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EDITORIAL

Protein and peptide probes for molecular imaging

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Molecular imaging (Weissleder et al. 2010), an emerging research field, with an emphasis on visualization and quantification of biological processes at the molecular and cellular levels, has shown great potential in early detection of diseases, stratifying patients into potential responders and non responders to a given treatment, designing personalized treatment, monitoring treatment efficacy, drug discovery, and development, as well as better understanding of biology and pathophysiology.

Molecular imaging requires biology to identify and validate the molecular imaging target(s); medical physics, and mathematics to develop high resolution, and high sensitivity imaging devices as well as the software algorithms for image acquisition, processing, and quantification; and chemistry/biochemistry to develop molecular imaging probes (Chen 2006).

Molecular imaging probes are one of the major driving forces of molecular imaging research (Chen and Chen 2010). Depending on the property of the molecular target (protein, ribonucleic acid, or deoxyribonucleic acid), location of the target (extracellular, cell surface, intracellular, or nuclear), and copies of the target per cell or tissue/organ of interest, there will be different requirements for the probe design. Clearly, the abundance and specificity of the target for the disease process under study is critical to make the molecular imaging assay successful. Ions, small organic molecules, peptides, aptamers, engineered proteins, nanoparticles, and even certain cell types are all possible molecular imaging probes. Several factors determine whether a molecular imaging probe is appropriate for a given biological process or disease state, such as its ability to traverse the cell membrane, the time involved for targeting and clearance from the body, the potential for non-specific interactions that would lead to the increased background signal, the ability to correlate between the probe signal and the levels of molecular target, and finally the safety of the probe.

This special issue of the *Amino Acids* was an outcome of a symposium of the 11th International Congress on amino acids, peptides, and proteins (Vienna, Austria, August 3rd–7th, 2009), which focuses on protein- and peptide-based molecular imaging probes and in some cases theranostics. This special issue starts with a review of protein-based molecular imaging probes for tumor imaging (Lin et al. 2010). It was pointed out that although the intact antibody probes have high binding affinity and specificity for the antigens, the relatively large size of antibodies (ca. 10 nm) leads to a long circulation half life, poor tissue penetration rate and thus compromises their use as diagnostic tools. However, they can be useful to guide therapeutics based on antibodies, antibody conjugates or radioimmunotherapy. On the other hand, various engineered protein probes, especially antibody fragments, protein scaffolds and natural protein ligands with more compact size, shorter clearance time, and better tumor penetration are finding their way in early stage diagnosis, therapeutic response monitoring, and personalized treatment. This review also emphasizes the importance of site-specific labeling (Wang and Chen 2008) and the so-called "imaging figure of merit" (IFOM) (Cai et al. 2007).

The second review article by Miao et al. (2010) touches bases on the protein scaffolds. A protein scaffold is a constrained polypeptide consisting of α-helix, β-sheet, or

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loops, which could be the derivatives from both the single domain immunoglobin (e.g., nanobody) and non-immunoglobin (e.g., affibody and knottin). Protein scaffolds can be applied for engineering and in vitro display selection against molecular targets. Protein scaffolds against various cancer biomarkers such as the Her-axis, carcinoembryonic antigen (CEA), tumor necrosis factor alpha (TNF-α), and integrin $\alpha v\beta 3$ with low nanomolar and even picomolar affinity have been discovered. The efforts of labeling these small proteins with various radionuclides, and near-infrared (NIR) fluorescent dyes were elaborated and some limitations of this class of probes were also discussed.

Regulatory peptide receptors are overexpressed in numerous cancers. Endogenous ligands for the regulatory receptors are potent low-molecular weight peptides that are mainly synthesized in the central nervous system and the GI tract. Roosenburg and her colleagues put cholecystokinin (CCK) receptor as an example to illustrate the power of peptide receptor radionuclide imaging (PRRI), and peptide receptor radionuclide therapy (PRRT) (Roosenburg et al. 2010). All the CCK/gastrin-related peptides (linear, cyclic, multimers, etc.) appear to have the C-terminal receptor-binding tetrapeptide sequence Trp-Met-Asp-Phe-NH_2. Some of the CCK analogs have been advanced to early phase clinical trials for tumor detection with the limited success. The major issues of these G-protein coupled receptor (GPCR) ligands, such as the poor metabolic stability and the unfavorable pharmacokinetics, need to be addressed before they can be realized as CCKR imaging agent and suitable candidates for PRRT.

Cell adhesion molecule, integrin $\alpha v\beta 3$ is a well-established angiogenesis target for the molecular imaging and therapy (Chen 2011a; Niu and Chen 2011). Cyclic RGD peptides, especially multimeric RGD peptides with different linker lengths have been developed to enhance integrin-binding affinity, increase tumor uptake, prolong tumor retention, and improve the pharmacokinetic profile. Shi et al. in their original research article pointed out that the bifunctional chelator also has profound effect on the biological properties of the resulting peptide conjugates (Shi et al. 2010). Among 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (DOTA), diethylenetriaminepentaacetic acid (DTPA), and 2-(*p*-thioureidobenzyl)-diethylenetriaminepentaacetic acid (DTPA-Bn), the ^{111}In-labeled DTPA-Bn conjugate showed more rapid tumor washout and poorer tumor contrast than the DOTA analog. It was concluded that the DTPA is preferred if high-specific activity is required while DOTA is the chelator of choice for the development of the therapeutic lanthanide radiotracers.

There are cases that the target is not located on the cell surface. For example, phosphatidylethanolamine (PE) is predominantly a constituent in the inner leaflet of the plasma membrane bilayer in a viable, typical mammalian cell. In apoptotic cells, PE is exposed to the cell surface, thus providing a molecular marker for detection. Zhao provided a recent account on the use of PE-specific probes derived from duramycin and cinnamycin, which are the members of type B lantibiotics (Zhao 2010). The author argued that for the detection of cell death, PE targeting is potentially superior to phosphatidylserine (PS) as the abundance of PE is even higher than PS. Furthermore, PE is also a critical anticoagulant. Some links between the anti-PET autoimmunity and the idiopathic thrombosis have been found, which suggest that the PE-targeted imaging may also be useful to fully understand the functional roles of the PE in hemostasis.

Although it is obvious that the peptides have many advantages over antibodies in molecular targeting, and issues of relatively low receptor affinity and short-retention time. Multivalent interactions account for the high affinities of antibodies. The same principle can also be applied to peptides. Both peptide homomultimers and heteromultimers have been designed for molecular targeting and imaging. Yan and Chen (2010)summarized the recent findings of peptide heterodimers, in which two different peptide ligands targeting different receptors are covalently linked by either a flexible or a rigid linker with adjustable length. RGD–BBN heterodimer structure is such an example that recognizes both integrin $\alpha v\beta 3$ (Niu and Chen 2011) and gastrin-releasing peptide receptor (GRPR) (Yang et al. 2011) were discussed in detail. Some other heterodimers such as melanocortin-4 (hMC4R) and δ-opioid (δ-OR), MSH(7) targeting hMC4R and CCK targeting CCK-2R, EMP-ERP heterodimers for c-Met and VEGFR-2, and peptide heterodimers binding different domains of VEGFR-2 were also briefly mentioned.

Sometimes it may not be obvious to have the prior knowledge of the target before one can use the peptide for imaging and targeting purposes. For example, Hao et al. (2010) in their recent study found that some cell-permeable peptides (CPPs), initially known as intracellular delivery vehicles for a variety of bioactive cargos, have unexpected preferential uptake in prostate tissue and prostate cancer cells. An arginine-rich CPP (NH_2GR_{11}) showed high prostate cancer-cell uptake and internalization with its subcellular localization in cytosol. Both fluorescent dye and radioisotope-labeled NH_2GR_{11} also had good tumor accumulation in vivo.

Phage display is a powerful technique that allows vast sequence space screening, providing a means to improve the peptide affinity and generate unique peptides that bind to any given target (Deutscher 2010). The distinctive advantage of this technique is that the targets may be unknown and non immunogenic, yet may serve as a delineating character for a particular cell type or tumor type (Sun et al. 2010). Cao et al. (2010) used a

bevacizumab-sensitive LS174T colorectal cancer model and a 12-mer bacteriophage (phage) display peptide library to identify a bevacizumab-responsive peptide (BRP). Both IRDye800 and ^{18}F-labeled BRP peptide had significantly higher uptake in tumors treated with anti-angiogenic therapeutic bevacizumab than in controls treated with PBS buffer. The changes in BRP uptake preceded the anatomical changes in ^{18}F-FDG. Although the target of BRP is unknown, this linear 12-mer peptide appears to bind specifically to endothelial cells exposed to bevacizumab. The same probe is also expected to bind to tumors treated with other anti-angiogenic drugs.

Hong et al. (2010) chose prostate cancer as the target and positron emission tomography (PET) as the imaging modality to review the current state-of-the-art of early detection of primary lesions and accurate imaging of bone metastasis. The tracers include small molecules, amino acids, peptides, and antibodies. Some tracers such as ^{18}F-choline and ^{18}F-FDHT (small molecules), ^{11}C-methionine (amino acids), and ^{18}F-BBN (peptides) already entered the clinical trial or have been extensively studied in the preclinical settings. The authors also stressed the point that PET alone may not be sufficient for prostate cancer staging, and monitoring the therapeutic efficacy.

For cell surface receptors, it is straightforward to properly label the receptor ligand, and apply the corresponding imaging techniques to visualize and quantify the target level based on the signal intensity measured from the probe accumulation. However, proteases such as matrix metalloproteases (MMPs) that are involved in a number of pathological conditions such as rheumatoid arthritis, osteoarthritis, and tumor metastasis (Zhu et al. 2011) may not be effectively imaged by the constitutively active probes as the enzyme level is intrinsically low. Optically activatable probes have been the choice for MMP activity measurement in vitro and in vivo. Ryu et al. (2010) used MMP-13 as an example and demonstrated the power of Föster resonance energy transfer (FRET) in designing the MMP-13 fluogenic probes that consisted of NIR dye Cy5.5, black hole quencher (BHQ-3), and MMP-13 substrate peptide for osteoarthritis detection in vivo. The same principle applied here can be extended to other proteases simply by changing the specific peptide substrate linker between the fluorophore and quencher.

Finally, Liu et al. (2010) further expanded the activatable probe concept and reviewed the optically "smart" probes for both NIR fluorescence imaging and photodynamic therapy (PDT). For PDT, the cytotoxic molecule, singlet oxygen ($^1O_2$), is generated by the light-activated photosensitizer (PS) to cause cell damage. The main issues of lipophilic PSs are their non-specific accumulation in the normal tissues, and the inability to know how much PS is actually accumulated in the target area. The authors highlight a molecular beacon that possesses a caspase-3 peptide linker holding a porphyrin-like PS, and a quencher in close proximity. The conjugate was further coupled with a folate molecule for tumor folate receptor targeting. The molecule is initially fluorescently silent. Once the light activates the PS, singlet oxygen is produced to damage the cell. If the apoptosis cascade is initiated, caspase-3 is processed to its active form indicating the initiation of the irreversible apoptotic death.

In summary, this special issue consists of 11 review articles and original papers, and was contributed by over 50 authors worldwide. Engineered proteins and peptides are of particular interest to both pharmaceutical industry and molecular imaging community, owing thanks to the recent advances in phage display technology, combinational peptide chemistry, and biology (Lee et al. 2010a, b). It is also of note that although many sophisticated peptide-based molecular imaging agents either directly measure the cell surface receptor expression level or indirectly assess the protease activity through activatable probes, only very few of them have been used in the clinic for the diagnosis of diseases. With concerted efforts from molecular imaging researchers, radiology and nuclear medicine physicians, and pharmaceutical industry, we expect to see more promising protein and peptide probes with the optimal targeting and favorable pharmacokinetics to be translated into the clinic for first-in-human trials. Eventually, the design of theranostic peptide probes (Chen 2011b; Xie et al. 2010) that allow simultaneous cancer imaging, smart therapy, and response monitoring will be the trend of personalized medicine.

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