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Progress of engineered antibody-targeted molecular imaging for solid tumors (Review)

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Received November 16, 2007; Accepted December 3, 2007

Abstract. Engineered antibodies, with their high specificity and affinity for their target antigens, as well as their reduced size and multivalent design, can be tailored to carry radionuclide magnetic, luciferase or fluorescent probes for specific attachment to tissue cells, extracellularly or intracellularly, for PET, SPECT, MRI, optical and ultrasonic imaging. The antigen-specific imaging agents of engineered antibodies have deep tissue penetration, high tissue retention and fast blood clearance, which are desirable properties for the rapid imaging of tumors with high specificity and resolution in pre-clinical or clinical imaging studies.

Contents

1. Introduction

Monoclonal antibodies have long been eyed as a potential new class of therapeutics targeting cancer and other diseases with their high-specificity targeting feature. Throughout the 1990's, innovative recombinant antibody technologies led to a wave of approvals by the FDA for therapeutic immunoglobulin (Ig) and Fab (fragment antigen binding) molecules. These developments have continued; there are presently 18 engineered antibody products on the market, and more than 100 are still being tested. It is predicted that by 2008 they will account for more than 30% of all revenues in the biotechnology market (1,2).

Molecular imaging is a powerful tool in the investigation of molecular interactions for drug discovery and development, and also provides important diagnostic and prognostic information affecting patient management in the clinical setting. However, the use of molecular imaging for diagnosis has not been widely adopted, in part due to a lack of suitable targeting agents (3).

Engineered antibodies, especially intermediate engineered antibody fragments such as single-chain variable fragments (scFv, 25 kDa), diabodies (dimers of scFvs, 55 kDa) and minibodies (scFvs fused to single Fc domains, 80 kDa) possess the unique properties of improved tumor penetration, faster clearance kinetics and increased tumor-to-blood ratios. They promise specific delivery to tissue cells for PET (positron emission tomography), SPECT (single photon emission computed tomography), MRI (magnetic resonance imaging), optical (fluorescence and bioluminescence) and ultrasound imaging, once tagged with different imaging probes.

This review will focus on the recent use of engineered antibodies for the non-invasive *in vivo* molecular imaging of solid tumors, and for the monitoring of tumor progression in response to cancer therapy in pre-clinical animal models and clinical trials.

2. PET imaging

Although most clinical PET studies (95%) are based on ^{18}F-FDG, recent interest has arisen regarding the development of specific imaging agents, such as probes with engineered antibodies, as they make it possible to visualize tumors that exhibit low metabolic activity and cannot therefore be detected by conventional ^{18}F-FDG PET.

Previous evaluation of the targeting and biodistribution of scFv fragments specific to carcinoembryonic antigen (CEA) or tumor-associated glycoprotein demonstrated localization to the tumor, but activity levels were insufficient (4,5). More recently, the results of Sundaresan *et al* (6) provided evidence that ^{124}I-radiolabeled anti-CEA diabody and minibody in CEA$^+$ LS174T xenografts have strong localization and low normal tissue background. Clinical imaging studies have also been conducted using ^{123}I-labeled anti-CEA minibody for pre-surgical colorectal cancer patients (7). The anti-CEA minibody demonstrated localization to seven of eight known lesions,

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Key words: engineered antibody, molecular imaging, solid tumor

including three that were not visible by computed tomography (7). scFv (25 kDa) for a given immunoglobulin exhibits faster clearance kinetics and deeper tumor penetration. However, the absolute dose deposition of scFv is much lower, primarily due to its monovalent binding nature, resulting in lower functional avidity. Diabodies (55 kDa), with their bivalent binding, exhibit better tumor deposition than scFv and are below the threshold for first-pass renal clearance (8). Larger fragments, such as minibodies (80 kDa), show intermediate clearance rates and reach higher tumor uptake levels (9). Diabodies and minibodies reach their maximum tumor uptakes within 1-6 h of administration in xenograft-bearing mice and, because of rapid blood clearance, tumor-to-blood ratios increase steadily over time and reach high values (>20:1) by 24 h, making these fragments prime candidates for imaging.

The *in vivo* properties of a slightly larger antibody fragment, scFv-Fc of 105 kDa (10), have been examined. It seems to behave in ways analogous to an intact antibody due to the presence of an intact Fc region. Moreover, certain mutations in the Fc region that mediate Fc receptor interactions modulate the clearance kinetics of this antibody fragment. Of particular importance to this work, it is also a variant containing two mutations (H310A/H435Q; Kabat numbering system) and shows similar pharmacokinetics to that of the minibody (10). In addition, high-resolution microPET images are obtained when this antibody fragment is labeled with ^{124}I (10) and ^{64}Cu positron emitters (11). This approach provides a means for tailoring the pharmacokinetics of engineered antibody fragments and should allow for the selection of versions optimized for imaging or therapeutic applications.

Recently, Cai *et al* applied ^{18}F-labeled T84.66 anti-CEA diabody in colorectal cancer xenograft-bearing mice (12). The results showed rapid and high tumor uptake and fast clearance from the circulation in the xenograft model, as evidenced by both small-animal PET imaging and bio-distribution studies. High-contrast small-animal PET images were obtained as early as 1 h after injection of the ^{18}F-T84.66 diabody, and only a background level of activity accumulation was found in CEA-negative tumors. ^{18}F-labeled diabodies therefore represent a new class of tumor-specific probes for PET, based on targeting cell surface antigen expression.

Some tumor-related antigens are readily internalized, which causes a hammering of imaging signals. Loss of the label in tumors as a result of dehalogenation and the metabolism of proteins labeled subsequent to internalization can be overcome by changing to a more stable conjugation chemistry (sulfosuccinimidyl-3-(4-hydroxyphenyl) propionate) (13). Additional work (14,15) has led to the development of anti-Her2 minibodies radiolabeled using ^{64}Cu-DOTA, permitting the microPET imaging of Her2-overexpressing xenografts in mice. In this case, internalization of the radiometal-labeled tracer resulted in metabolism and trapping of the radioactivity at the tumor site, maintaining the signal.

Visualization and quantification of Her2 expression by *in vivo* imaging will probably prove to be an important approach, not only for assessing target availability but also for monitoring response to treatment. Others have also used PET with ^{68}Ga-radiolabeled F(ab')2 fragments of trastuzumab to monitor Her2 expression in animal tumors during the course of treatment with 7-allylaminogeldanamycin (Hsp90 inhibitor), showing loss and subsequent recovery of Her2 expression *in vivo* (16).

3. SPECT imaging

SPECT is another type of nuclear medical examination. In some cases, PET may be more sensitive than SPECT, but the latter is more widely available because radioisotope generation technology is longer lasting and far less expensive in SPECT, as is the γ-scanning equipment.

Angiogenesis or vasculargenesis is very important during the maintenance and development of tumor growth. *In vivo* SPECT imaging has been used for vascular evaluation with engineered antibodies as agents. SPECT imaging of tumor neo-vascularization with ^{123}I-labeled anti-fibronectin ED-B scFv dimer in cancer patients has proven the potential of engineered antibodies as radiotracers (12,17). SPECT imaging with iodine-125 or technetium-99m radiolabeling has shown promising results, providing important information. For example, within 30 min of injecting engineered anti-VEGF antibody (TX3.833) in the tail vein of rats, >80% of the dose was transported across the vascular membrane and accumulated in lung tissue (17).

Various SPECT radionuclides have different energies, making it possible to distinguish a panel of biomarker antigens at the same time. On the other hand, PET isotopes all have the same emission, 511 keV annihilation photons. Serial imaging is therefore necessary for the evaluation of different markers. The availability of radionuclides for clinical and research purposes continues to expand, along with their potential applications, and the radioimmunodetection of tumor growth is essential and widely used. A radiolabeled immunoconstruct with enhanced properties for imaging must be of potentially intermediate molecular mass with tumor uptake comparable to intact antibodies, but with clearance times more rapid than the intact IgG, providing improved imaging capabilities.

4. MRI imaging

Even though MRI has been regarded as a powerful imaging tool as a result of its non-invasive nature, high spatial resolution and tomographic capabilities, its low signal sensitivity has been a major limitation. When ultra-sensitive magnetic nanoprobes, like magnetism-engineered iron oxide (MEIO), are conjugated with Herceptin, enhanced MRI sensitivity is demonstrated in the detection of breast cancer marker Her2/neu in tumors as small as ~50 mg (18). If such nanoprobes can be integrated with engineered antibody fragments that show versatile functions, including targeting moiety, engineered antibody-magnetic nanoprobes will definitely become promising candidates for high-performance MRI imaging agents. As for toxicity, the results of Lee *et al* (18) show that Mn-doped $MnFe_2O_4$ (MnMEIO), which was the nanoparticle with the highest relativity value (the strongest MR contrast effect), along with MnMEIO-Herceptin, were found to be biologically nontoxic in HeLa and HepG2 cell lines at 200 μg/ml. However, *in vivo* toxicity should be further investigated as no literature exists that well proves the point.

al fluorescence and bioluminescence imaging

In vivo optical imaging has emerged as a relatively simple, inexpensive method for non-invasively observing biological processes inside a small living animal. For example, a bifunctional anti-CEA diabody-Renilla luciferase fusion protein (Db-Rluc8) was systemically administered in tumor-bearing mice (19). The results show that this fusion protein preferentially localized antigen-positive tumors and generated a bioluminescent signal that could be detected in the living mouse. More recently, the anti-CEA diabody was fused to Gaussia luciferase, which offers a fusion protein of a smaller size (Da-Gluc, 90 kDa) with potentially brighter light output that improves targeting and imaging properties (20).

Near-infrared fluorescent dye conjugated to antibodies against EGFR has also shown promise for the detection of pre-cancers (21), as it provides deeper tissue penetration. Molecular optical-specific imaging using fluorescence-labeled antibodies (22) and indocyanine green N-hydroxysulfo-succinimide ester (ICG-sulfo-Osu)-labeled antibodies (23) has also been demonstrated. Even though no official reviews have been published in this field, we can predict that engineered antibody fragments will give better results for specific fluorescent imaging.

Nanocrystalline semi-conductor materials, named quantum dots (QDs), have become a new class of fluorescent probes for optical imaging. Many efforts have been undertaken to image ligand-receptor interactions utilizing QD-conjugated antibodies to target the receptors (24-27). The primary antibody targets the cell-surface receptors and is then recognized by a biotinylated secondary antibody, which binds a streptavidin-coated QD (28). This sandwich-imaging format leads to a very large molecular complex that may hamper accurate imaging of receptor densities and receptor dynamics. Engineered antibody fragments might be one strategy to minimize the size of the QD-label complex, and can also be covalently conjugated to a QD surface. Intact antibodies, on the other hand, have to be non-covalently complexed to the QDs, usually through biotin-streptavidin conjugates. Although QDs are so far considered to be safe, recent work done by Shiohara *et al* (29) shows that water-soluble QDs covered with mercapto-undecanoic acid (also known as MUA-QD) affect the cell variability of Vero, HeLa, and primary human hepatocyte cells even at low concentrations. This suggests that further studies on cell type versus QD cell damage must be conducted, since there is currently not enough information about the discharge of QDs from living organisms or on their long-term toxicity.

6. Ultrasound imaging

Even though ultrasound is accessible, portable and widely available, it has received less attention than other imaging techniques. However, the development of microbubble contrast agents (the intravenous injection of small amounts of air or gas bubbles that enhance the Doppler signal) (30) makes it a competitive alternative as it has enabled many new possibilities, including molecularly-targeted tumor imaging. For example, a lipid-shelled microbubble engineered with a monoclonal antibody to ICAM-1 can be used as an early indicator of atherosclerosis when expressed on the surface of activated endothelial cells, and gives better results as non-targeted microbubbles (31). However, ultrasound microbubble contrast agents have recently been black-labeled by the FDA because of reports of deaths and serious cardiopulmonary reactions following their administration.

7. Conclusion

Antibody engineering has changed significantly in the past decade, making smaller recombinant antibody fragments and engineered variants (like diabodies, triabodies, minibodies and single-domain antibodies) a promising alternative to monoclonal antibodies. With innovative technologies, these engineered antibody fragments retain the targeting specificity of whole mAbs, but are more economically available. In addition, by forging them into multivalent and multispecific reagents or linking them to therapeutic payloads for many diagnostic and therapeutic applications, they can posses other superior properties. As these properties allow them to be used for real-time imaging with higher specificity, they will doubtless become very important in this field and, in years to come, will be indispensable as both clinical and research reagents.

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