

PET Imaging of GRP Receptor Expression in Prostate Cancer

In the United States alone, 1 in 6 men will be diagnosed with prostate cancer over the course of their lifetimes, with 31,000 dying each year from the disease. Imaging prostate cancer remains a major challenge in nuclear medicine. ^{18}F -FDG, the most commonly used radiotracer for the detection of tumors, is not helpful in identifying primary prostate tumors because of the slow glucose metabolism and attendant low cellular uptake of such lesions (1). However, unlike the primary tumors, metastases can be more metabolically active in prostate cancer and the use of ^{18}F -FDG may be warranted. Accordingly, as with other imaging techniques applied to prostate cancer, ^{18}F -FDG imaging will likely be used primarily in patients who have had radical, local therapy and present with a rising prostate-specific antigen (PSA) level (2).

Other positron-emitting radiotracers—such as ^{18}F -choline, ^{11}C -choline, ^{11}C -acetate, and ^{18}F -fluorodihydrotestosterone—have recently been introduced as imaging agents for the detection of prostate (and other) tumors. Clinical evaluation of those radiotracers is presently underway (3–5).

The only receptor-based prostate cancer imaging agent currently approved for clinical use is ProstaScint, an ^{111}In -labeled monoclonal antibody to the prostate-specific membrane antigen (PSMA) (6). This SPECT agent has proved difficult to use in standard clinical practice—that is, without anatomic coregistration—and controversy surrounds its clinical utility (7). Other radiolabeled monoclonal antibodies to PSMA are being explored and one has recently en-

tered clinical trials (8). Nevertheless, improvements in prostate tumor imaging are badly needed.

A different approach to prostate cancer imaging is based on targeting neuropeptide receptors that are overexpressed on a variety of carcinomas. An important discovery in 1999 was that the bombesin (BN)/gastrin-releasing peptide (GRP) receptor is overexpressed on prostate cancer cells (9). Moreover, GRP receptor overexpression was noted only in human neoplastic prostate tissue; it was not seen in prostate hyperplasias (10). Several high-affinity BN analogs, radiolabeled with $^{99\text{m}}\text{Tc}$ and ^{111}In , have recently been developed for γ -ray scintigraphy of GRP-positive tumors and have been tested in animals and humans. Various BN analogs have also been labeled with β -emitting radionuclides (^{177}Lu , ^{188}Re , ^{131}I , and ^{149}Pm) for potential radiotherapeutic application (11).

Although other GRP receptor-based SPECT imaging agents for prostate cancer have been developed (12), last year Rogers et al. (11) introduced the radiolabeled BN analog ^{64}Cu -DOTA-Aoc-BN(7–14) (DOTA is 1,4,7,10-tetraazadodecane-*N,N',N'',N'''*-tetraacetic acid; Aoc is 8-aminooctanoic acid) as the first such radiopharmaceutical for PET. microPET images showed good tumor localization of ^{64}Cu -DOTA-Aoc-BN in a PC-3 xenograft mouse model. As noted by the authors, relatively high retention in normal tissues may, however, prevent the clinical application of that particular BN radiotracer.

In this issue of *The Journal of Nuclear Medicine*, on pages 1390–1397, Chen et al. (13) report on the synthesis and pharmacologic evaluation of another ^{64}Cu -labeled BN analog—that is, ^{64}Cu -DOTA-[Lys³]-BN for targeting GRP receptor expression in prostate cancer. The results of studies in GRP receptor-positive, androgen-independent (AI), PC-3

human prostate cancer cells and in vivo in PC-3 solid tumors in a mouse model showed ^{64}Cu -DOTA-[Lys³]-BN accumulation to be specific and saturable. Clear delineation of the tumors was achieved because of high tumor-to-background ratios. By contrast, binding of ^{64}Cu -DOTA-[Lys³]-BN to androgen-dependent (AD) CWR-22 tumors appeared to be nonspecific. ^{64}Cu -DOTA-[Lys³]-BN accumulation in normal tissues—particularly in the liver—was lower than that of ^{64}Cu -DOTA-Aoc-BN, and clearance from the body was faster and occurred predominantly via urinary excretion. Whether ^{64}Cu -DOTA-[Lys³]-BN will be applied in clinical imaging studies will depend to a certain degree on the outcome of future dosimetry estimates. Overall, the article presents an important step toward the development of a PET agent for noninvasive localization of GRP receptors in human prostate cancer and may be a marker for AI disease.

There are, however, some shortfalls in this investigation that limit the validity of the conclusions drawn by the authors—in particular, with regard to the degree of tracer binding to AI PC-3 tumors compared with that in AD CWR-22 tumors.

In vitro receptor binding and tracer internalization studies were performed in PC-3 tumor cells only and not in the CWR-22 cells. A clear differentiation between ^{125}I -[Tyr⁴]-BN binding in the 2 cell types would have confirmed the differences in specific versus nonspecific binding that were observed in vivo in the biodistribution studies.

A major concern with this and other studies that attempt to image small animals with receptor-based PET radiopharmaceuticals is the fact that the carrier that is invariably introduced may begin to occupy a significant portion of tumor receptors in the mouse. In this case, microPET and quantitative autoradiographic receptor (QAR) imaging

Received Mar. 4, 2004; revision accepted Mar. 19, 2004.

For correspondence or reprints contact: Ursula Scheffel, ScD, Department of Radiology, The Johns Hopkins Medical Institutions, 2202 Deerpark Crescent, Baltimore, MD 21209.

E-mail: uscheffel@erols.com

studies were performed with doses of ^{64}Cu -DOTA-[Lys³]-BN (14.8 MBq [400 μCi]; approximately 100 μg BN/kg) that were able to block partially radiotracer binding to the GRP receptors on the PC-3 tumors (and in other GRP receptor-rich areas such as the pancreas). Therefore, the difference in uptake between AI (PC-3) and AD (CWR-22) tumors was not remarkable (Fig. 7 in (13)). Nevertheless, effective blocking of receptor binding by additional carrier (10 mg BN/kg) was demonstrated by microPET in the PC-3 tumor model (Fig. 8 in (13)). Unfortunately, negative controls for blockade were not presented (for the CWR-22 tumor model).

The question of the low-capacity, saturable characteristics of GRP receptors for the radiotracer in the PC-3 tumor model should be investigated further. Saturation of the GRP receptors with the radiotracer must be avoided to optimize tumor detection and to quantify receptor density. Radiotracer preparations with a specific activity that is considerably higher than 18.5 GBq/ μmol (500 mCi/ μmol) will be needed to perform quantitative microPET studies in small animals as well as for future clinical studies. The need to use particularly high specific activity tracers for imaging receptors in small laboratory animals with dedicated small animal PET scanners has been discussed eloquently by Hume et al. (14).

Another question concerns the PC-3 tumor model itself and the influence of blood flow to the tumor on the binding of ^{64}Cu -DOTA-[Lys³]-BN. Rogers et al. (11) found that the number of GRP receptors in solid PC-3 tumors is much lower than was expected from the quantity of receptors measured on single tumor cells in vitro. In addition, blood flow to the PC-3 tumor has been shown to be low, thus limiting tracer diffusion to the tumor and its subsequent localization (11). An alternative GRP receptor-containing prostate tumor model with adequate blood flow may be helpful in future studies with radiolabeled BN analogs.

An additional concern involves the fact that the washout of ^{64}Cu radioactivity from the PC-3 tumors in vivo occurred at a relatively fast rate (Fig. 4 in (13)), although the radiotracer demonstrated high levels of tumor cell internalization (Fig. 3 in (13)). As suggested by Chen et al. (13), further investigations into the metabolism of the radiotracer, as well as possible modification of the BN analog to achieve prolonged tumor retention, will be necessary before clinical application can be realized.

The question as to which peptide agents may be best for imaging AI prostate cancers has not been resolved. The suitability of BN-like compounds for imaging GRP receptors in AI prostate cancer has yet to be substantiated. BN and its analogs promote the growth of AI prostate cancers (through an AI-independent mechanism) (15). The mechanism for GRP-mediated tumor growth in AI disease is not clear but is being investigated (16). On the other hand, Chen et al. (13) claim that androgen receptor-based agents cannot be used to study AI disease. Although that seems logical, and is true in the context of antiandrogen therapy, there may be exceptions. A recent article by Isaacs and Isaacs (17) stated that a 2- to 5-fold increase in androgen receptor messenger RNA is the only change in gene expression consistently associated with AI disease. As a result, cells become supersensitive to androgen, rather than independent of it, suggesting that they may bind increased levels of androgen receptor-based radiopharmaceuticals. Clearly, more work is needed to understand fully receptor expression on AI cancer cells. Only then can successful imaging and therapeutic agents for AI disease be realized.

Ursula Scheffel, ScD

Martin G. Pomper, MD, PhD

*The Johns Hopkins Medical Institutions
Baltimore, Maryland*

REFERENCES

1. Liu JJ, Zafar MB, Lai YH, Segall GM, Terris MK. Fluorodeoxyglucose positron emission tomography studies in diagnosis and staging of clinically organ-confined prostate cancer. *Urology*. 2001;57:108–111.

2. Chang CH, Wu HC, Tsai JJ, Shen YY, Changlai SP, Kao A. Detecting metastatic pelvic lymph nodes by ^{18}F -2-deoxyglucose positron emission tomography in patients with prostate-specific antigen relapse after treatment for localized prostate cancer. *Urol Int*. 2003;70:311–315.
3. Kotzerke J, Gschwend JE, Neumaier B. PET for prostate cancer imaging: still a quandary or the ultimate solution? *J Nucl Med*. 2002;43:200–202.
4. Liu A, Dence CB, Welch MJ, Katzenellenbogen JA. Fluorine-18-labeled androgens: radiochemical synthesis and tissue distribution studies on six fluorine-substituted androgens, potential imaging agents for prostate cancer. *J Nucl Med*. 1992;33:724–734.
5. Larson SM, Morris M, Gunther I, et al. Tumor localization of $^{16}\beta$ - ^{18}F -fluoro-5 α -dihydrotestosterone versus ^{18}F -FDG in patients with progressive, metastatic prostate cancer. *J Nucl Med*. 2004;45:366–373.
6. Haseman MK, Rosenthal SA, Polascik TJ. Capromab pentetide imaging of prostate cancer. *Cancer Biother Radiopharm*. 2000;15:127–130.
7. Sodee DB, Faulhaber PF, Nelson AD, Bakale G. The prognostic significance of indium-111-capromab pentetide. *J Clin Oncol*. 2004;22:379–380.
8. Yao D, Trabulsky EJ, Kostakoglu L, et al. The utility of monoclonal antibodies in the imaging of prostate cancer. *Semin Urol Oncol*. 2002;20:211–218.
9. Reile H, Armatis PE, Schally AV. Characterization of high-affinity receptors for bombesin/gastrin-releasing peptide on the human prostate cancer cell lines PC-3 and DU-145: internalization of receptor bound ^{125}I -(Tyr⁴)bombesin by tumor cells. *Prostate*. 1994;25:29–38.
10. Markwalder R, Reubi JC. Gastrin-releasing peptide receptors in the human prostate: relation to neoplastic transformation. *Cancer Res*. 1999;59:1152–1159.
11. Rogers BE, Bigott HM, McCarthy DW, et al. MicroPET imaging of a gastrin-releasing peptide receptor-positive tumor in a mouse model of human prostate cancer using a ^{64}Cu -labeled bombesin analogue. *Bioconjug Chem*. 2003;14:756–763.
12. Hoffman TJ, Gali H, Smith CJ, et al. Novel series of ^{111}In -labeled bombesin analogs as potential radiopharmaceuticals for specific targeting of gastrin-releasing peptide receptors expressed on human prostate cancer cells. *J Nucl Med*. 2003;44:823–831.
13. Chen X, Park R, Hou Y, et al. microPET and autoradiographic imaging of GRP receptor expression with ^{64}Cu -DOTA-[Lys³]-bombesin in human prostate adenocarcinoma xenografts. *J Nucl Med*. 2004;45:1390–1397.
14. Hume SP, Gunn RN, Jones T. Pharmacological constraints associated with positron emission tomographic scanning of small laboratory animals. *Eur J Nucl Med*. 1998;25:173–176.
15. Yashi M, Muraishi O, Kobayashi Y, Tokue A, Nanjo H. Elevated serum progastrin-releasing peptide (31-98) in metastatic and androgen-independent prostate cancer patients. *Prostate*. 2002;51:84–97.
16. Lee LF, Guan J, Qiui Y, Kung HJ. Neuropeptide-induced androgen independence in prostate cancer cells: roles of nonreceptor tyrosine kinases Etk/Bmx, Src, and focal adhesion kinase. *Mol Cell Biol*. 2001;21:8385–8397.
17. Isaacs JT, Isaacs WB. Androgen receptor outwits prostate cancer drugs. *Nat Med*. 2004;10:26–27.