VPAC1 targeted ^{64}Cu-TP3805 PET imaging of prostate cancer: preliminary evaluation in man

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Abstract

Objectives—To evaluate ^{64}Cu-TP3805 as a novel biomolecule, to PET image prostate cancer (PC), at the onset of which VPAC1, the superfamily of G-protein coupled receptors, is expressed in high density on PC cells, but not on normal cells.

Methods—25 patients undergoing radical prostatectomy were PET/CT imaged preoperatively with ^{64}Cu-TP3805. Standardized uptake values (SUVmax) were determined, malignant lesions (SUV > 1.0) counted, and compared with histologic findings. Whole mount pathology slides from 6 VPAC1 PET imaged patients, 3 BPH patients, one malignant and one benign lymph node underwent digital autoradiography (DAR) after ^{64}Cu-TP3805 incubation and compared to H&E stained slides.

Results—In 25 patient PET imaging, 212 prostate gland lesions had SUVmax > 1.0 vs.127 lesions identified by histology of biopsy tissues. The status of the additional 85 PET identified prostate lesions remains to be determined. In 68 histological slides from 6 PET imaged patients, DAR identified 105/107 PC foci, 19/19 HGPIN, and ejaculatory ducts and verumontanum involved with cancer.

Additionally, DAR found 9 PC lesions not previously identified histologically. The positive and negative lymph nodes were correctly identified and in 3/3 BPH patients and 5/5 cysts, DAR was negative.

Conclusion—This feasibility study demonstrated that ^{64}Cu-TP3805 delineates PC in vivo and ex vivo, provided normal images for benign masses, and is worthy of further studies.

Keywords

PET Imaging; Prostate Cancer; VPAC1 Receptors; Cu-64-TP3805; Diagnosis

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Introduction

Prostate cancer (PC) is the most common non-skin cancer in men. In 2015, in North America alone, there will be 220,800 new cases of PC and 27,540 men will die of it[1]. PC is also increasing worldwide[2]. There is considerable controversy over PSA based screening for PC, with no consistent recommendations from major medical organizations on the best approach to screening [3,4]. While many biomarkers are in development to identify PC, non-invasively using blood and urine assays, the definitive diagnosis of PC relies on the histologic identification of cancer cells on invasive prostate tissue biopsy[5]. The standard biopsy procedure, transrectal ultrasound (TRUS) with 10 to 12 needle cores of the prostate gland, can be associated with morbidity, and over 2/3 of the time may not identify all malignant lesions[6,7].

Radiologic examination such as TRUS, computerized x-ray tomography (CT), multiparametric magnetic resonance imaging (MRI), and nuclear scans such as single photon emission computerized tomography (SPECT) and positron emission tomography (PET), using such radiopharmaceuticals as, In-111-ProstaScint™, F-18-FDG and C-11-Choline, are available, but suffer from limitations[8]. Therefore, there is a compelling need for continuation of the development of a biomolecule, which will detect PC and its metastatic lesions with high sensitivity and specificity.

Recent approaches to imaging PC have generally been directed to targeting prostate-specific membrane antigen (PSMA)[9–12]. We have chosen to target VPAC1, which belongs to the superfamily of G-protein coupled surface receptors that are expressed in high density on certain cancer cells, including PC cells at the onset of oncogenesis and prior to the alterations in cell morphology[13,14]. VPAC1 receptors (combined for vasoactive intestinal and pituitary adenylate cyclase activating peptide, PACAP) are involved in cell proliferation, cell differentiations, and survival of PC cells, and are overexpressed in cancer of the prostate, breast, bladder, and lungs[13]. On stroma, normal cells and benign masses, VPAC1 receptors are minimally present[13–19].

We hypothesized that a radiolabeled biomolecule with a high affinity for VPAC1 would not only image PC, but also distinguish malignant lesions from benign prostatic hyperplasia (BPH) and contribute to the management of patients on surveillance. To validate this hypothesis, a large body of preclinical data has been generated in our laboratory[20–24]. This included PET imaging of spontaneously grown PC in TRAMP (Transgenic adenocarcinoma of the mouse prostate) mice that mimicked the pathophysiology of human PC[23] and a feasibility study of PET and PEM (positron emission mammography) imaging of breast cancer (BC) in humans, on which the VPAC1 receptors are also expressed in high density[24].

The biomolecule we chose to target VPAC1 receptors was chosen out of four such compounds designed and evaluated extensively in our laboratory[20–24]. It consists of 28 amino acid PACAP analogue that is conjugated to a N_2S_2 (diaminedithiol(N_2S_2-Benzoyl)$_2$) chelating agent at the C terminus of the peptide and labeled with ^{64}Copper (t ½ = 12.8 hrs.), a positron emitting (β^+, 19%, 656 KeV) radionuclide produced using a cyclotron. The agent was named ^{64}Cu-TP3805. Our preclinical evaluation demonstrated that ^{64}Cu-TP3805 has

not only a strong affinity ($K_d = 3.1 \times 10^{-9}$M) for VPAC1, is receptor specific, and is stable in vivo, but also has <2% urinary excretion, a virtue favorable for imaging PC[23]. In this article, we describe our preliminary findings in PET imaging of 25 males known to have PC and that were scheduled for radical prostatectomy. Our in vivo data were substantiated by using digital autoradiography (DAR) on whole mount histologic slides from 6 PET imaged patients. In addition, DAR was also performed on slides obtained from three BPH patients not imaged by PET and two excised lymph nodes from institutional tissue bank.

Materials and Methods

TP3805 Synthesis and Kit Preparation

The peptide was synthesized and kits were prepared as described previously[24]. The 28-amino acid peptide, the amino acid sequence of which is published previously, was synthesized by American Peptide Co (Sunnyvale, CA) on a Wang resin, purified and characterized by electrospray mass spectroscopy[20]. The peptide stock (M.W. 3805) was stored at $-80°C$, 20μg peptide kits under sterile N_2 were prepared aseptically, and stored at $-10°C$ until use[24]. Stability of the kits was checked by their ability to label them with ^{64}Cu as measured by high-pressure liquid chromatography (HPLC).

Preparation of ^{64}Cu-TP3805

On the day of preparation, a kit vial was removed, brought to room temperature, and a required quantity of ^{64}Cu solution (Washington University, St. Louis, MO) was added to the vial (usually 222 MBq, 6 mCi in 30μl of 0.1 M HCl), followed by the addition of 220 μl of sterile water. The vial was incubated at 50°C for ninety minutes. The solution was then diluted for I.V. injection by the addition of 3 ml sterile 0.9% NaCl.

The radiochemical purity was determined by HPLC, with reverse phase Zorbax 300SB.C18, 4.6 mm × 250mm column (Agilent), eluted with a linear 23 min. gradient from 0% to 100% acetonitrile in 0.1% aqueous TFA in 7 min and then to 90% in 15 min; ran for 23 min. (Labeling efficiency of 95% was considered as the criterion for kit stability). This procedure rendered a ^{64}Cu-TP3805 specific activity of 44.4 GBq (1.2 Ci/μmol). Sterility of each preparation was determined as described previously[24].

Patient Inclusion

Exploratory investigational new drug (eIND) number 101550 was assigned by the Food and Drug Administration (FDA). Approvals were also obtained, prospectively, from the Institutional Review Board (IRB), Clinical Cancer Research Review Committee (CCRRC), and Radioactive Drug Research Committee (RDRC). Patients (n=25, 6 African Americans, 19 Caucasian, age ranges: 44–77 yrs., mean 63.4±7.6 yrs.) known to have PC and scheduled for radical prostatectomy (Gleason score 6 to 8), and who signed informed consent form, were recruited in consecutive order and imaged 1–3 weeks prior to the surgery. Prior to imaging, each patient underwent US guided, 12 core needle biopsy, 4 each from the apex, midsection, and base, each subdivided into four subsections: L lateral, L medial, R medial, and R lateral. Standard H&E staining was performed and Gleason score was assigned.

Statistical analysis assumed that there will be three PC lesions per patient and ^{64}Cu-TP3805 will correctly detect 80% of the lesions, with 87% power.

PET/CT Imaging

PET/CT images were obtained in supine position with a 4 min. bed time using a Biograph-6 PET/CT scanner (Siemens, Inc.). For ^{64}Cu-TP3805 imaging, patients neither fasted nor had their blood glycemic levels determined. ^{64}Cu-TP3805 was injected I.V. (148±10% MBq, 4±10% mCi) through an indwelling catheter. The quantity of ^{64}Cu was determined by the dose escalation study performed previously[24] and was approved by the FDA. Whole body scans were then obtained at 30 min. and 2 hr. after injection. The 30 min. time was based upon our breast cancer imaging study, in which optimal uptake was observed in 30 min. post injection[24]. Following I.V. administration, each patient was carefully observed, during and after the imaging procedure, by monitoring and recording their vital signs. Twenty-four hours later, each patient was also contacted for any delayed adverse events.

Image Analysis

All PET/CT images were read by two board certified nuclear medicine physicians (SK,CI) who determined standardized (maximum) uptake values (SUVmax) in 12 sectors (4 each of the apex, middle, and base) of each prostate as seen on the PET image. These sections approximated the standard biopsy sites.

Digital Autoradiography (DAR)

From all PET imaged patients, prostatectomy specimens received fresh from the operating room were fixed in neutral-buffered 10% formalin solution for 24 hours. Post fixation, the glands were serially sectioned at 4 mm from apex to base. Tissue sections were processed for paraffin infiltration and embedded to construct whole mount tissue slices. The paraffin blocks were sectioned at 3μm and mounted on large format glass slides, dried at 60°C, de-paraffinized and stained with hematoxylin and eosin. The stained slides were examined microscopically and areas of tumor were mapped. Using paraffin blocks from six of the PET imaged patients, slides were cut in duplicate. From each of these six PET imaged patients, 9–15 deparaffinized 3μm thick histological sections (n=66) were incubated at room temperature with ^{64}Cu-TP3805 solution, washed thoroughly with phosphate buffered saline (PBS), dried and subjected to 15 sec. digital autoradiography (DAR, Imaging 4000, Kodak/Bruker Inc.). These slides were then H&E stained and foci were marked by a urologic pathologist (PM, RB) as PC, benign, cystic, or prostatic intraepithelial neoplasia (PIN). In addition, three histological slices each from 3 BPH patients (n=9), from one malignant lymph node (n=3) and from one benign lymph node (n=3) were also examined with DAR followed by H&E staining. The histologic findings were then analyzed with the autoradiographic findings for match or mismatch.

Results

Peptide and Kit Stability

As determined by mass spectrometric analysis, TP3805 was stable for more than 3 years. The kits were stable for longer than 18 months and labeled ^{64}Cu with >95% efficiency[20–24].

All preparations were sterile. The total ^{64}Cu dose remaining in the syringe and the intravenous line averaged less than 5.5 MBq (150 μCi). Following administration of ^{64}Cu-TP3805, none of the patients had any adverse events of any kind.

PET Image Analysis

PET images acquired both at 30 min. and 2 hours post injection of ^{64}Cu-TP3805 revealed liver uptake and mild GI uptake on 2 hour images. Although none of the organ distribution data were quantified in humans, in animal tissue distribution data, liver uptake was 25.4 ± 1.74% at 4 hours post injection, and that 7% of radioactivity was eliminated in 24 hours via feces[22]. During 24 hours post injection, urinary uptake in mice was <2%[22]. Consistent with this, no bladder uptake of ^{64}Cu was noted in any image in any of the patients (Fig. 1). For malignant PC lesions, generally, no significantly or consistently greater SUVs were noted at 2hr images than those acquired at 30 min. post injection.

PET images in all patients revealed more than one malignant lesion in the prostate. In one patient, a lymph node was delineated by the ^{64}Cu-TP3805 PET scan (Fig. 1C).

The lymph node was dissected and was found to be malignant by histology. There were no known distant metastatic bony lesions in any of the patients, and none were detected by the PET scan. In two patient (Fig. 1B & 1C), ^{64}Cu activity was noted in iliac crest. The bone scans, performed, 2 weeks before were negative.

SUVmax for lesions in each of the 12 biopsy sectors, for each patient, were determined, the range for which was 0.7–8.8. These were corroborated with the sectional histology results. Lesions with SUVmax ≤ 1.0 were considered normal (n=88). In 25 patients, PET images identified more lesions (n=212) with SUVmax >1.0, than the lesions histologically considered to be malignant (n=127, Table 1).

DAR for histological tissue examination is a well-established technique[13]. The DAR data from 6 patients using 66 histological slides, each of which were H&E stained post-DAR, read by a pathologist, marked with a color code, and compared visually, provided better insight into the greater number of malignant lesions seen on PET images with >1.0 SUVmax than determined by histology of core biopsy (Table 2).

Table 2 shows that, in 66 slices, there were a total of 107 PC foci, as determined by histology (Fig. 2). Out of these, 105 (98%) were identified by DAR. DAR missed 2 (1.8%) PC lesions due to technical artifact. For 3 BPH patients without PC (Fig. 3A and B), DAR was negative. The malignant lymph node and benign lymph node (Fig. 3C and D) were correctly identified by DAR. Additionally, 9 (8.5%) small PC lesions not previously noted by histology were identified by DAR. Furthermore, DAR identified 19 lesions corresponding to high grade prostatic intraepithelial neoplasia (HGPIN) (Fig. 4A and B), 2 ejaculatory duct and 5 urethra verumontanum not previously noted by histology. For 5 intraprostatic cysts, DAR was negative (Fig. 4C)

Comment

Although great strides have been made over the past few years to diagnose PC with minimally invasive procedures, ultrasound guided transrectal prostate biopsy for histologic examination of prostate tissue is considered the gold standard approach[7].

As the controversy due, primarily to, the non-specificity of the PSA screening for early detection of PC continues, the need is even greater to have reliable tools that can provide information about the patient's disease. The anatomical diffusion MR reliably images PC with Gleason score 7 and when combined with T_2 weighted imaging, PC lesions with Gleason score 6 can also be imaged with high sensitivity. In the era of molecular imaging, novel blood and urine markers and a large number of novel radiopharmaceuticals have been developed[8–12,25–29]. ProstaScint™ (^{111}In-capromab pendetide) is a radiolabeled murine monoclonal antibody against intracellular epitope of PSMA, initially approved in 1996, has the largest published clinical data to date. ProstaScint™ binds to an intracellular portion of PSMA, reducing its ability to image living tumor cells[8]. A recent study concluded that ProstaScint™ can be used to detect lymph node metastases from PC but the test had limited utility in diagnosing tumors in the prostate gland[8]. Another agent is ionic $^{64}CuCl_2$ shown to be taken up in human PC grown in experimental mice[25]. The uptake is thought to be mediated by human Copper Transporter 1 (hCtr1). A newer agent ^{68}Ga labeled Prostate Specific Membrane Antigen (PSMA) ligand, in humans, demonstrated high sensitivity, but low specificity, and presents both false positive and false negative results[26,27]. Anti-3-[(18)F]FACBC is a relatively new tracer primarily studied in the setting of recurrent disease. In comparison, the detection for extraprostatic recurrence with this agent was superior to ProstaScint™ but at present, studies of this F-18 agent in the primary detection setting are limited[30]. Although results of these investigations have generally been encouraging, much remains unknown about their applicability in routine clinical practice.

We have chosen to target VPAC1 receptors with ^{64}Cu-TP3805, the early evaluation of which demonstrated that it has the ability to target only malignant, but not normal or benign lesions, indicating a virtue of much needed specificity. ^{64}Cu-TP3805 has a high affinity ($Kd=3.1\times10^{-9}M$) for VPAC1, which is over-expressed in high density on PC cells at the onset of oncogenesis. Furthermore, ^{64}Cu-TP3805 has excellent (97%) stability in mouse and human serum. Urine collected in metabolic cages, at 4 hrs. and 24 hrs. post-injection, $98\pm1.8\%$ and $85\pm3\%$ of ^{64}Cu, respectively, was associated with TP3805. Its firm stability in vivo was also evident by receptor blocking studies (tumor uptake, unblocked $5.78\pm0.66\%$ ID/g vs. blocked $1.84\pm0.44\%$ ID/g, n=25, P<0.01)[21]. These characteristics, together with its repeated ability to correctly image only malignant breast cancer, but not benign, lesions in MMTV neu transgenic mice and in humans, as well as spontaneously grown PC in TRAMP mice, and the lack of its urinary excretion, prompted us to hypothesize that ^{64}Cu-TP3805 would permit us to image PC early and accurately, and perhaps allow one to distinguish PC from other non-malignant conditions[21–24]. The objective here was to perform a feasibility study to determine if ^{64}Cu-TP3805 shall image known PC, a multifocal disease, with high efficacy.

The SUVmax, determined from PET images, indicated that ^{64}Cu-TP3805 imaged more lesions in vivo than those observed by histology of biopsy tissues. Since the routine histological examinations were performed using 3μm thick slices, cut from a few of the 4 mm thick interval sections, it is conceivable that some of the PET positive PC foci may not have been detectable by histology. This observation needs to be confirmed using further histologic examination of several slices obtained from whole mount excised prostate tissues. However, the in vitro data from six of the PET imaged patients, in whom we performed DAR and corroborated its findings with whole mount histologic examinations of the same slides ex-vivo, lead us, in part, to the validation of our in vivo imaging data, in that, not only did ^{64}Cu-TP3805 imaging identify all malignant lesions, but it also identified the HGPINs, the ejaculatory ducts, and urethra verumontanum, but not the BPH or cysts (Fig. 2–4). If such a trend persisted in the remaining 19 patients, it at least in part, may explain the additional number of malignant lesions observed in the prostate by ^{64}Cu-TP3805 PET imaging. Detection of HGPIN is consistent with early expression of VPAC1 prior to the modulation of cell morphology. Although this probable sensitivity could be a problem in the patient management, HGPIN has a high predicative value as a marker for adeno carcinoma. Similarly, ejaculatory ducts and urethra verumontanum (UV), in which PC cells are present, were also seen by DAR, but not cysts in which VPAC1 expression does not exist (Fig. 4C). DAR was positive for malignant lymph node, but was negative for normal lymph node (Fig. 3C&D). However, the reasons for the bone marrow uptake seen in a patient (Fig. 1) with negative bone scan are not yet clear.

These data consistently demonstrated positive results for malignant lesions and negative results for benign tissues indicating high specificity of ^{64}Cu-TP3805 to image malignant lesions, but not benign masses. However, these observations need to be evaluated on a large number of patients. The data are also consistent with our previous observations in which we have targeted VPAC1 biomarker to image breast cancer in humans using ^{64}Cu-TP3805[24]. Although preliminary and need to be substantiated in a large number of cases with different prostatic conditions, ^{64}Cu-TP3805 identified more lesions by PET imaging than seen by histology (212/127, Table 1) in 25 patients and by DAR than the corresponding H&E staining (43+32=75/43 Table 2). These data exceeded our goal of 80% positivity and provided us with real insight into imaging many malignant and precancerous PC lesions and rendered ^{64}Cu-TP3805 worthy of further evaluation in imaging PC early and accurately.

Conclusion

There is a compelling need for a biomolecule that will image PC, early and accurately, but not benign prostate conditions, and that will minimize the number of unnecessary biopsies, reduce patient anxiety, and reduce health care cost. Although our data are preliminary, they demonstrated that ^{64}Cu-TP3805 delineated PC and HGPIN lesions, with excellent specificity for VPAC1, and call for further studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Glossary

PET	Positron Emission Tomography
CT	X-Ray Computerized Tomography
SUV	Standardized Uptake Value
BPH	Benign Prostatic Hyperplasia
DAR	Digital Autoradiography
HGPIN	High Grade Prostatic Intraepithelial Neoplasia
PSA	Prostate Specific Antigen
ED	Ejaculatory Duct
PSMA	Prostate Specific Membrane Antigen
UV	Urethra Verumontanum
H&E	Hematoxylin and Eosin
GI	Gastrointestinal Tract
HPLC	High Pressure Liquid Chromatography
CCRRC	Clinical Cancer Research Review Committee
IRB	Institutional Review Board
FDA	Food and Drug Administration
RDRC	Radioactive Drug Research Committee
TRUS	Transrectal Ultrasound
MRI	Magnetic Resonance Imaging
SPECT	Single Photon Emission Computerized Tomography
PC	Prostate Cancer
BC	Breast Cancer
TRAMP	Transgenic Adrenocarcinoma of the Mouse Prostate
PEM	Positron Emission Mammography

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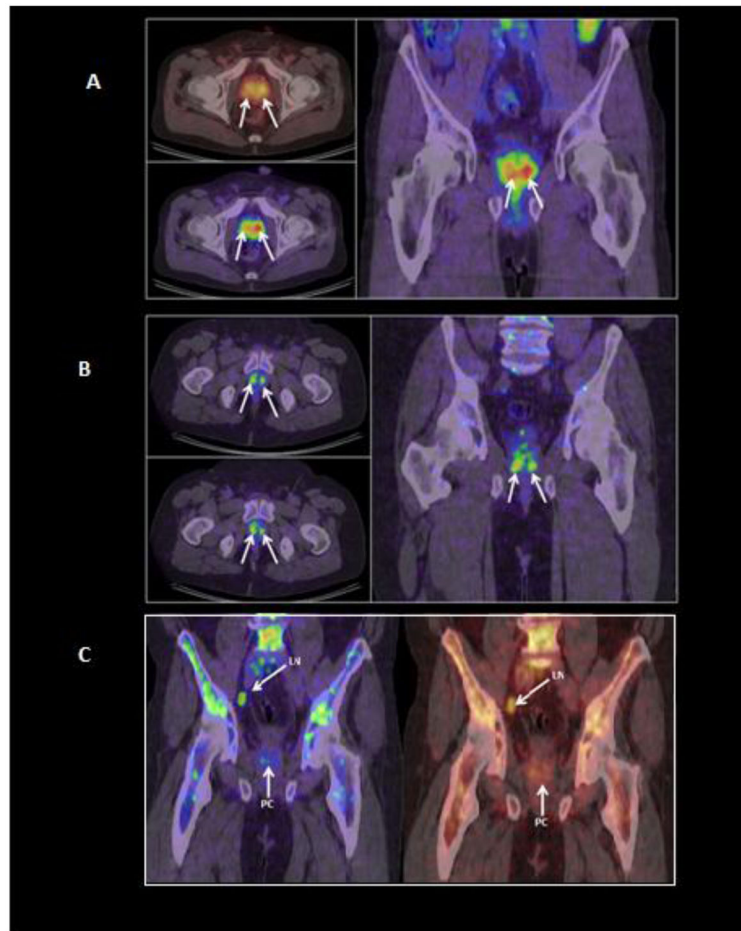
Fig. 1.
A composite of cross-sectional and coronal PET/CT images of two patients (1A: 68 yrs, Gleason score 7. Fig. 1B: 51 yrs, Gleason score 6) obtained 30 min. post-injection of 4±10% ^{64}Cu-TP3805. Multiple PC lesions are delineated (arrow). Radioactivity in the bladder is negligible. Some bone marrow uptake is visible. A Tc-99m-MDP bone scan was negative. Fig. 1C: A 58 yr old male with PC (Gleason score 7) received 4mCi ^{64}Cu-TP3805, 30 min. prior to PET/CT imaging. The coronal images in different colors demonstrated a lymph node (LN) and prostate cancer (PC). The lymph node was dissected and found to be malignant by histology. Bone marrow uptake of ^{64}Cu-TP3805 is apparent. A standard of care Tc-99m-MDP bone scan was negative for metastatic bone lesions.
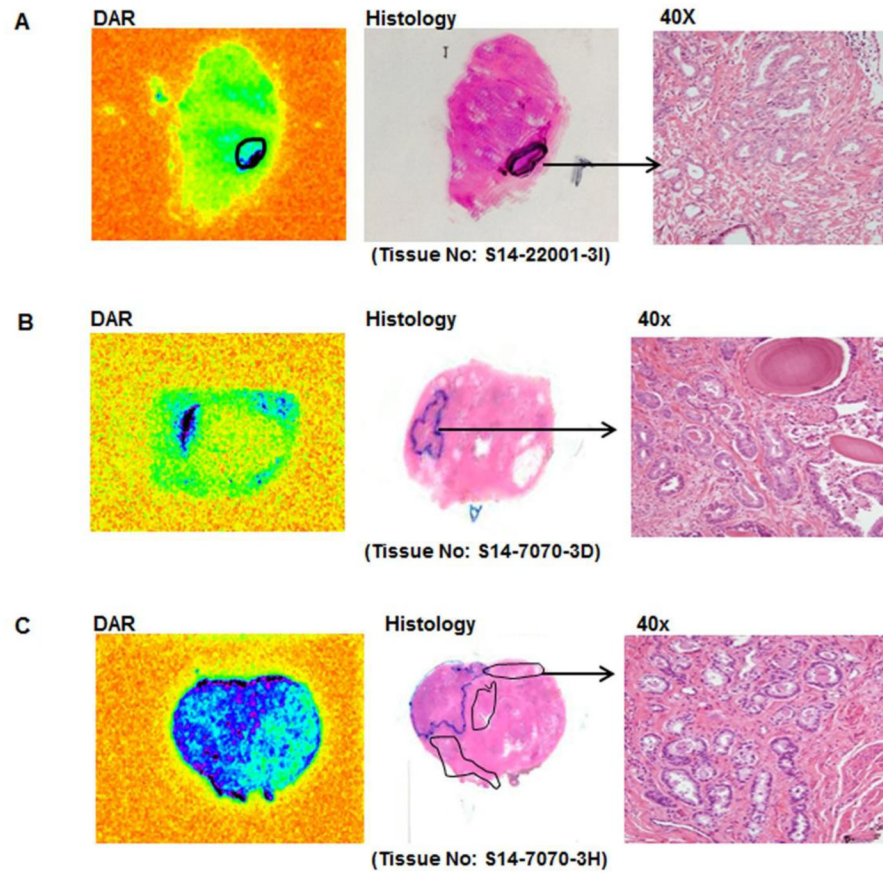
Fig. 2.
Examples of digital audioradiography (DAR) and H&E stained of the same slide (histology as marked by the pathologist) are shown for two different patients (2A is patient #1, and 2B & 2C are patient #2) who received ^{64}Cu-TP3805 prior to radical prostatectomy. The malignant lesions are marked on histology slides and corroborate well with DAR.
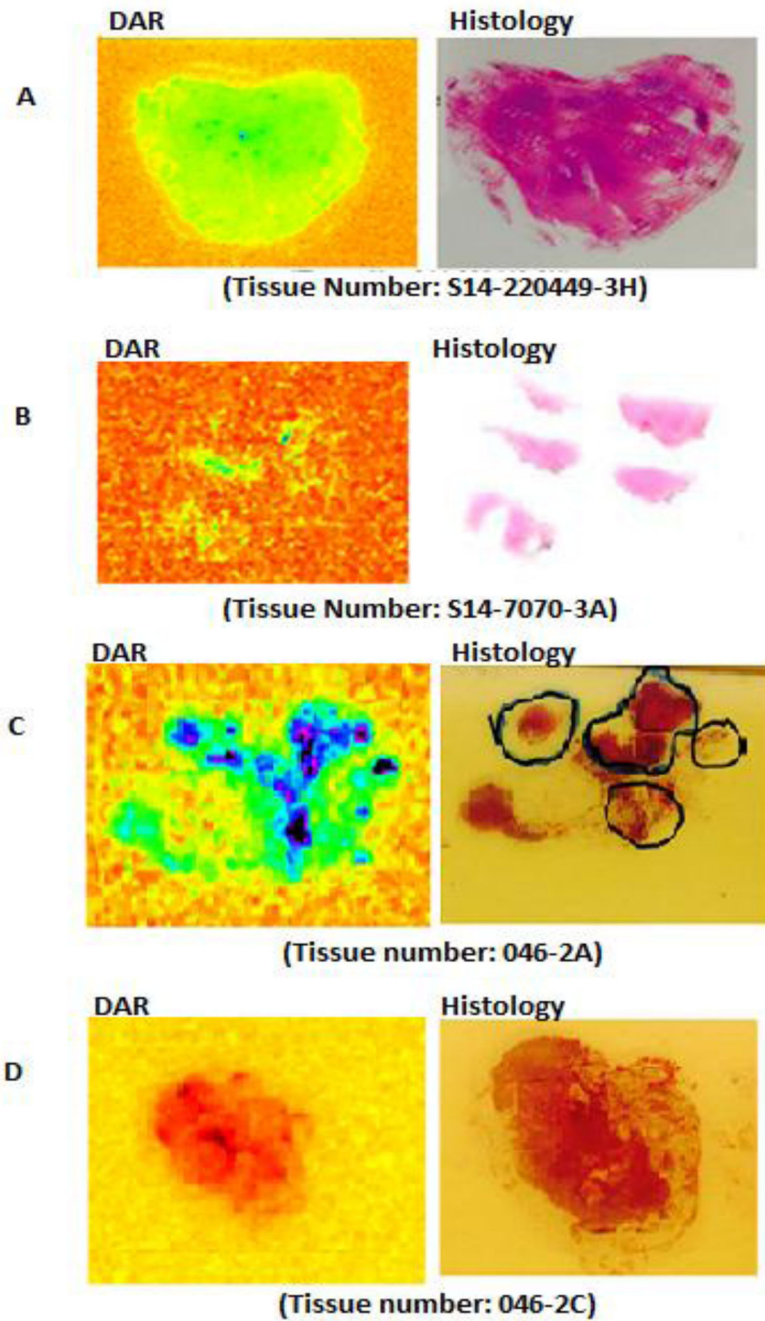
Fig. 3.
A and B show the absence of ^{64}Cu radioactivity on the slides examined from three separate patients with benign prostatic hyperplasia (BPH) as determined by Histology of the same three slides. Examples of histologically, delineated malignant lymph node (3C on the right) and benign lymph node (3D on the right). Note the uptake of ^{64}Cu-TP3805 in the malignant lesions (3C on the left) but not in the benign node (3D on the left).
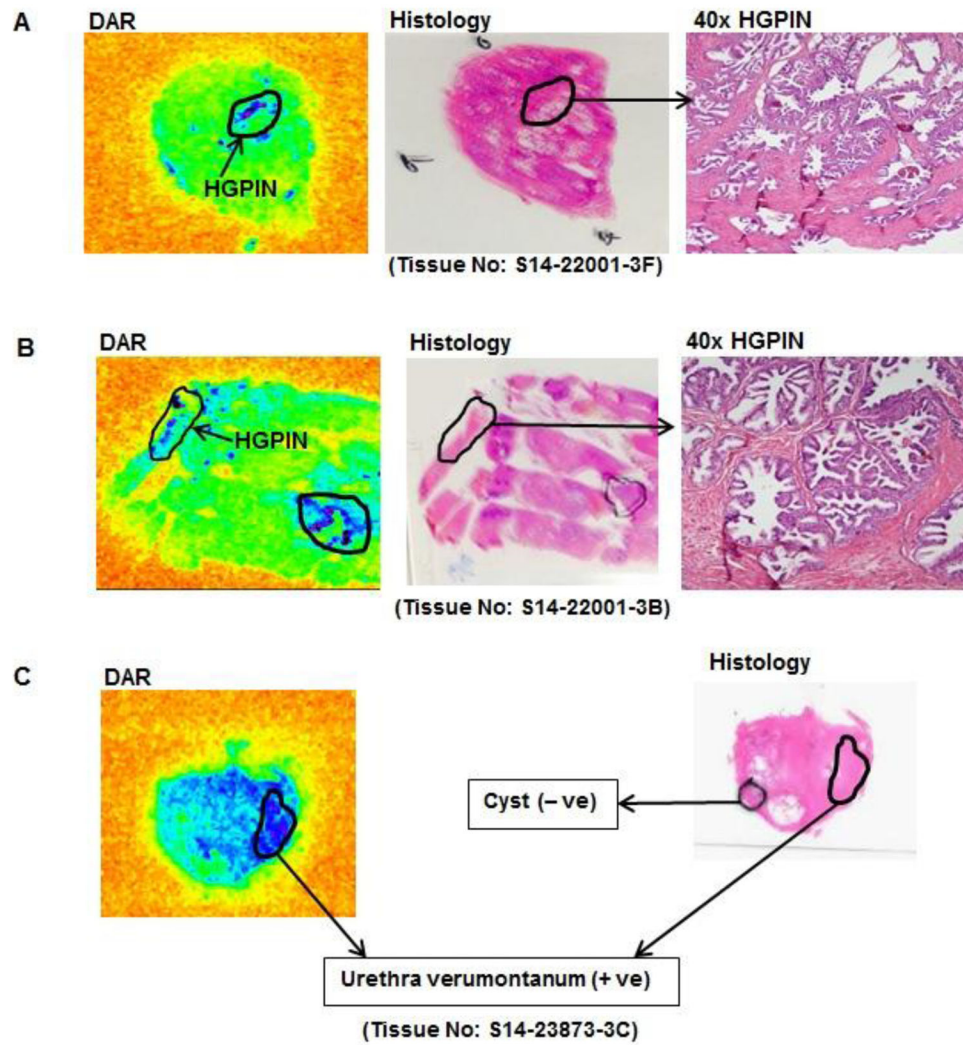
Fig. 4.
Digital audioradiography (DAR) of histological tissues from three separate patients demonstrates the ^{64}Cu-TP3805 uptake on the lesions of HGPIN (4A and 4B) and on urethra verumontanum (4C), but not on the cyst (4C) where VPAC1 receptors are not expressed (all lesions were marked by the pathologist).