Glypican-3–Targeted ^{89}Zr PET Imaging of Hepatocellular Carcinoma: Where Antibody Imaging Dares to Tread

Recently we have seen an increase in PET imaging of ^{89}Zr-radiolabeled antibodies in preclinical and clinical investigations, particularly in oncology. ^{89}Zr-antibodies are examples of the immuno-PET imaging class, in which antibodies equipped with a PET isotope are detected and quantified in vivo. ^{89}Zr, with a half-life of 78.4 h, is an excellent PET isotope to pair with antibodies to address the limitations of antibodies' long blood circulation, requiring several days to clear from the bloodstream to achieve optimal imaging. The comprehensive groundwork conducted to make ^{89}Zr more widely available, along with published procedures for radiolabeling and quality control, has led to a relatively straightforward set of instructions that can be performed at many radiochemistry centers (*1–4*). Many studies have been conducted with ^{89}Zr-radiolabeled Food and Drug Administration–approved therapeutic antibodies for development as companion diagnostics for their therapeutic counterparts toward a "look before you treat" approach. These immuno-PET tracers illustrate that they still hit their target in well-defined xenograft models, and several have been successful in clinical trials (*5–11*). Now, as with many other imaging construct classes, we have entered the next phase in the development of targeted imaging, to go beyond just hitting the target in subcutaneous tumor models to investigating the imaging characteristics of these agents in complex orthotopic tumor models.

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Antibodies can be raised to be specific for a wide variety of antigens, including ones that are presented on the cell surface, are within the cell, or are secreted. Notably, antibodies can be raised against antigens that either are overexpressed on cancerous tissue or are unique to the cancer cells. Clearly, targeting a uniquely expressed antigen is preferable to eliminate antibody binding to normally expressed antigens. However, one of the many challenges in oncologic imaging is that most of the tumor antigens are overexpressed in cancer cells but are also present in normal tissues.

The study in this issue of *The Journal of Nuclear Medicine* by Sham et al. (*12*) is an excellent example of a highly specific molecular imaging agent. This paper describes the assessment of an ^{89}Zr-labeled monoclonal antibody against glypican-3 (GPC3), a marker of hepatocellular carcinoma (HCC). GPC3 is a transmembrane protein of the heparin sulfate proteoglycan family expressed primarily during gestation (*13*). Mutations in the GPC3 gene are the leading cause of the Simpson-Golabi-Behmel syndrome, an X-linked disease generally characterized by overgrowth (*14,15*), suggesting a role in growth factor and morphogen biology. Sham et al. evaluated ^{89}Zr-labeled monoclonal antibody against GPC3 (^{89}Zr-αGPC3) in xenograft models with orthotopic liver tumors from GPC3-expressing and non–GPC3-expressing cells imaged from 1 to 7 d after injection. Immuno-PET imaging of HCC is a daunting task because the liver is the main clearance organ for most antibodies. Typically, liver uptake values range from 5 to 10 percentage injected dose (%ID)/g (*11,16,17*), which is persistent over several days, resulting in low tumor-to-liver ratios. However, the remarkable accumulation of ^{89}Zr-αGPC3 in HCC tumors—greater than 800 %ID/g—overshadows liver uptake, yielding tumor-to-liver ratios greater than 30 in the larger (3.8-mm-diameter) tumor model. This ratio is substantially higher than that achieved with most other antibody probes, which have tumor-to-liver ratios ranging from 1.5 to 4 (*10,16,18–20*) at their optimal time points after injection of ^{89}Zr-labeled antibody. These values are consistent with ^{89}Zr-αGPC3 uptake in smaller (<1-mm-diameter) tumors of the liver with a tumor-to-liver ratio of 1.49–1.57 at 3–6 d after injection. Furthermore, the ^{89}Zr-labeled antibodies referenced above accumulate in their respective tumors, with either increasing or constant uptake over several days as a function of the residualizing property (one that is trapped in the cell after internalization) of ^{89}Zr, and likely contribute to enhanced contrast as well (*13*). It is interesting to observe that ^{89}Zr-αGPC3 does not follow the same trend as the other ^{89}Zr-labeled antibodies discussed above. In the larger tumor model, there is almost a 2-fold reduction in tumor uptake between 3 and 7 d after injection, from 836.6 and 443.9 %ID/g, respectively, and in the smaller tumor models the uptake is reduced from 42.5 to 21.6 %ID/g between 1 and 7 d after injection. Although more investigations are needed to determine the tumor phenotype of HCC and its relationship to GPC3 expression over time, the work by Sham et al. shows how a carefully designed imaging construct targeting an overexpressed antigen can provide meaningful and interpretable images with excellent contrast.

Sham et al. also further characterize the tumor lesions using histology, which confirms the high expression of this receptor in liver tumors. Interestingly, lesions that were smaller than 1 mm were undetected by histology, suggesting that the sensitivity of this immuno-PET can greatly contribute to the existing diagnostic techniques for HCC, with histology remaining the gold standard (*21–23*). Furthermore, the authors used luciferase-transfected tumor cell lines implanted orthotopically for bioluminescence imaging with luciferin substrate as a secondary imaging modality to confirm tumor growth in the liver independently of PET imaging. They also tested ^{89}Zr-αGPC3 in large, GPC3-negative tumors to determine any enhanced permeability and retention effects notorious for trapping macromolecules such as antibodies. ^{89}Zr-αGPC3 did not accumulate in these tumors, demonstrating the small impact of the enhanced permeability and retention effect in this study. Sham et al. also denatured ^{89}Zr-αGPC3 by

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heating this antibody as an aggregate control, which also did not accumulate in the GPC3-positive tumors of similar size to the xenografts that received nondenatured ^{89}Zr-αGPC3, further confirming the specificity of this probe. Another negative control antibody to consider would be a nonspecific IgG, the same isotype as ^{89}Zr-αGPC3, so that the sizes and the amino acid sequence of ^{89}Zr-αGPC3 would be similar with the exception of the amino acid sequence of the antigen-binding domain. Although large tumors may often develop necrosis that can prevent access of imaging agents, bioluminescence imaging in these luciferase-transfected tumors shows that the luciferin substrate can still access the tumors, demonstrating that the lack of ^{89}Zr-αGPC3 uptake is not due to necrotic tumors. However, future work that investigates blood flow, vascularization, and the presence of hypoxia in these orthotopic tumor models may shed more insight on possible mechanisms of antibody penetration in these complex systems. Additionally, blocking studies in the presence of excess unlabeled ^{89}Zr-αGPC3 in GPC3-expressing tumors resulted in a 16-fold reduction in tumor uptake of ^{89}Zr-αGPC3, further illustrating antigen specificity.

Although the authors state that preliminary in vitro studies showed that this antibody internalized within 24 h, it would be interesting to see the kinetics of this process to examine the role of total number of receptors versus internalization on tumor uptake. Such an examination would follow a similar vein to looking at "presence–number of receptors" versus "function–internalization" of the target as has been discussed with respect to imaging with small molecules (*24*)—in this case, "function" of the target to take up more of the imaging agent. Greater characterization of the target such as the rate of receptor recycling will provide valuable information especially for advancing this probe as an antibody–drug conjugate for therapy if ^{89}Zr-αGPC3 internalization is receptor-mediated.

Lastly, this study illustrates the potential use of antibodies that may be highly specific for their target but may have no known therapeutic function. Indeed, this study shows that this antibody can be used as a noninvasive imaging agent with potential to be used for therapy as an antibody–drug conjugate. Other humanized or fully human antibodies targeting GPC3 are being investigated for therapy as a single agent or in combination with chemotherapy (*25*). It would be interesting to compare these antibodies with ^{89}Zr-αGPC3 as discussed by Sham et al. Perhaps the targeting of receptors without blocking of downstream signaling pathways may even be advantageous, because the cell is less likely to become resistant as is the case with therapeutic antibodies.

Now with several clinical trials moving forward, we are seeing the advancement of immuno-PET (*9,26*). These new compounds may be used for identification of micrometastasis, stratification of patients for response to radioimmunotherapy, and validation of patient response to therapy. If clinical utility of radiopharmaceuticals such as this one can be realized, we are truly on the way to personalized medicine.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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