INVITED COMMENTARY

Genetically Engineered Antibody Fragments and PET Imaging: A New Era of Radioimmunodiagnosis

The emerging interest in antibody-based pharmaceuticals and diagnostics is evident from the fact that currently 12 antibodies are available in the market as therapeutics (*1*) and another 5 are being exploited for clinical diagnosis of several malignancies. Because antibodies have high specificity toward their cognate antigens, tumor-antigen–specific antibodies are considered to be attractive vehicles for targeted delivery of imaging agents for the diagnosis of both primary and metastatic malignancies. The use of intact antibodies for imaging is hampered, however, by their large molecular size, which leads to prolonged serum half-life. Furthermore, because of the slow diffusion from vasculature into the tumor, intact antibodies take longer for maximal dose deposition in the target tissue (*2*,*3*). The combination of prolonged clearance kinetics and slow tumor uptake results in low radiolocalization indices and high systemic background (*4*). The simplest approach to circumvent these problems is to tailor the antibody molecules either by enzymatic digestion or genetic engineering to yield smaller-molecular-weight fragments with unaltered specificity and improved pharmacokinetics. Enzymatically produced antibody fragments $F(ab)'_2$ and Fab′ exhibit more favorable pharmacokinetics and produce better images when radiolabeled with suitable radionuclide; however, they are tedious to prepare reproducibly.

Antibody engineering is an emerging field that has made it possible to tailor antigen-binding domains into a single polypeptide with a much smaller size than intact immunoglobulin. A single-chain Fv (scFv) recombinant protein for a given monoclonal antibody can be prepared by connecting genes encoding for heavy-chain and light-chain variable regions at the DNA level by an appropriate oligonucleotide linker. The resulting translation product forms a single polypeptide chain with a linker bridging the 2 variable domains. Compared with intact parental antibody (150 kDa), 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 for scFv (*4*). This is primarily due to its monovalent binding nature, which results in lower functional avidity. Divalent and multivalent scFvs have recently been described and result either from the spontaneous dimerization or covalent association of 2 or more monovalent scFvs (*5*). There is increasing evidence that recombinant antibody fragments with di- and multivalent binding with intermediate molecular weight (60–120 kDa) are an ideal compromise between slow-clearing high-localizing immunoglobulins and fast-clearing low-localizing monovalent scFvs (*4*,*6*,*7*).

Diabodies result from noncovalent cross-pairing of scFv fragments, which is mediated by short linker peptides (*8*). With their bivalent binding, they exhibit better tumor deposition than do their scFv counterparts yet clear faster from circulation than do intact antibodies because of their smaller molecular size (55 kDa). Recently, the crystal structure of T84.66 diabody was described, and it appears that this design is suitable for enhanced flexibility and in vivo stability (*9*). The fusion proteins of scFv with the hinge region and C_H3 domain of immunoglobulin molecule are called minibodies (*10*). The hinge region facilitates dimerization mediated by disulphide bond formation and imparts flexibility to the antigen-binding domain. With a molecular weight of 80 kDa, minibodies persist longer than diabodies in the serum, hence allowing higher dose deposition in the tumors (*8*,*10*,*11*).

Advances in nuclear medicine have allowed the development of several imaging modalities that are being used for diagnosis, classification, characterization, preoperative evaluation of the extent of tumor, and postoperative monitoring of recurrence of the various malignancies. These techniques are based on differential uptake of radiolabeled tracers by tumor cells. The resulting signal is detected and computed to reconstruct a planar or tomographic image corresponding to the spatial distribution of tracer. Nuclear imaging has enabled not only morphologic but also metabolic (glucose uptake), antigenic (antibody-mediated), and physiologic (vascular imaging agents, hypoxia, etc.) characterization of tumors.

PET is a powerful imaging technique (*12*) that has been used in the staging and management of several malignancies, including lung cancer, colorectal cancer, melanoma, and lymphoma. Positrons emitted from tracer isotopes collide with the neighboring electrons, after which both particles are annihilated. This results in the re-

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lease of energy in the form of 2 γ-rays of 511 keV, which are emitted in opposite directions and are recorded coincidently by a ring of external nuclear detectors. The signals are computed to reconstruct a tomographic image of the emitting source. The high sensitivity of PET, which is about 10-fold greater than that of conventional SPECT scanners, allows the detection of tracer isotopes at a much lower concentration (10^{-11}–10^{-12} mol/L), and its higher resolution of $(3–9)^3$ mm^3 makes it possible to image tumors of very small dimensions (*12*). Although most of the clinical PET studies (~95%) are based on ^{18}F-FDG, recent interest has emerged to develop specific probes, such as antibodies and receptor ligands. ^{124}I-labeled monoclonal antibodies have been evaluated for colon, breast, and ovarian cancer in animal models (*13–15*) and in humans for breast cancer (*16*). Recently, PET imaging using ^{124}I-labeled anti–vascular endothelial growth factor (VEGF) antibodies was performed to evaluate the utility of VEGF as a target in animal models (*17*) and in a patient group with various solid tumors (*18*). However, because of long serum persistence of intact immunoglobulin, optimal images are obtained 3–10 d after administration, which is a clinical disadvantage.

In the current issue of *The Journal of Nuclear Medicine*, Sundaresan et al. (*19*) describe antigen-specific PET imaging of tumor xenografts in the murine model using engineered antibodies. The authors have combined the high sensitivity and resolution of PET with the high specificity, selectivity, and improved pharmacokinetics of diabody and minibody forms of anti-CEA antibody T84.66 to obtain high-quality images of LS174T xenotransplants. Both minibody and diabody exhibited rapid blood clearance and specific retention in the tumor, which resulted in high ratios of tumor to normal tissue and tumor to background and enabled excellent visualization of the tumors at 18 h after administration. Despite lower absolute accumulation in the tumors, diabody yielded images with good contrast as early as 4 h after injection, primarily because of rapid clearing from the nontarget tissues. On the other hand, minibody exhibited nearly 5-fold-higher peak uptake and took longer to clear from the circulation, resulting in images with good contrast after 18 h after injection. It was possible to image tumors as small as 3 mm in diameter with minibody because of the high sensitivity. Comparing the PET images obtained by ^{18}F-FDG in the same animals, Sundaresen et al. have also established the superiority of a more specific immunoPET over metabolic PET imaging. In a previous study, Wu et al. performed similar studies with minibody using ^{64}Cu as the tracer positron emitter (*20*). Although the decay-half-life of ^{64}Cu (12.7 h) is well suited for molecules exhibiting clearance kinetics similar to those of diabodies or minibodies, the major limitation of the use of copper is its nonspecific accumulation in nontarget tissues, particularly the liver and kidneys. This results in images with a nonspecific background in these tissues and makes copper, as a tracer, ineffectual for imaging hepatic lesions and metastases. PET using ^{18}F-FDG, which images glucose use and accumulation, also suffers from a similar limitation because of the high metabolic activity in the liver, leading to a significant accumulation and causing nonspecific background. Additionally, labeling of antibodies with radiometals involves modification of the molecules by coupling them chemically to chelating agents. This often leads to alteration of the binding characteristics of the antibodies. These difficulties are not encountered when the proteins are labeled with the isotopes of iodine. The decay half-life of ^{124}I (4.2 d) allows sufficient time for quality assessment of the radiolabeled proteins before their administration. Because of the longer physical half-life of ^{124}I and the prolonged background persistence of intact immunoglobulins, ^{124}I-labeled monoclonal antibodies directed against several tumor antigens have been exploited for PET imaging. As mentioned earlier, it usually takes anywhere from 3 to 10 d to image the tumors. In a recent study, bispecific anti-MUC1/anti-Ga chelate antibodies were used for PET imaging of breast cancer (*21*). Though the shorter interval for optimal imaging (~20 h) using the pretargeting approach was comparable to that observed using minibodies (*19*), the former approach involved injections with antibody, blocking agent, and tracer (^{86}Ga) chelate, in contrast to the single injection of the ^{124}I-minibody.

The results of Sundaresan et al. (*19*) have provided evidence that engineered antibody fragments labeled with ^{124}I can serve as excellent tools for rapid imaging of tumors with high specificity and resolution using positron emitters as tracers. Such antigen-specific novel imaging agents can be of immense importance for visualizing tumors that exhibit low metabolic activity and, thus, cannot be visualized by conventional ^{18}F-FDG PET. At present, many tumor-specific antigens have been identified and are being explored as targets for therapy and diagnosis. The best examples include carcinoembryonic antigen, MUC1, TAG-72, VEGF, EGFRvIII, and HER2/neu. Also, many monoclonal antibodies directed against such tumor antigens have been engineered and are available in formats similar to diabody and minibody. With the lower cost and increasing availability of PET scanners in most of the clinical settings, it is time to explore and use these engineered antibodies for the antigen-specific imaging of several classes of tumors (and micrometastases) with high spatial resolution. The compatibility of recombinant antibodies with appropriate positron-emitting tracers has set the stage for the transition of PET from a relatively less specific metabolic imaging modality to a more specific, antigen-based imaging technique.

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