

Research Paper

Expression of Prostate-specific Membrane Antigen (PSMA) in Papillary Renal Cell Carcinoma - Overview and Report on a Large Multicenter Cohort

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Abstract

Prostate specific membrane antigen (PSMA) is an emerging diagnostic and therapeutic target in prostate cancer. ⁶⁸Ga-PSMA-labeled hybrid imaging is used for the detection of prostate primary tumors and metastases. Therapeutic applications such as Lutetium-177 PSMA radionuclide therapy or bispecific antibodies that target PSMA are currently under investigation within clinical trials. The expression of PSMA, however, is not specific to prostate-tissue. It has been described in the neovascular endothelium of different types of cancer such as breast cancer, and clear cell renal cell carcinoma (ccRCC). The aim of this study was to analyze PSMA expression in papillary RCC (pRCC) type 1 and type 2, the most common non-ccRCC subtypes, and to evaluate the potential of PSMA-targeted imaging and treatment in pRCC. Formalin-fixed, paraffin-embedded tissue samples of primary tumors were analyzed for PSMA expression

by immunohistochemistry. Out of n=374 pRCC specimens from the multicenter PANZAR consortium, n=197 pRCC type 1 and n=110 type 2 specimens were eligible for analysis and correlated with clinical data. In pRCC type 1 PSMA staining was positive in 4 of 197 (2.0%) samples whereas none (0/110) of the pRCC type 2 samples were positive for PSMA in this large cohort of pRCC patients. No significant PSMA expression was detected in pRCC. Reflecting current clinical evaluation of PSMA expression in RCC do not encourage further analysis in papillary subtypes.

Key words: prostate specific membrane antigen; papillary renal cell carcinoma; kidney cancer

Introduction

Papillary renal cell carcinoma (pRCC) is the second most common type of RCC according to the current (2016) classification of the World Health organization (WHO) and attributes to approximately 10-15% of RCC cases (1, 2). It is divided into two subtypes, type 1 and type 2. Type 2 tumors are associated with unfavorable clinical outcomes compared to type 1 pRCC (3-5). Comprehensive molecular characterization revealed that alterations in the MET (mesenchymal-epithelial transition) gene are a common feature of type 1 pRCC whereas type 2 pRCC is a heterogeneous disease with many subtypes. In pRCC type 2 mutations in SETD2, fumarate hydratase (FH) and CKDN2A silencing as well as TFE3 fusions have been described (6).

Prostate specific membrane antigen (PSMA; glutamate carboxypeptidase II) is a type 2 transmembrane glycoprotein that consists of three parts: an 18 amino acid intracellular part, an 24 amino acid transmembrane part, and an 707 amino acid external part (7). PSMA is located on the short arm of chromosome 11 and has *in vitro* neuropeptidase and folate hydrolase activity (8). Initially, PSMA was considered to be exclusively expressed in prostatic tissue. An upregulation of PSMA in prostate cancer specimens can be observed and there is growing evidence that androgen deprivation drives this increase of PSMA (9). The extent of immunohistochemical PSMA expression in prostate cancer tissue is correlated to tumor uptake on ⁶⁸Ga-PSMA positron emission tomography (PET)/ computer tomography (CT) (10). ⁶⁸Ga-PSMA-labeled hybrid imaging is nowadays widely used in Europe and Australia to

detect recurrent prostate cancer as it outperforms conventional imaging techniques (11, 12). Although not currently approved, PSMA directed radioligand therapies with Lutetium-177 (¹⁷⁷Lu-PSMA-617; ¹⁷⁷Lu-PSMA-I&T) or Actinium-225 (²²⁵Ac-PSMA-617) or others are under investigation within clinical trials and are also used off-label for treatment of refractory metastatic prostate cancer (13-19).

Despite its name PSMA is not specific to prostate tissue. Physiologic expression was described in salivary glands, proximal renal tubules, brain, and small intestine tissues (20, 21). PSMA expression has been found in the neovascular endothelium of several types of cancer, e.g. breast cancer, colorectal cancer, non-small cell lung carcinoma, and RCC (21-24). Among RCC samples, PSMA expression has been studied in larger cohorts of clear cell RCC (ccRCC). Only small sample sizes of non-ccRCC were included in those analyses and results were inconclusive. Within ccRCC PSMA expression was found to be increased in vena cava tumor thrombi compared to renal tumor mass suggesting a potential mechanism for progression and malignant neovascularization (25).

Small case series have reported promising results of PET/CT in RCC patients using different PSMA directed radiotracers potentially outperforming conventional imaging modalities (26-29). Clinical trials are underway exploring the use of ⁶⁸Ga-PSMA-labeled hybrid imaging for patients with PSMA positive tumors other than prostate cancer (Table 1).

Table 1. Overview of prospective clinical trials (diagnostic) targeting Prostate Specific Membrane Antigen (PSMA) in Renal Cell Carcinoma.

NCT identifier	Tumor entity	Tracer, mode of imaging	Trial phase	Status
NCT03427476	Metastatic RCC	¹⁸ F-CIT1057 PET/CT or MRT	1	Completed
NCT02687139	Clear cell RCC	¹⁸ F-DCFPyL PET/CT	1	Completed, (30)
NCT03387514	Metastatic clear cell RCC	¹⁸ F-DCFPyL PET/CT	2	Recruiting
NCT03073395	Metastatic RCC	⁶⁸ Ga-P16-093 PET/CT	1	Recruiting
NCT02978586	breast cancer, lung cancer, and other tumor types know to express "PSMA"	⁶⁸ Ga-PSMA PET/CT	-	Recruiting
NCT03453528	Advanced/metastatic solid tumors	⁶⁸ Ga-PSMA PET/CT	-	Recruiting
NCT03073395	Metastatic RCC	⁶⁸ Ga-P16-093 PET/CT	1	Recruiting
NCT03841760	PSMA-expressing non-prostate tumor	¹⁸ F-DCFPyL PET/CT or ⁶⁸ Ga-PSMA-11 PET/CT	2	Recruiting
NCT04147494	RCC, solid cancer	⁶⁸ Ga-FAPI-46 PET/CT or ⁶⁸ Ga-PSMA PET/CT	1	Recruiting

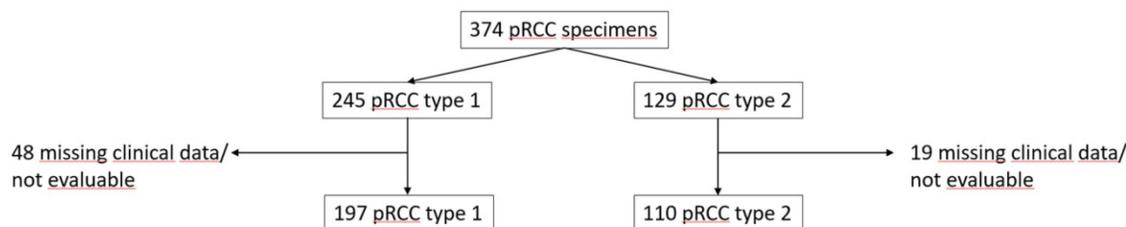


Figure 1. Consortia diagram of PANZAR cohort.

The aim of our investigation was to determine the expression of PSMA in pRCC type 1 and 2 and to evaluate PSMA as a potential diagnostic or therapeutic target in these RCC subtypes.

Material and Methods

Patient cohort

Routine kidney surgery due to kidney tumor was performed between 1994 and 2007. A total of 374 pRCC type 1 and 2 specimens, 245 (65.5%) type 1 and 129 (34.5%) type 2, from the multicentric PANZAR consortium were analyzed (Figure 1).

Primary tumor samples were perceived from the PANZAR contributing partners (in alphabetical order: Erlangen, Heidelberg, Herne, Homburg, Mainz, Mannheim, Marburg, Münster, LMU Munich, TU Munich and Regensburg). Detailed clinical data of the cohort have been previously published (31). For the analysis of PSMA expression samples of $n=307$ patients were available. Papillary subtype and pathological TNM staging were determined by an experienced uropathologist (AH). For each case, the papillary subtype was defined according to 2004 WHO tumor classification, and pathological TNM was performed according to the 2002 TNM classification. Grading was performed according to ISUP and WHO. Papillary type 1, or type 2 RCC were defined as previously reported (32). Patients and pathological data were accessed retrospectively. The study was performed according to the standards established in the Declaration of Helsinki and in concordance with each local ethic committee recommendations.

Tissue preparation and Immunohistochemistry

One representative area of the pRCC tumors was selected to construct tissue microarrays. PSMA expression was determined by immunohistochemistry (IHC). 2 μm TMA slides were stained for PSMA (Anti-PSMA, clone 3E6, M3620, DAKO, dilution 1:20) with a fully automated Dako Autostainer (Dako, Agilent pathology systems). The antibody is established in routine pathological diagnostic. Antigen retrieval was accomplished at

pH=7.2. The bound antibody was visualized with an HRP-conjugated secondary antibody (Dako) and the diaminobenzidine chromogen (Dako). Sections were briefly rinsed in tap water, counterstained with Mayer's Hematoxylin solution and then mounted. All stained tissue samples were assessed in a blind way by pathologists (FE, AH). The evaluation was performed under a Leitz ARISTOPLAN light microscope (Leica Microsystems, Germany) with a $\times 10$ eyepiece, a 22-mm field of view and $\times 40$ objective lens (Plan FLUOTAR $\times 40/0.70$). All microarrays were stained in the same batch, and positive and negative controls were included according to the antibody manufacturer's instructions. Prostate cancer tissue was used as positive control. The immunohistochemical results were reported as staining intensity and percentage of positively staining cells following the immunoreactive score. The staining reaction was classified as positive ($\geq 1\%$).

Statistical Analyses

Descriptive statistics were performed with SPSS v25 (Armonk, NY, USA). Two-sided p-values below 0.005 were considered statistically significant.

Results

Tissue microarrays of $n=307$ patients with pRCC type 1 ($n=197$ (64.2%)) and type 2 ($n=110$ (35.8%)) were eligible for analysis. Clinical and pathological parameters of the analyzed cohort are displayed in Table 2.

In pRCC type 1 PSMA staining was positive in 4/197 (2.0%) of specimens, and in none (0.0%) of type 2 specimens (Figure 2A and 2B). In these four samples intensity of PSMA staining was strong in almost 100% of the area. PSMA staining was detected primarily in the cell membrane and cytoplasm of tumor cells. Furthermore, a weak staining reaction was detected in single endothelial cells of the neovasculature in these cases. Therefore, we used a binary cutoff system with positive or negative staining reaction.

In all four PSMA positive tumors stage was pT1 cN0 cM0 and grading was G2. No significant association between clinicopathological parameters nor follow up parameters and PSMA positivity were observed (Table 2, outcome parameters not shown).

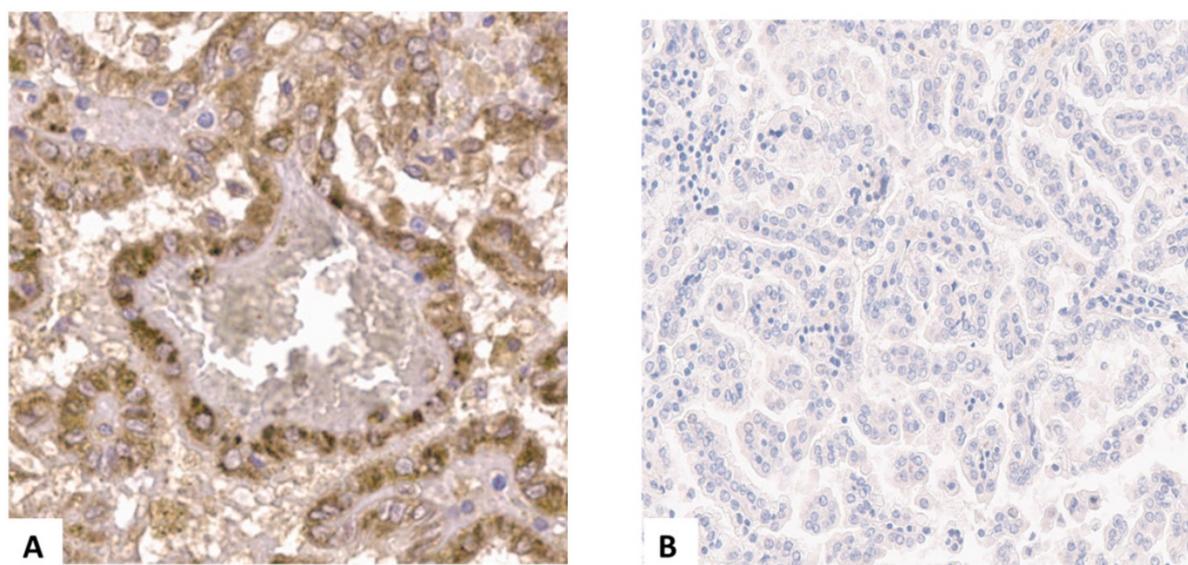


Figure 2. (A) Positive prostate specific membrane antigen (PSMA) staining in a papillary renal cell carcinoma type I specimen. **(B)** negative PSMA staining in a papillary renal cell carcinoma type I specimen.

Table 2. Clinical and pathological parameters of the analyzed cohort.

Variable	pRCC Type 1 PSMA ⁻ n=193 (98.0%)	pRCC Type 1 PSMA ⁺ n= 4 (2.0%)	p-value	pRCC Type 2 PSMA ⁻ n=110 (100.0%)	pRCC Type 2 PSMA ⁺ n=0 (0%)	p-value
Age [years] median (range)	63.0 (15-86)	66.5 (57-77)	p=.400	66.0 (18-85)		
NE (n)	34	0		26		
Sex			p=.816			
Male, n (%)	126 (65.3)	3 (75.0)		64 (58.2)		
Female, n (%)	32 (16.6)	1 (25.0)		20 (18.2)		
NE, n (%)	35 (18.1)	0 (0.0)		26 (23.6)		
TNM Stage			p=.382			
pT1, n (%)	106 (54.9)	2 (50.0)		37 (33.6)		
pT2, n (%)	35 (18.1)	2 (50.0)		14 (12.7)		
pT3, n (%)	17 (8.8)	0 (0.0)		30 (27.3)		
pT4, n (%)	0 (0.0)	0 (0.0)		1 (0.9)		
NE, n (%)	35 (18.1)	0 (0.0)		28 (25.5)		
Grade			p=.367			
G1, n (%)	45 (23.3)	0 (0.0)		9 (8.2)		
G2, n (%)	107 (55.4)	4 (100.0)		52 (47.3)		
G3, n (%)	4 (2.1)	0 (0.0)		19 (17.3)		
G4, n (%)	0 (0.0)	0 (0.0)		0 (0.0)		
NE, n (%)	37 (19.2)	0 (0.0)		30 (27.3)		
Lymph node metastasis #			p=.751			
N-, n (%)	187 (94.9)	4 (100.0)		93 (84.5)		
N+, n (%)	6 (3.1)	0 (0.0)		17 (15.5)		
NE, n (%)	0 (0.0)	0 (0.0)		0 (0.0)		
Distant metastasis#			p=.819			
M-, n (%)	153 (79.3)	4 (100.0)		65 (59.1)		
M+, n (%)	2 (1.0)	0 (0.0)		14 (12.7)		
NE, n (%)	38 (19.7)	0 (0.0)		31 (28.2)		
Locally Advanced disease			p=.471			
T1/T2 N0M0, n (%)	138 (71.5)	4 (100.0)		48 (43.6)		
T3/4 and/or N+ and/or M+, n (%)	18 (9.3)	0 (0.0)		31 (28.2)		
NE, n (%)	37 (19.2)	0 (0.0)		31 (28.2)		

at time of renal surgery. Legend: N- = lymph node status unknown or tumor cells absent from regional lymph nodes, N+ = regional lymph node metastasis present, NE = not evaluable.

Discussion

PSMA expression by immunohistochemistry has been explored for many non-prostatic tumor types and among them RCC. Diagnostic, as well as therapeutic trials are ongoing in RCC addressing PSMA as target. In non-malignant renal tissue PSMA

is found in the proximal renal tubules (20, 23). Upregulation of PSMA in the neovasculature of ccRCC samples has been described whereas the overall expression level in RCC is low (21, 22, 33). Since biology of ccRCC and non-ccRCC is different, data on PSMA expression in non-ccRCC, like pRCC is important to evaluate, to address the potential role of

PSMA in this subtype of RCC.

Data on PSMA expression in pRCC has been reported, however reported sample sizes are considerably small, and results are inconclusive. Spatz et al. report on 257 RCC samples of all subtypes analyzing PSMA expression in the vasculature of renal primary tumors, wherein 22 (8.6%) papillary subtypes were included (24). Herein, in particular PSMA expression was found in 82% of the ccRCC specimen and only in 13.6% of the pRCC specimen (n=3; weakly positive (n=2), strongly positive (n=1)). An association with PSMA expression and clinicopathological risk parameters was primarily found for ccRCC only, not in non-ccRCC. Al-Ahamdie et al. investigated 75 nephrectomy specimens, among them 15 pRCC samples (22). Whereas ccRCC specimen showed a diffuse (24/30, 80%) and mostly a strong (25/30, 83%) PSMA staining pattern, in pRCC specimen no diffuse PSMA expression and only focal expression (11/15, 73%) with minimal strong expression (5/15, 33%) was identified. Among all RCC subtypes analyzed ccRCC showed the most, non-ccRCC lesser and pRCC the least intense and extensive PSMA expression. No association of clinicopathological parameters and PSMA expression was found in pRCC, neither in type 1 nor 2. Kinoshita et al. analyzed 18 renal tumor samples. While 11 samples were PSMA negative, they found a moderate staining intensity in 2 of 2 pRCC and 2 of 9 ccRCC samples and a weak PSMA staining in 3 of 9 ccRCC samples (21). Baccala et al found that positive PSMA staining was detectable in 76.2% of ccRCC, 31.2% of chromophobe RCC, 52.6% of oncocytoma, and 0% of pRCC samples (23). Evangelista et al give an overview on PSMA PET imaging in RCC (34) that has been used in small case series or proof-of-concept trials. While for ccRCC results indicate that further usage should be explored no statement can be made regarding pRCC due to small sample size.

PANZAR is the largest pRCC cohort reported to date. To elucidate whether PSMA might play a role in pRCC, due to the heterogenous data about this histological subset we analyzed PSMA expression on tissue microarrays of 197 patients with pRCC type 1 and 110 patients with pRCC type 2. Our analysis showed a rare expression in pRCC type 1 and no PSMA expression in pRCC type 2 samples. Accordingly, no association with clinicopathological parameter could be observed. Our results are not consistent with all previously published data. However, in concordance to prior reports e.g. (23), we could strongly underline a limited impact of PSMA expression in pRCC. Differences in amount of positivity among the different analyses remain elusive, but limitations of all analyzes were the same:

methodology of IHC, different ages of specimen cohorts, as well as their retrospective character. A further limitation is the use of tissue microarrays, because only a small part of tumor can be analyzed. This small section often shows only several neovasculature vessels, which leads to a limited significance. However, the tissue micro-arrays (TMAs) of the PANZAR cohort have successfully been used in several former analyses and we consider those samples as representative for pRCC. Also, thickness of slides of the TMA used in our analysis is within standard range (2-7 μ m). Analysis of several samples per tumor specimen would have been possible. Considering missing clinical relevance in the case of heterogenous expression (i.e. missing target for application of theranostic/ therapeutic approaches) we decided against further analyses.

Furthermore, PSMA is an unspecific antigen. It is expressed in prostate tissue and, to a lesser extent, in peripheral and central nervous system, small intestinal and salivary gland tissues. Cytoplasmatic and, to a lesser degree, membranous PSMA expression has been recently documented in 11% of analyzed urinary bladder adenocarcinomas (35). The lack of specificity could explain the positive staining reaction in several cases.

What to consider from our findings from bench side to bed side? Case series on ^{68}Ga -PSMA targeted PET imaging in RCC patients showed encouraging results for ccRCC in detecting metastatic lesions that were not revealed in conventional imaging ((30, 36); Reviews: (37, 38)). In pRCC patients, however, ^{68}Ga -PSMA-PET-CT did not detect additional metastases compared to conventional imaging modalities (39). SUV_{max} in pRCC was also reported to be lower than in other RCC types. As sample sizes were small (number of pRCC patients between 1-3 patients) results were discussed not to be representative (39). The missing expression of PSMA as reported for the PANZAR cohort, however, explains that finding well. Larger trials to investigate PSMA hybrid imaging in RCC should probably exclude pRCC.

Therapeutic applications targeting PSMA have found entrance into the treatment of prostate cancer and are currently investigated for other cancer entities (40). Besides Lutetium-177-PSMA or Actinium-225-PSMA radionuclide therapy bispecific antibodies that target PSMA have been investigated in prostate cancer (41). PSMA targeted CAR-T cell therapy might offer an additional treatment option for PSMA positive tumors and probably will enter clinic in the near future (42). However, as for imaging we do not consider PSMA directed therapeutics promising for patients suffering from pRCC.

Conclusions

No relevant PSMA expression and consequently no relevant clinicopathological association with PMSA was detected in the pRCC PANZAR consortium cohort. Based on our data, a prognostic value of PMSA in pRCC is unlikely. Further on, due to missing PSMA expression in pRCC our findings point out that neither in pRCC type 1 nor type 2 PSMA targeted PET imaging or PMSA targeted therapeutical approaches seem to be reasonable. Whether PSMA targeted PET imaging and treatment modalities might expand the diagnostic and therapeutic landscape in other RCC subtypes needs to be further elucidated.

Abbreviations

ccRCC: clear cell renal cell carcinoma; CT: computer tomography; PET: positron emission tomography; pRCC: papillary renal cell carcinoma; PSMA: prostate specific membrane antigen; RCC: renal cell carcinoma; SUV: standardized uptake value.

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Institutional Review Board Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author Contributions

Conceptualization, Stefanie Zschäbitz, Franziska Erlmeier, Philipp Ivanyi and Sandra Steffens; Data curation, Franziska Erlmeier and Sandra Steffens; Formal analysis, Stefanie Zschäbitz, Franziska Erlmeier, Abbas Agaimy, Yvonne Mondorf, Philipp Ivanyi and Sandra Steffens; Funding acquisition, Sandra Steffens; Investigation, Stefanie Zschäbitz, Franziska Erlmeier and Sandra Steffens; Methodology, Franziska Erlmeier, Christine Stöhr, Iris Polifka, Abbas Agaimy, Arndt Hartmann and Sandra Steffens; Resources, Edwin Herrmann, Lutz Trojan, Frank Becker, Christian Wülfig, Peter Barth, Michael

Stöckle, Michael Staehler, Christian Stief, Axel Haferkamp, Markus Hohenfellner, Bernd Wullich, Joachim Noldus, Walburgis Brenner, Frederik Roos, Bernhard Walter, Wolfgang Otto, Maximilian Burger, Andres Schrader, Arndt Hartmann and Sandra Steffens; Supervision, Sandra Steffens; Validation, Franziska Erlmeier and Sandra Steffens; Visualization, Stefanie Zschäbitz and Franziska Erlmeier; Writing - original draft, Stefanie Zschäbitz, Franziska Erlmeier, Philipp Ivanyi and Sandra Steffens; Writing - review & editing, Stefanie Zschäbitz, Franziska Erlmeier, Christine Stöhr, Edwin Herrmann, Iris Polifka, Abbas Agaimy, Lutz Trojan, Philipp Ströbel, Frank Becker, Christian Wülfig, Peter Barth, Michael Stöckle, Michael Staehler, Christian Stief, Axel Haferkamp, Markus Hohenfellner, Stephan Macher-Göppinger, Bernd Wullich, Joachim Noldus, Walburgis Brenner, Frederik Roos, Bernhard Walter, Wolfgang Otto, Maximilian Burger, Andres Schrader, Yvonne Mondorf, Arndt Hartmann, Philipp Ivanyi and Sandra Steffens. All authors have read and agreed to the published version of the manuscript.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Competing Interests

The authors have declared that no competing interest exists.

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