Histol Histopathol (2020) 35: 919-927

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

Review

PSMA expression on neovasculature of solid tumors

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Summary. The use of prostate specific membrane antigen (PSMA) binding agents, labelled with diagnostic and therapeutic radio-isotopes is opening the potential for a new era of personalized management of prostate carcinoma. A wide variety of immuno-histochemistry studies have shown PSMA also to be upregulated on the endothelial cells of the neovasculature of a wide variety of other solid tumors where it may facilitate endothelial cell sprouting and invasion through its regulation of lytic proteases that have the ability to cleave the extracellular matrix. Similar to the introduction of PSMA-targeting theranostics in prostate carcinoma, overexpression of PSMA on newly formed tumor vessels may serve as a target for imaging and subsequent treatment of cancer through the use of agents that are capable of blocking PSMA in its function or through PSMA-mediated delivery of chemotherapeutics or radiation agents. In this review, the available data on PSMA expression on tumor neovasculature in human solid tumors assessed by using immunohistochemistry are discussed.

Key words: PSMA, Neovasculature, Solid tumors

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DOI: 10.14670/HH-18-215

Introduction

Prostate specific membrane antigen (PSMA), an integral membrane glycoprotein ectopeptidase with both folate hydrolase and N-acylated, a-linked dipeptidase activities, was first characterized by the murine monoclonal antibody 7E11, derived from mice immunized with partially purified cell membrane fractions isolated from the human prostate adenocarcinoma cell line LNCap in 1986 (Horoszewicz et al., 1987; Evans et al., 2016). A 2.65 kb cDNA fragment encoding the PSMA protein was subsequently cloned and mapped to chromosome 11p11.2 (Israeli et al., 1993). The PSMA protein is made up of a 19 amino-acid internal portion, a 24 amino-acid transmembrane portion and a 707 amino-acid external portion. Initial immunohistochemical analysis showed PSMA to be highly expressed in the epithelial cells of the prostate with an intense over-expression in prostate cancer, where its increased expression was shown to correlate with advanced disease stage and the presence of distant metastases (Horoszewicz et al., 1987; Chang et al., 2000; Virgolini et al., 2018). Low expression of PSMA was early-on also identified in kidney, salivary glands, the duodenum and the central and peripheral nervous system (Chang et al., 2000; Evans et al., 2016). Subsequently, a wide variety of IHC studies showed PSMA to be upregulated on the endothelial cells of the neovasculature of a wide variety of other solid tumors where it may facilitate endothelial cell sprouting and invasion through its regulation of lytic proteases that have the ability to cleave the extracellular matrix. Similar to the introduction of PSMA-targeting

theranostiscs in prostate carcinoma, overexpression of PSMA on newly formed tumor vessels may serve as a target for imaging and subsequent treatment of cancer through the use of agents that are capable of blocking PSMA in its function or through PSMA-mediated delivery of chemotherapeutics or radiation agents.

The available data of PSMA expression on tumor neovasculature in human solid tumors assessed by using immunohistochemistry are reviewed, including only studies in which the methodology used for PSMA staining was clearly described.

Renal cell carcinoma

Data on PSMA expression on neovasculature of renal cell carcinoma assessed using IHC are summarized in Table 1. Silver et al. (1997) observed intense PSMA staining (mAb 7E11) in endothelial cells of capillary vessels in peritumoral and endotumoral areas of a number of malignancies, including 8 out of 17 renal cell carcinoma studied, the subtypes of which were not characterized (Silver et al., 1997). Chang et al. (2001) studied 20 metastatic clear cell renal carcinoma (CRCC) in various anatomic sites, including bone, lymph nodes, liver, lung and soft tissue, and found that all lesions studied consistently expressed PSMA (mAbs 7E11 and PM2J004.5) making it an effective target for mAb-based anti-neovasculature therapy in metastatic renal carcinoma (Chang et al., 2001). Using diffuse strong or weak or focal strong PSMA staining (mAb PM2J004.5) as positive for PSMA staining, Baccala et al. (2007) found 16 out of 21 CRCC (76.2%), 0% of papillary renal cell carcinoma (PRCC), in 5 out of 16 chromophobe renal cell carcinoma (ChRCC) (31.2%) and in 10 out of 19 oncocytomas (52.6%) to be PSMA-endothelial positive, demonstrating that PSMA is differentially expressed in the tumor associated neovasculature (Baccala et al., 2007). In a comparable study by Al-Ahmadie et al. (2008) including 30 CRCC, 15 PRCC, 15 ChRCC and 15 oncocytoma, neovasculature staining positive for PSMA (MoAb 13D6) was identified in 24/30 CRCC (80%), in 0/15 PRCC, in 9/15 ChRCC (60%) and in 5/15 oncocytoma (33%) (Al-Ahmadie et al., 2008). As evidenced by additional CD31 staining, CRCC was also found to be the most vascularized and PRCC the least vascularized tumor type. More recently, Spatz et al. (2018) studied the prognostic value of PSMA expression on RCC neovasculature using both immunohistochemistry (IHC) (mAb 3E6) and northern blotting in a series of 257 RCC patients (228 CRCC, 22 PRCC and 7 ChRCC). It was found that higher grade and stage, metastatic and lethal clear cell carcinoma showed higher PSMA expression in tumor vessels. Of 30 CRCC patients presenting with metastasis, the primary tumor vessels were positive for PSMA in 29 of whom 60% showed immunohistochemically moderate to intense staining. On univariate and multivariate analysis, the intensity of positive versus negative endothelial PSMA expression was significantly associated with overall survival (Spatz et al., 2018).

Transitional cell carcinoma of the bladder

Campbell et al. (2018) performed 18F-DCFPyL PET/CT imaging in three patients suffering from metastasized urothelial carcinoma. While 18F-DCFPyL PET/CT allowed for the detection of sites of urothelial carcinoma in all three patients (respectively a recurrent prostatic urethra lesion, a bladder mass and pelvic lymph

Table 1. Table demonstrating the heterogeneity in methodology and data interpretation used in e.g. renal cell carcinoma.

PSMA IHC	IHC	Metastases	Primary tumor	Results
Silver et al., 1997	- Paraffine embedded - 7E11/CD45 - + if > 20% of the tumor showed reactivity		17 RCC (subtypes not specified)	8/17 (47%)
Chang et al., 2001	- Snap-frozen tissue - 7E11 and PM2J005.5/CD34 - Positive versus negative	20 patients (bone, lymph nodes, liver, lung and soft tissue)		15/20 (7E11, 75%) 20/20 (PM2J004.5, 100%)
Baccala et al., 2007	- Tissue micro-arrays - PM2J004.5/CD34 - 4-tiers scoring (score 3 and 4 considered positive)		21 CRCC 20 PRCC 16 ChRCC 19 oncocytoma	16/21 (76%) 0/20 (0%) 5/16 (31%) 10/19 (53 %)
Al-Ahmadie et al., 2008	- Paraffin embedded - 1D6/CD31 - 3-tiers scoring (score 2 and 3 = string) + diffuse versus focal (extent, 50% cut-off (focal versus diffuse)		30 CRCC 15 PRCC 15 ChRCC 15 onoccytoma	24/5 /25/4 (D/f/Str/W) 0/11/5/6 0/4/9/4 5/9/8/6
Spatz et al., 2017	- Tissue micro-arrays - 3E6 - 4-tiers score (absent, weak, moderate, strong, in maximal 2 tumor spots)		228 CRCC 22 PRCC 7 ChRCC 11 oncocytoma	40/82/90/16 (score 1 to 4) 19/2/0/1 2/2/3/0 (2/11 positive)

IHC, immunhistochemistry; RCC, renal cell carcinoma; CRCC, clear cell renal cell carcinoma; PRCC, papillary renal cell carcinoma; ChRCC, chromophobe renal cell carcinoma.

nodes and retroperitoneal lymph nodes and lung metastases), overall levels of radiotracer uptake were low. Consistent with this observation, IHC staining of tissue from one of the imaged patients demonstrated a low level of neovascularization and nearly absent PSMA expression (Campbell et al., 2018).

Primary brain tumors

Data on PSMA expression on neovasculature of primary brain tumors assessed using IHC are summarized in Table 2. Mhawech-Fauceglia et al. (2007) reported on IHC results using microarrays tested with the Y-PSMA-1 antibody in a series of 52 glioblastoma patients and found positive PSMA staining in glioblastoma neovasculature in only 3 patients. The authors ascribed the lack of staining in the remaining 49 patients to the lack of stroma in their samples (Mhawech-Fauceglia et al., 2007). Inversely, Wernicke et al. (2011) reported on IHC results in a series of 32 glioblastoma patients using the 3E6 mAb which has been compared to frozen section immunohistochemistry using the reference mAbs 7E11 and J591, the results of which proved identical. In their study, 3 glioblastomas displayed a faint, 15 a moderate and 14 a strong intensity PSMA-staining on their neovasculature. Inversely, none of the vessels of normal brain tissue seen in the specimens analyzed stained positive for PSMA (Wernicke et al., 2011). Nomura et al. (2014) performed IHC on a series of 19 gliomas; 5 grade I gliomas, 4 grade II gliomas, 5 grade III gliomas and 5 grade IV gliomas (glioblastoma). Whereas glioma blood vessels stained heavily for PSMA in glioblastoma, grade III and II glioma exhibited little vessel staining and grade I glioma exhibited moderate PSMA staining at tumor blood vessels. The authors suggested that the differential expression of PSMA on neovasculature of gliomas of different grades as found in their series may relate to the fact that glioblastomas are highly angiogenic compared with lower grades and that PSMA is only expressed on angiogenic vessels or to a differential expression of PSMA splice variants (Nomura et al., 2014). It is well known that the gene encoding for PSMA generates several splice variants, including amongst others, PSMA, PSME, PSM', PSMA-C, PSMA-D, and grade I through 4 gliomas which exhibit different behavior and gene signatures may also have differential expression of PSMA-gene splice variants. Saffar et al. (2018) evaluated 10 grade I, 26 grade II, 9 grade III and 27 grade IV gliomas for their expression of PSMA using the monoclonal clone1D6 antibody (37). Positive PSMA staining was observed in 12 (33.3%) of high grade gliomas (1 grade III and 11 grade IV gliomas) and 3 (8.3%) of low grade gliomas (2 grade I gliomas and 1 grade II glioma). Of interest, while high grade tumors more commonly had positive results for PSMA, the intensity of staining was significantly stronger in low-grade tumors (Saffar et al., 2018). Matsuda et al. (2018) assessed PSMA-expression using IHC on glioma using the monoclonal rabbit antibody EPR6253 as well as CD34 endothelial staining to confirm the endothelial origin of the PSMA staining in 65 gliomas, respectively 41 grade IV, 15 grade II and 7 grade II gliomas. Out of these respectively 40, 10 and 1 stained positive for PSMA on their endothelium (Matsuda et al., 2018).

Table 2. Table demonstrating the heterogeneity in methodology and data interpretation used in gliomas.

PSMA IHC	IHC	Primary tumor	Results
Mhawech-Fauceglia et al., 2007	- Paraffin embedded - Y-PSMA-1 - positive if > 5 % of the vessels showed reactivity - intensity score: weak/moderate/strong	52 GBM	3/52 (6%)
Wernicke et al., 2011	- Paraffin-embedded - 3E6 - % staining; positive if > 5 % of the vessels showed reactivity, 6-25% (mild), 26-50% (moderate), 51-75% (strong), 76-100% (very strong) - intensity of staining: weak, moderate, intense	32 GBM	% staining: 8 mild, 2 moderate, 15 strong, 7 very strong Intensity: 3 faint, 15 moderate, 14 intense
Nomura et al., 2014	- Paraffin-embedded - 3E6 - software-analysis (average intensity, versus normal brain tissue)	gr I : 5 pts gr II: 4 pts gr III: 5 pts gr IV: 5 pts	grade IV (GBM): average intensity > 3 normal
Saffar et al., 2018	- Paraffin embedded - 1D6 - % cells: 1-9% mild, 10-39% moderate, 40-69% strong, > 70% very strong	gr I : 10 pts gr II: 26 pts gr III: 9 pts gr IV: 27 pts	Positive staining: -12/36 grII/IV -3/36 gr I/II
Matsuda et al., 2018	- Paraffin embedded - EPR6253 - % cells ; 1-5% (low), 5-10% (moderate), > 10% (high)	gr II: 7 pts gr III: 15 pts gr IV: 41 pts	1/7 (low) 10/15 (2 low, 3 moderate, 5 high) 40/41 (3 low, 5 moderate, 32 high)

IHC, immunhistochemistry; GBM, glioblastoma multiforme; gr, grade; pts, patients.

Thyroid carcinoma

Using the monoclonal antibody 3E6, Bychkov et al. (2017) studied PSMA expression in the microvessels of 267 thyroid tumor samples, including 24 lymph node metastases, and found PSMA to be expressed in tumor capillaries and rarely of larger vessels of 19% of follicular adenomas (8/43), in 46% of follicular thyroid carcinoma (24/52), in 51% of papillary carcinomas (61/120), in 40-50% of poorly differentiated and anaplastic carcinomas and in 54% (13/24) of lymph node metastases. CD31/34 and CD105 staining were also performed as endothelial markers and endothelial cell proliferation respectively. Most often PSMA immunoreactivity was noted in radioactive iodine (RAI)-resistant thyroid cancer (63% of cases) suggesting that endothelial expression increases in parallel with tumor progression. Of interest, while PSMA immunostaining was not directly related to endothelial cell proliferation it was significantly associated with the size of the thyroid carcinoma (Bychkov et al., 2017). Using a similar study set-up (3E6 mAb and CD31/CD34 staining), Heitkötter et al. (2018) studied 101 thyroid lesions, 63 malignant of which 19 showed strong PSMA expression (mAb 3E6) in the neovasculature and 38 benign, of which only 1 showed strong PSMA-expression in its neovasculature, respectively a Grave's disease. Of interest, none of the 12 medullary thyroid carcinoma studied strongly expressed PSMA whereas 4/4 anaplastic carcinoma and 4/6 poorly differentiated thyroid carcinoma did so (Heitkötter et al., 2018). Finally, Moore et al. (2017) evaluated 91 thyroid carcinoma samples derived from 68 patients, including 37 primary differentiated thyroid cancers (DTCs) of which 6 were RAI-refractory, 5 anaplastic carcinoma, 9 distant and 12 lymph node metastases for PSMA (MoAb3E6) and CD31 expression. DTC tumors demonstrated a significantly higher PSMA expression when compared to benign tumors. Furthermore, PSMA expression was seen in all of the distant metastases studied and in 8 out of 12 lymph node metastases (Moore et al., 2017).

Breast carcinoma

In a study by Wernicke et al. (2014) including 106 breast carcinoma patients with AJCC stage 0-IV, out of which 92 primary breast carcinoma, tumor-associated vasculature (antiCD31 positive) were positive for PSMA (mAb 3E6) in 68/92 (74%) primary breast cancers and in 14/14 of breast cancer metastatic to the brain. In all but two cases, absence of PSMA expression in normal breast-tissue associated vasculature was found. Tumors that displayed more than 50% of PSMA-positive tumor vessels were significantly larger, of higher nuclear grade, more proliferative and more likely to be progesterone receptor negative (Wernicke et al., 2014). Comparable results were obtained in a study by Tolkach et al. (2018) comprising 315 cases of invasive breast carcinoma of which 60% exhibited PSMA-positive endothelia, as assessed by IHC (mAb 3E6) and Northern blotting, with higher expression rates in tumors with higher grade, HER2-positivity and lack of hormone receptors. Of interest, the highest PSMA rates were observed in triple-negative carcinomas (4.5 times higher than in other tumors) (Tolkach et al., 2018). Finally, in a study by Kasoha et al. (2017), including primary breast carcinoma and distant metastases, distant metastases displayed a higher PSMA expression (mAb 3E6) in tumor-associated neovasculature when compared to primary breast carcinoma and brain metastases neovasculature had significantly higher expression of PSMA compared to bone-tumor associated neovasculature (Kasoha et al., 2017). These findings are in disagreement with an earlier report by Nomura et al. (2014) who studied 5 cases of brain metastases of breast carcinoma, 3 of which exhibited staining less than that of the primary breast carcinoma (Nomura et al., 2014).

Miscellaneous

Results obtained on PSMA expression using IHC in other tumor types are limited in number as summarized in Table 3 and described below.

Squamous cell carcinoma of the oral cavity (SCCOC)

Using the 3E6 mAb, in a series of 96 SCCOC patients by Haffner et al. (2012), 24 cases showed no detectable PSMA staining, 48 demonstrated positive immunoreactivity for PSMA in less than 50% of tumor associated microvessels and 24 showed strong endothelial PSMA expression in more than 50% of tumor-associated microvessels (Haffner et al., 2012). PSMA expression proved strongly associated with microvesseldensity as assessed by CD31 staining and an independent predictor for overall survival in both univariate and multivariate analysis (77 months versus 18 months).

Lung carcinoma

Schmidt et al. (2017) studied 275 samples of non-small cell lung carcinoma (NSCLC) tissue specimens and found readily apparent expression at low power magnification or strong PSMA-expression using the 3E6 mAb in tumor cells of only 6 % of the samples studied whereas positive staining of the neovasculature was found in 49% of NSCLC (Schmidt et al., 2017). High neovascular PSMA expression was associated with higher tumor grading. Wang et al. (2015) studied 150 lung specimens of patients with lung cancer, 120 NSCLC and 30 small cell lung carcinoma (SCLC), and found high PSMA expression rates (non-specified monoclonal antibody) for both tumor neovasculature endothelial cells (85%) and for tumor cells (54%). A significantly higher percentage of early-stage NSCLC patients had PSMA-positive endothelial cells when compared to those with advanced NSCLC. PSMA-

positivity on endothelial cells of neovascularization proved not related to tumor size or the presence of lymph node metastases (Wang et al., 2015).

Adrenocortical carcinoma

In a series of 50 adrenal samples (16 normal glands (NMNL), 16 adrenocortical adenomas (AA), 15 primary and 3 metastases of adrenocortical carcinoma (ACC) using the monoclonal antibody 3E6, Crowley et al. (2016) found that 87% of ACCs were found to have a moderate to high expression of PSMA, whereas none of either the NML or AA did. Of interest, using the vascular marker CD34, a significant reduction in vascular density was found in ACC when compared to both NML and AA (Crowley et al., 2016).

Colorectal carcinoma

Using mAb SP29 targeting the extra-cellular C-terminal domain of PSMA (aa 592-750), Abdel-Hadi et al. (2014) found expression of PSMA in 75 out of a hundred colorectal carcinoma, results comparable to those reported by Haffner et al. (2009) who found expression of PSMA (monoclonal antibody 3E6) in the neovasculature of 110 out of 130 cases (Abdel-Hadi et al., 2014). Furthermore in the series by Haffner et al. (2009) the neovasculature of 16 out of 19 liver and 4 out of 5 nodal metastases proved PSMA positive, while PSMA expression did not correlate with any of the clinicopathologic parameters studied (TNMstage, grade and overall survival), attributed by the authors to the high proportion of PSMA-positive tumors leading to failure of PSMA-expression to subset cases into those with a relatively high and low risk (Haffner et al., 2009).

Gastric carcinoma

Haffner et al. (2009) found PSMA expression (monoclonal antibody 3E6) in 79 of 119 gastric

Table 3. Table demonstrating the heterogeneity in methodology and data interpretation used in other tumor types.

Type of Cancer	Authors	IHC	Primary tumor (pr)/LN/M+	Results
Squamous Cell Carcinoma of the Head and Neck (SCCHN)	Haffner et al., 2012	- 3E6 Ab - % positive (pos) microvessels (mv); 5-50% (1), > 50% (2)	- 96 pr SCCHN	- 50% score 1 - 25% score 2 - 25% neg
Lung carcinoma	Schmidt et al., 2017	- 3E6 Ab - 3-tiers intensity score - % pos mv; <5% (negative (neg)), > 5% (pos)	- 275 pr Non Small Cell Lung Ca. (NSCLC)	- 49% pos, - related to tumorgrading
	Wang et al., 2015	- Ab not described - 3-tiers intensity score - % pos mv; < 5% (0), 6-30% (1), 31-60% (2), > 60% (3)	- 150 pr - 120 NSCLC - 30 Small Cell Lung Ca. (SCLCC)	- 85% pos on mv - 50% pos on tumorcells
Adrenocortical carcinoma (ACC)	Crowly et al., 2016	- 3E6 Ab - 3-tiers intensity score - % pos mv; 0-25% (1), 26-50% (2), 51-75% (3), > 76% (4)	- 15 pr ACC - 16 Adrenocortical Adenoma (AA) - 16 Normal surrenal glands (NSG)	- 87% of ACC pos - 0% of AA and NSG pos
Colorectal Carcinoma	Abdel-Hadi et al., 2014	- SP29 Ab - % pos mv; none (0), 1-9% (1), 10-50% (2), >50% (3)	- 100 pr	- 75% pos (score 1)
	Haffner et al., 2009	- 3E6 Ab - score 1: < 10% of mv pos, score 2: 10-50% of mv or high intensity in up to 25% of mv, score 3: >50% of mv pos or >25% high intensity	- 130 pr - 5 LN - 19 liver M+	- 85% pos - 80% pos - 84% pos
Gastric carcinoma	Haffner et al., 2009	- 3E6 Ab + see supra	- 119 pr	- 66% pos
Pancreatic carcinoma	Ren et al., 2014	- Ab19071 - 3-tiers intensity score - % pos mv; 1-25% (1), 26-50% (2), 51-100% (3)	- 147 pr	- 84% pos
	Stock et al., 2017	- 3E6Ab and 4-tiers intensity score	- 81 pr	- 63% pos
Gynaecological carcinoma	Wernicke et al., 2017	- 3E6 Ab - Pos or neg	- 21 pr ovarian ca. - 25 m+ of ovarian ca. - 20 pr vulva ca.	- 100%pos - 100% pos - 75% pos
High grade sarcomas	Heitkötter et al., 2017	- 3E6Ab - 4-tiers intensity staining - < 5 or > 5% of mv	- 779 soft tissue and bone tumors	- 19% pos
	Zeng et al., 2012	- Ab not specified - pos or neg mv staining	- 45 pr osteosarcoma	- 47% pos

IHC, immunohistochemistry; mv, microvessels; pos, positive; neg, negative.

carcinoma (66%) which proved unrelated to TNM stage, tumor grade and overall survival (Haffner et al., 2009).

Pancreatic carcinoma

Using the monoclonal antibody ab19071 targeting aa716-723 in the extracellular domain of PSMA, Ren et al. (2014) found that out of 147 pancreatic andenocarcinoma samples studied, only 23 cases did not express PSMA (16%) whereas 100 cases (68%) showed medium to high expression. Of the ten cases of intra-epithelial pancreatic neoplasias also studied, none proved PSMA positive. PSMA expression proved significantly higher in TNM stage III and IV when compared to I and II as well as in the less differentiated tumor group (Ren et al., 2014). Inversely, using the monoclonal antibody 3E6, Stock et al. (2017) found only weak PSMA expression in pancreatic ductal adenocarcinoma (4 out of 81 samples studied) while PSMA expression in tumor-associated neovasculature could be identified in 51of the 81 samples studied. There was no significant relationship between neovascular PSMA expression and TNM stage, K-ras mutation status, primary or metastatic tumor or tumor cell budding in the investigated cohort (Stock et al., 2017).

Gynecologic malignancies

Using the monoclonal antibody 3E6, Wernicke et al. (2017) found predominantly diffuse PSMA staining on the tumor neovasculature (CD31 staining) of all 21 primary and 25 metastatic ovarian carcinoma studied. Furthermore, PSMA staining proved positive in 15 out of 20 of vulvar carcinoma and in 15% of cervical squamous cell carcinoma and 50% of cervical adenocarcinoma (Wernicke et al., 2017).

High grade sarcomas

Using mAb 3E6, Heitkötter et al. (2017) found PSMA positive tumor-associated neovasculature in 151 of 779 soft tissue/bone tumors, the majority of which (108 tumors) showed low expression whilst the remaining 43 (5.52%) showed strong expression, predominantly observed in subsets of different sarcomas, including some rhabodymyosarcomas, malignant peripheral nerve sheath tumors, synovial sarcomas and undifferentiated pleiomorphic sarcomas (Heitkötter et al., 2017). Similar results were obtained by Zeng et al. (2012) (mAb non-specified) in a series of 45 osteosarcoma, 21 of which (46.7%) showed strong PSMA expression in the cytoplasm of endothelial cells in tumor-associated neovasculature. Of interest, expression of PSMA was correlated with tumor size, the presence of pulmonary metastases and predictive for overall survival in univariate analysis (5-year survival rate of 63.2% for PSMA negative patients versus 36.6% in PSMA positive patients) (Zeng et al., 2012).

Discussion

Using immunohistochemistry and a wide variety of

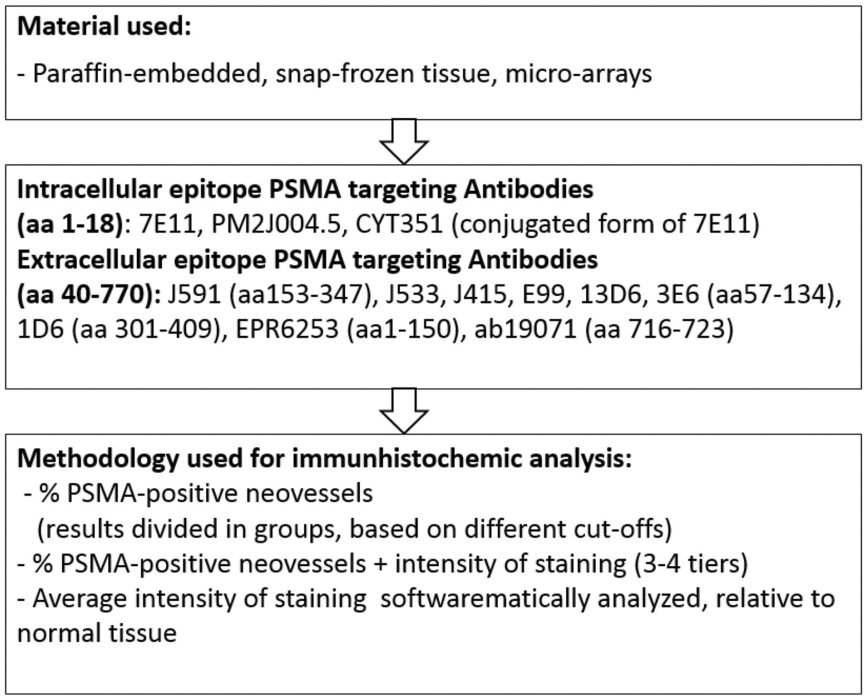

Fig. 1. Figure summarizing the various materials, antibobies and methodologies used for assessing immunohistochemical expression of PSMA in human malignancies.

monoclonal PSMA targeting antibodies (Fig. 1), targeting both the extracellular (amino-acid 44-770) and intracellular domain (amino-acid 1-18), PSMA expression has been identified on endothelial cells of newly formed vessels in a wide variety of human solid malignancies other than prostate carcinoma by most but not all authors. For instance, Mhawech-Fauceglia et al. (2007) using the monoclonal antibody Y-PSMA found relatively widespread expression of PSMA in several normal tissues and cancer cells but virtually no neovascular expression, which the authors attributed to the presence of a limited amount of stroma in their microarrays. However, when validated against the "gold standard" established using validated anti-PSMA antibodies (e.g. 7E11, J591, J415) on comparable frozen tissue sections, the antibody did not yield a staining profile on paraffin sections that paralleled previous reports of PSMA expression on frozen sections. These findings stress the need for a very thorough validation of an anti-PSMA antibody either by the vendor or the user prior to its use on tumor samples (Mhawech-Fauceglia et al., 2007).

Whereas initial IHC studies were performed on frozen tissues, later on, solely paraffin-embedded tissues, including micro-arrays, were used (Fig. 1). During the fixation/embedding process some loss of PSMA reactivity may occur. This may for instance explain the fact that using the 7E11 antibody, striated muscle stained positively on frozen sections but not on paraffin-embedded sections. Furthermore, over time, novel antibodies have been introduced for PSMA IHC which have been largely selected for among other features, their strong immunohistochemical reactivity. Utilization of the latter antibodies is likely to result in a more intense staining pattern and a higher percentage of positive staining samples as for instance evidenced in a study by Chang et al. (2001) who using the monoclonal antibody PM2J004.5 and the original 7E11 antibody found respectively 20/20 versus 15/20 renal metastatic lesions to be positive on IHC PSMA staining. In some studies, faint antibody staining also occurred on tumor cells in addition to a high expression on endothelial cell of newly formed vessels. Several factors may have contributed to this finding. First, this finding may be due to cross-reaction between the human transferrin-receptor (TfR) which is homologous with the residues 418-567 in the extracellular epitope of PSMA and which has been shown to be upregulated in brain-, breast-, colon-, liver-, ovarian-, prostate and lung cancer (Shen et al., 2018). While the epitope (amino-acid sequence) targeted by some of the mAbs used for IHC targeting PSMA is known, this is not the case for all antibodies used (Table 4). Furthermore, it is possible that different PSMA targeting antibodies recognize different splicing forms of PSMA that could be differentially expressed in different tumors on tumor-associated neovasculature (Williams and Kole, 2006; Cao et al., 2012). Alternatively, cytoplasmatic tumor cell staining might be a technical artifact as tumor cell staining in the same type of pathology using the same antibody is not a systematic finding.

Overall, in the various primary tumor types studied using IHC, the strongest intensity of PSMA-staining was predominantly, but not systematically, found in those primary tumors or subtypes of primary tumors known from literature to be hypervascularized. For instance, in RCC, PSMA-positivity on IHC was most pronounced and most frequently positive in clear cell carcinoma. Using dynamic volume CT perfusion in renal cell carcinoma, equivalent blood volume and blood flow were shown to be significantly higher in CRCC when compared to PRCC and chRCC (Chen et al., 2014). Likewise, using dynamic contrast-enhanced perfusion MRI, the mean enhancement ratio of clear cell carcinoma proved significantly higher than that of PRCC and chRCC (Razek et al., 2016). Also, in line with perfusion and arterial spin labeling MRI findings showing significantly higher flow in high grade gliomas when compared to low grade gliomas, high grade gliomas and especially glioblastoma multiforme or glioma grade IV were reported to have the highest incidence and intensity of PSMA staining on their neovasculature (Gao et al., 2015; Tietze et al., 2015). In part, this finding may result from the presence of intra-tumoral hypoxia resulting from the rapid growth of these tumors whereby PSMA facilitates endothelial cell invasion during angiogenic sprouting. On the other hand, non-hypoxia dependent mechanisms may also have contributed to this finding. For instance, in CRCC, it has been shown that mutated or hypermethylated forms of the Von-Hippel-Lindau (VHL) gene-product are no longer capable of binding HIF-1alpha whereby the latter is no longer routed towards proteosomal degradation and its intracellular build-up results in transcription and activation of various downstream growth factors, including vascular endothelial growth factor (VEGF) (Kumar et al., 2018). Finally, the high expression of PSMA as found on the microvessels of adrenocortical carcinoma and anaplastic

Table 4. Antibodies used for immunhistochemic analysis of PSMA expression on human solid malignancies.

Intracellular epitope PSMA targeting Ab (aa 1-18)	Extracellular epitope PSMA targeting Ab (aa 40-770)
7E11, PM2J004.5, CYT351 (conjugated form of 7E11)	J591 (aa153-347), J533, J415, E99, 13D6, 3E6 (aa57-134), 1D6(aa 301-409), EPR6253 (aa1-150), ab19071 (aa 716-723)

Aa, amino-acid sequence, specified if known from literature.

thyroid carcinoma, both of which are known from literature to be poorly vascularized, suggest that other, at present unidentified mechanisms, may also drive PSMA expression and upregulation stressing the need for additional studies unravelling the role of PSMA in tumor neovascularization (Diaz-Cano et al., 2001; Bernini et al., 2002; Crowley et al., 2016; Yang and Fried, 2017).

A limited number of studies, respectively in the field of breast and ovarian carcinoma, have tried to assess whether the expression of PSMA by distant metastases can be predicted from its expression in the primary tumor, using IHC, and whether this expression is differently presented depending on the site of metastases and this with conflicting results, in part likely related to the differences in scoring methodology used and to the limited number of samples/patients included. Obviously, if PSMA IHC of the primary tumor is going to be used to select patients for PSMA-PET/CT (Positron Emission Tomography/Computed Tomography) imaging, as suggested by some authors, then a uniform standardized staining and scoring protocol is mandatory.

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Accepted April 13, 2020