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Title: Correlation of PSMA-targeted ^{18}F -DCFPyL PET/CT Findings with Immunohistochemical and Genomic Data in a Patient with Metastatic Neuroendocrine Prostate Cancer

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Introduction

Prostate cancer (PCa) remains the second-leading cause of cancer death in Western men.¹ At the present time, the most commonly used imaging methods to detect sites of metastatic PCa are x-ray computed tomography (CT) and bone scintigraphy with ^{99m}Tc-methylene diphosphonate. In an effort to improve upon the sensitivity and specificity of these tests, many in the field have pursued the development of molecularly-targeted imaging agents that utilize positron emission tomography (PET).^{2,3} Currently, the most promising PET radiotracers for PCa imaging target prostate-specific membrane antigen (PSMA), a type II membrane glycoprotein that is highly expressed on PCa epithelial cells.⁴ A potential drawback of PSMA-targeted imaging of PCa, however, is that tumors with neuroendocrine differentiation do not reliably express PSMA,⁵ thus providing a potential source of false negative tests. Here we report a case in which PSMA-targeted PET/CT imaging results were compared with lesion-specific genomic and histologic data, advancing our understanding of this imaging modality in the context of neuroendocrine PCa.

Case Presentation

We report the case of a 69-year-old man who presented in December 2012 with persistent gross hematuria. Cystoscopy demonstrated tumor in the prostatic urethra, and digital rectal examination revealed an indurated and fixed prostate consistent with clinical stage T4 PCa. Serum PSA was 0.7 ng/ml, and prostate biopsy confirmed Gleason score 5+4=9 cancer in 6 of 12 cores. Staging imaging with CT and bone scan demonstrated no evidence of metastatic disease and so in April 2013 the patient underwent a robotic radical prostatectomy at an outside institution. Surgical pathology revealed adenocarcinoma with extraprostatic extension and

negative lymph nodes (pT3aN0). Initial post-surgical serum PSA was undetectable, but PSA was detected at 0.24 ng/mL six months following surgery. CT and bone scan in October 2013 demonstrated multiple vertebral lesions consistent with metastases, and treatment was initiated with leuprolide, bicalutamide, and sipuleucel-T. In November 2014, radium-223 was added due to PSA progression. Subsequent imaging demonstrated multiple lesions of the spine, liver, peritoneum, and left cerebellar hemisphere. Biopsy of the spinal and peritoneal lesions were determined to be metastatic PCa. The patient next underwent stereotactic body radiation therapy of the spinal lesions and initiated docetaxel, which was then substituted with carboplatin and etoposide due to neuropathy. He then developed a pathologic fracture of the left ileum requiring surgical fixation. Follow-up magnetic resonance imaging in October 2015 revealed widespread metastatic disease.

The patient next elected to enroll in a study of PSMA-targeted ^{18}F -DCFPyL PET/CT. Interestingly, the patient's metastatic lesions demonstrated highly variable radiotracer uptake on this exam (Figure 1). Overall, the patient's pattern of metastatic disease was highly atypical with peritoneal, liver, and cerebellar parenchymal lesions. A pelvic lesion was highly avid (lean body mass corrected $\text{SUV}_{\text{max}} = 10.2$), a sternal lesion was moderately avid ($\text{SUV}_{\text{max}} = 3.3$), peritoneal lesions had moderate avidity ($\text{SUV}_{\text{max}} = 3.1$), and the liver lesions had no perceptible radiotracer uptake. A lesion in the right cerebellar hemisphere also demonstrated mild to moderate radiotracer uptake ($\text{SUV}_{\text{max}} = 1.7$, Figure 2). The patient subsequently underwent core-needle biopsy of the peritoneal and liver lesions. Histology revealed metastatic adenocarcinoma consistent with the known prostate primary and poorly differentiated metastatic carcinoma with neuroendocrine features, respectively. Due to the patient's poor response to conventional

therapies and findings consistent with a neuroendocrine phenotype, we elected to perform genomic sequencing and immunohistochemical (IHC) staining of the tissue obtained from the liver biopsy to potentially identify therapeutic targets.

Genomic data revealed that the liver lesion harbored the *RBI* mutation E413X (nonsense; mutant fraction 53%) and *TP53* mutation I195T (missense; mutant fraction 85%); alterations consistent with neuroendocrine differentiation.^{6,7} Moreover, IHC staining for PSMA and the androgen receptor (AR) were negative, and staining for synaptophysin was strongly positive (Figure 3). The peritoneal lesion was also largely negative for PSMA and AR, and showed overexpression of p53 protein. In contrast, the moderately PET-avid peritoneal lesion was negative for synaptophysin and positive for NKX3.1 and CD56 (data not shown), findings consistent with a degree of neuroendocrine differentiation but with retained features of adenocarcinoma.

Following imaging the patient underwent stereotactic radiotherapy of his cerebellar lesion, radio-embolization of the liver lesions, and continued systemic therapy with carboplatin and etoposide. CT imaging in March 2016 showed mild progression of bony and liver metastases, an enlarging omental mass, and new pelvic lymphadenopathy. The patient was subsequently transitioned to low-dose etoposide and cyclophosphamide.

Discussion

PSMA is overexpressed in >90% of local and distant PCa lesions,^{8,9} making it an ideal molecular target for imaging PCa.⁴ Initial clinical experience with ¹⁸F-DCFPyL, a fluorinated urea-based small molecule targeting PSMA, has yielded promising results to this point.^{10–13} One potential

pitfall of PSMA imaging is in the setting of neuroendocrine differentiation, whereby PSMA expression is downregulated on tumor cells.⁵ This relationship was invoked to explain the absence of radiotracer uptake in one recent case report of imaging neuroendocrine PCa with a gallium-68 labeled PSMA-targeted PET radiotracer, although that case lacked tumor immunohistochemistry or genomic data.¹⁴ In the present case, we have demonstrated a direct relationship between radiotracer uptake on ¹⁸F-DCFPyL PET/CT imaging and the intensity of PSMA expression on immunohistochemical staining. Specifically, we obtained tissue from a peritoneal metastasis with moderate radiotracer uptake and a liver metastasis with no uptake, and we confirmed that PSMA was moderately expressed in the peritoneal metastasis and not expressed in the liver metastasis.

Genomic analysis of the liver lesion further supports the association of PSMA downregulation with neuroendocrine differentiation, as the liver lesion harbored mutations of *RBI* and *TP53*, alterations consistent with known pathways of neuroendocrine progression.^{6,7} Interestingly, additional staining of the peritoneal lesion implicated these pathways as well. IHC revealed overexpression of p53 protein, likely due to an underlying missense mutation (such as I195T) stabilizing the protein, and loss of cyclin d1 protein, likely secondary to *RBI* loss-of-function (such as E413X).¹⁵ In light of this, the peritoneal metastasis appears to be clonally related to that biopsied from the liver. At the same time, the peritoneal lesion was negative for synaptophysin and positive for CD56 (data not shown), findings consistent with adenocarcinoma. Thus, features of the peritoneal lesion suggest a primary adenocarcinoma undergoing transition to the neuroendocrine phenotype.¹⁶

Although PSMA-targeted imaging has several advantages relative to conventional modalities for PCa detection, our findings support the hypothesis that neuroendocrine differentiation of PCa results in downregulation of PSMA expression and thus absence of radiotracer localization in vivo. As such, care must be taken in evaluating advanced tumors or those with neuroendocrine differentiation. Suspected cases may necessitate continued use of conventional imaging in combination with emerging modalities when expected findings are not well-established. Alternatively, heterobivalent ligands targeting both PSMA and neuroendocrine markers may prove most useful, although such radiotracers are not yet clinically available.

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Figure Legends

Figure 1. Axial PSMA-targeted ^{18}F -DCFPyL PET/CT images showing A) a liver metastasis without radiotracer uptake and B) a peritoneal metastasis with moderate radiotracer uptake.

Figure 2. Right cerebellar hemisphere lesion suspected to represent metastatic prostate cancer as demonstrated on A) axial ^{18}F -DCFPyL PET/CT image with focal radiotracer uptake that correlates to a lesion with associated vasogenic edema on B) an axial T2-weighted fat saturation magnetic resonance (MR) image and enhancement on C) an axial T1-weighted post-contrast MR image.

Figure 3. Immunohistochemical staining of the patient's primary tumor, peritoneal metastasis and liver metastasis. Moderate staining for PSMA was observed in the primary tumor and peritoneal metastasis. In contrast, the liver metastasis had no perceptible PSMA staining. Both the androgen receptor (AR) and cyclin D1 (CCND1) were found to be expressed in the primary tumor but subsequently lost in the two metastases. The pattern of staining for CCND1 suggests that the *RBI* gene was intact in the primary lesion and that loss occurred early in the development of metastases. Synaptophysin (a marker of neuroendocrine differentiation) was only found in the liver metastasis. Finally, p53 over-expression consistent with *TP53* mutation was observed in both the primary tumor and metastases, suggesting the possibility that the observed mutation occurred as an early event in the course of the patient's disease.

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Primary Tumor
Adenocarcinoma

Peritoneal Metastasis
Adenocarcinoma

Liver Metastasis
Neuroendocrine PCa

H&E

PSMA

AR

CCND1

Synaptophysin

p53