacceptable for the patient in future; it results in the death of the target cells. Thus, this system cannot be applied to gene therapy for the treatment of functional deficit. In addition, low lipophilicity and limited membrane permeability of radiolabeled acycloguanocine is not suitable for brain studies. Another approach has been done with dopamine D-2 receptor (D2R) protein. D2R protein can be detected using F-18-fluorospiperone, a dopamine antagonist. Advantage of this approach is that the labeled probe is freely penetrated through the cell membrane including BBB, and can be applied also for gene expression monitoring in the brain. However, endogenous D2R expression in the brain might induce misreading of the expression of transfected gene. Another possible problem is that transfected D2R might bind with endogenous dopamine, change the physiological levels of dopamine in the target tissue, and induce some biological effect. Transfected receptor proteins should be modified to biologically inactive form, and this modification might bring immunogenicity. Cell membrane receptors might be faced with this problem rather than intracellular proteins.

At present, I might be able to say that there still be no ideal molecular probe-reporter protein system for gene expression monitoring in vivo. However, large number of efforts and trials being done now will solve the problems to realize the truly useful system near future.

CONCLUSION

Biomedical imaging has revolved not only with clinical medicine but chemistry, physics, mathematics, computer sciences, and of course, molecular biology/gene technology. It is an interdisciplinary research field inborn and can be merged with any kind of life sciences. Molecular imaging is a promising example of merging for clinical practice as well as basic understanding of life.

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