

Prostate-specific membrane antigen: Molecular functions and emerging roles as a therapeutic target (Review)

RYUTA WATANABE¹, TOMOHISA SAKAUE², NORIYOSHI MIURA¹,
TADAHIKO KIKUGAWA¹ and TAKASHI SAIKA¹

¹Department of Urology, Ehime University Hospital, Toon, Ehime 791-0295, Japan; ²Department of Cardiovascular and Thoracic Surgery, Ehime University Graduate School of Medicine, Toon, Ehime 791-0295, Japan

Received October 21, 2025; Accepted January 20, 2026

DOI: 10.3892/mmr.2026.13832

Abstract. The present review focuses on the molecular functions of prostate-specific membrane antigen (PSMA) as a biologically active protein. Its clinical use as a positron emission tomography imaging marker or radioligand therapy target is beyond the scope of the current review. The role of PSMA (also known as folate hydrolase 1/glutamate carboxypeptidase II/N-acetylated- α -linked acidic dipeptidase) has progressed from that of a prostate cancer biomarker to a functional driver of tumor biology. Structurally, PSMA is a type II transmembrane glycoprotein with glutamate carboxypeptidase and folate hydrolase activities, linking glutamate and one-carbon metabolism to proliferation, redox balance and epigenetic regulation. PSMA undergoes clathrin-dependent endocytosis and interacts with various scaffolding proteins, such as filamin A and receptor for activated C kinase 1, which are properties

that underlie its functional role as a molecular signaling hub, in addition to being a therapeutic entry point. Its expression is dynamically regulated by androgen receptor signaling, NF- κ B activation and epigenetic modifiers, contributing to intra-patient heterogeneity and treatment resistance. PSMA expression is not restricted to prostate epithelium but is also expressed in tumor-associated endothelium across multiple malignancies, where it can promote angiogenesis through integrin/PI3K-AKT-mTOR signaling and paracrine induction by extracellular vesicles. These molecular functions can result in immune exclusion, stromal activation and neuronal interactions, positioning PSMA as a key regulator of the tumor microenvironment. Although PSMA-targeted imaging and therapies have demonstrated substantial clinical utility, understanding the biological basis of the function of PSMA is essential for interpreting the heterogeneous clinical responses and for designing next-generation therapeutic strategies in association with this protein. By integrating enzymatic activity, non-enzymatic scaffold signaling and tumor microenvironmental regulatory information, the present review provides a functional framework in the PSMA biology field and discusses how these molecular properties can be leveraged to develop novel rational and effective PSMA-targeted interventions.

Correspondence to: Dr Ryuta Watanabe, Department of Urology, Ehime University Hospital, 454 Shitsukawa, Toon, Ehime 791-0295, Japan
E-mail: watanabe.ryuta.cu@ehime-u.ac.jp

Abbreviations: ADC, antibody-drug conjugate; ADT, androgen deprivation therapy; AR, androgen receptor; BiTE, bispecific T-cell engager; CAF, cancer-associated fibroblast; CAR-T, chimeric antigen receptor T cell; CRPC, castration-resistant prostate cancer; ECM, extracellular matrix; EV, extracellular vesicle; FDG, fluorodeoxyglucose; FOLH1, folate hydrolase 1; GCPH, glutamate carboxypeptidase II; HDAC, histone deacetylase; ICI, immune checkpoint inhibitor; mGluR1, metabotropic glutamate receptor 1; NAAG, N-acetylaspartylglutamate; NAALADase I, N-acetylated- α -linked acidic dipeptidase; PARP, poly(ADP-ribose) polymerase; PCFT, proton-coupled folate transporter; PET/CT, positron emission tomography/computed tomography; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; RFC, reduced folate carrier; RLT, radioligand therapy; RPT, radiopharmaceutical therapy; TAM, tumor-associated macrophage; TCA cycle, tricarboxylic acid cycle; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; UTUC, upper tract urothelial carcinoma

Key words: PSMA, tumor angiogenesis, RLT, TME, therapeutic target

Contents

1. Introduction
2. Structure and basic molecular functions of PSMA
3. Intracellular trafficking and endocytosis of PSMA
4. Regulation of PSMA expression and transcriptional control
5. Tumor angiogenesis and TME
6. Immunological roles of PSMA
7. Novel and exploratory functions of PSMA
8. Clinical applications and therapeutic implications
9. Conclusions

1. Introduction

Prostate-specific membrane antigen (PSMA, also known as FOLH1/GCPH/NAALADase) is a type II transmembrane glycoprotein that is highly expressed in prostate cancer

cells and now widely exploited as a molecular imaging and therapeutic target. PSMA PET/CT has transformed prostate cancer staging and restaging, enabling the detection of micro-metastatic disease and guiding metastasis-directed therapies. In particular, radioligand therapy (RLT) has emerged as a life-prolonging treatment for advanced prostate cancer (1). Despite these clinical advances, the biological mechanisms regulating PSMA expression, its heterogeneity across metastatic sites and its functional consequences remain poorly understood (2). Therefore, a comprehensive overview of PSMA PET imaging or RLT is beyond the scope of this review. Instead, it focuses on the molecular functions of PSMA that underlie its biological and therapeutic relevance. A unified conceptual framework integrating the metabolic enzymatic activity of PSMA, non-enzymatic scaffold signaling, tumor-associated angiogenesis and immunological consequences within tumor biology is the focus of this review.

Recent studies have revealed that PSMA is not merely a passive marker but a multifunctional protein involved in glutamate and folate metabolism, clathrin-mediated internalization, potentially in signaling processes that influence tumor progression. Importantly, PSMA expression is not restricted to prostate epithelial cells but can also be found in tumor-associated endothelium across a diverse range of malignancies, including renal cell carcinoma, colorectal cancer and glioblastoma (3,4). This endothelial expression profile implicates PSMA in angiogenesis and tumor microenvironment (TME) remodeling. Consistent with this, paracrine mechanisms have been proposed in which PSMA-positive extracellular vesicles (EVs) or membrane fragments induce PSMA expression in endothelial cells to enhance angiogenesis, suggesting a positive feedback loop for reinforcing tumor vascularization (5). Additional evidence from renal cell carcinoma and other urological malignancies further supports the concept of PSMA as a pan-tumor neovascular marker (6).

This review will provide a comprehensive summary of current knowledge on PSMA, covering its structural and enzymatic features, transcriptional and epigenetic regulatory profile, endothelial expression and roles in angiogenesis, interactions with immune and metabolic pathways, and clinical applications and future therapeutic opportunities (Fig. 1). Our goal is to shift the perspective on PSMA from a mere diagnostic biomarker to a potential driver of tumor biology and a versatile pan-cancer therapeutic target.

2. Structure and basic molecular functions of PSMA

Features as a membrane protein. PSMA is encoded by the FOLH1 gene on chromosome 11p11.2 and was first cloned in the early 1990s as a prostate cancer-associated surface antigen (7). Shortly thereafter, Bzdega *et al.* (8) and Pinto *et al.* (9) identified its enzymatic identity to be a glutamate carboxypeptidase II (GCPII; NAALADase I).

Structurally, PSMA is a type II transmembrane glycoprotein with a short N-terminal cytoplasmic tail [~19 (amino acids)] containing a canonical MWNLL internalization motif, a single transmembrane helix (~24 aa) and a large extracellular ectodomain (~700 aa) that harbors the catalytic machinery (10). The ectodomain is organized into protease, apical and dimerization domains, where the protein exists predominantly as

a non-covalent homodimer, which is essential for optimal substrate binding and catalytic efficiency (10).

PSMA is heavily N-glycosylated at up to 10 sites, where proper glycosylation is required for stability, surface expression and enzymatic activity. By contrast deglycosylation leads to ER retention and loss of function (11). Internalization is constitutive and clathrin-dependent, which is further accelerated by ligand or antibody binding (12). Interaction with filamin A (FLNa) restrains constitutive endocytosis and has been reported to attenuate NAALADase activity (13). These properties underpin the use of PSMA as an entry point for antibody-drug conjugates (ADCs) and RLT.

Enzymatic activities: General view. PSMA is a member of the M28 metallopeptidase family, functioning as a glutamate-preferring carboxypeptidase. Its binuclear zinc active site is located within the protease domain, coordinated by a number of key residues, such as His377, Asp387, Asp453, Glu425 and His553. A positively-charged substrate-binding cavity (Arg210, Arg463, Arg534 and Arg536) stabilizes the γ -carboxylate group of glutamate (10). Pharmacologically, PSMA can be inhibited by phosphonate analogs [including 2-(Phosphonomethyl)pentanedioic acid] and can be clinically targeted by a range of urea-based ligands, such as PSMA-617 and DCFPyL, for imaging and therapy (11).

GCPII activity and glutamate signaling. As NAALADase I/GCPII, PSMA hydrolyzes N-acetylaspartylglutamate (NAAG) into N-acetylaspartate (NAA) and glutamate. In the central nervous system, this reaction regulates extracellular glutamate levels to modulate metabotropic and ionotropic glutamate receptor signaling, thereby serving a key role in neuronal excitability (14). Consistent with this, genetic knockout or pharmacologic inhibition of GCPII reduces susceptibility to seizures and excitotoxic injury by limiting glutamate release, supporting a physiological role of NAAG metabolism in neuroprotection (8,15).

In prostate cancer cells, PSMA-derived glutamate exerts a distinct biological role beyond neurotransmitter regulation. Increased pericellular glutamate generated by GCPII activity can activate metabotropic glutamate receptor 1 (mGluR1), leading to the downstream stimulation of PI3K β -AKT signaling and subsequent phosphorylation of mTOR effectors, such as ribosomal protein S6 kinase β 1 (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1), ultimately promoting cell proliferation and survival (16,17). In particular, this aforementioned signaling cascade is initiated by enzymatic substrate hydrolysis and represents a metabolically-mediated mechanism by which PSMA indirectly engages oncogenic signaling pathways, rather than a direct receptor-scaffolding function.

Folate hydrolase activity. PSMA also exhibits folate hydrolase activity, cleaving poly- γ -glutamylated folates into monoglutamate forms that are competent for cellular uptake through the proton-coupled folate transporter (PCFT) and the reduced folate carrier (RFC). Whilst this function is more physiologically relevant in the jejunum, it is markedly upregulated in prostate cancer, where the increased folate availability supports nucleotide synthesis and one-carbon metabolism (18).

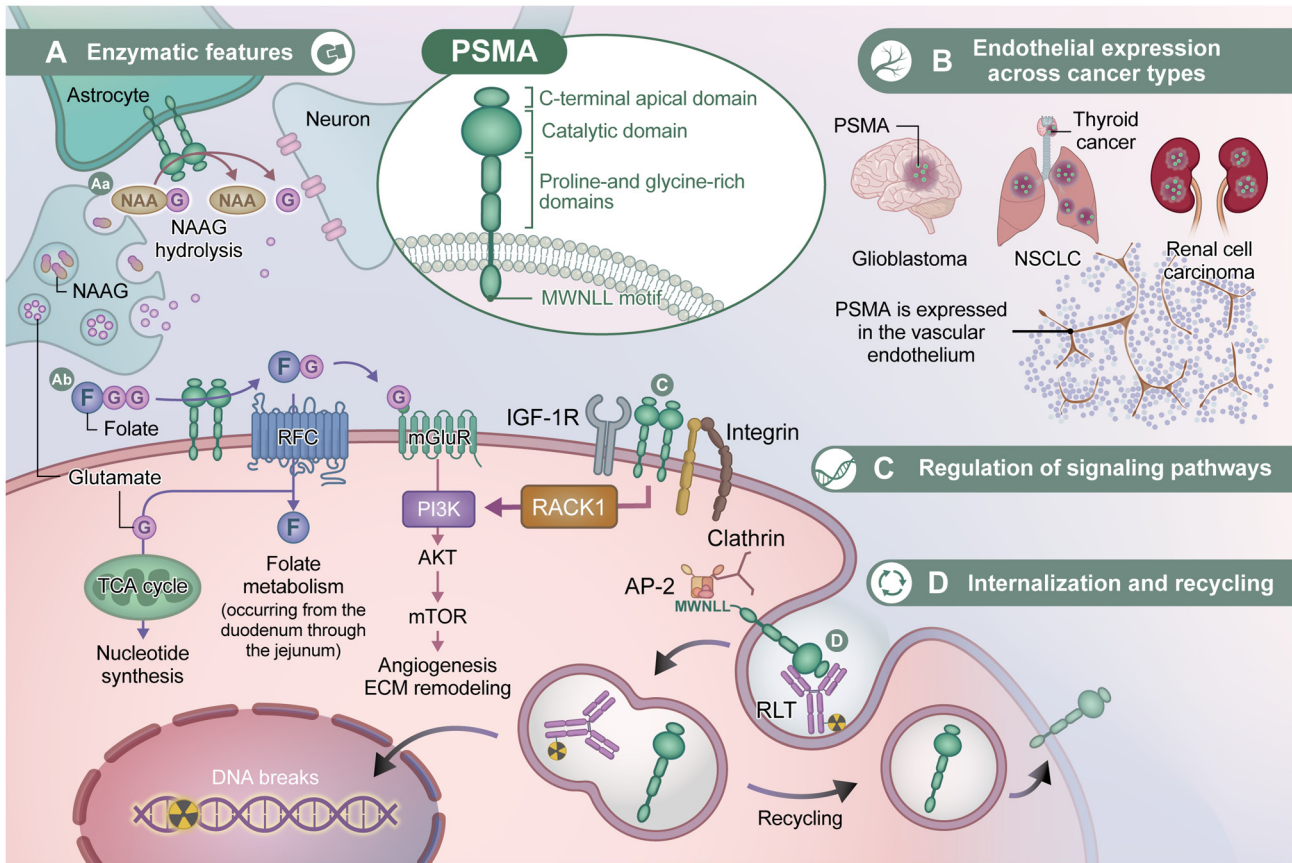


Figure 1. Integrated schematic of PSMA molecular functions and therapeutic implications. (A) Enzymatic features. PSMA structure and enzymatic activities in the (Aa) brain and (Ab) intestine, linking glutamate signaling and folate metabolism. (B) Endothelial expression across cancer types. PSMA expression in tumor-associated vascular endothelium and its role in tumor angiogenesis. (C) Regulation of signaling pathways. PSMA functions as a signaling hub through interactions with integrins and other membrane proteins. (D) Internalization and recycling. Ligand-induced internalization and recycling of PSMA, enabling targeted therapy and sustained surface signaling. This schematic illustration was created with the support of Medical Fig (<https://medicalfig.medicaleducation.co.jp/>). AP-2, adaptor protein 2; ECM, extracellular matrix; F, folate; G, glutamate; IGF-1R, insulin-like growth factor 1 receptor; mGluR, metabotropic glutamate receptor; NAAG, N-acetylaspartylglutamate; NSCLC, non-small cell lung cancer; PSMA, prostate-specific membrane antigen; RACK1, receptor for activated C kinase 1; RFC, reduced folate carrier; RLT, radioligand therapy; TCA cycle, tricarboxylic acid cycle.

Previous transcriptomic analyses have demonstrated that FOLH1 expression is positively correlated with that of genes involved in one-carbon metabolism, including MTHFD1L and SHMT2, suggesting a coordinated metabolic program in PSMA-high tumors (17). Through this enzymatic activity, PSMA increases the intracellular pool of folate-derived one-carbon units, thereby supporting DNA synthesis, redox balance and epigenetic regulation through S-adenosylmethionine-dependent methylation reactions.

The contribution of folate hydrolase activity to tumor progression is however primarily metabolic in nature and does not require the direct participation of PSMA in receptor-associated signaling complexes. Together with GCPII-mediated glutamate production, this function positions PSMA as a metabolic gatekeeper that aligns nutrient availability with the biosynthetic and epigenetic demands of proliferating cancer cells.

Substrate specificity and signaling scaffolding. Beyond its enzymatic activities, PSMA also confers non-enzymatic functions as a signaling scaffold that reorganizes membrane-associated receptor complexes. Structural and biochemical studies have shown that PSMA can interact with β 1-integrin

and insulin-like growth factor-1 receptor through various adaptor proteins, such as receptor for activated C kinase 1, thereby forming macromolecular signaling assemblies at the plasma membrane (16).

Through these interactions, PSMA redirects downstream signaling from the MAPK pathway towards the PI3K-AKT axis, a process referred to as ‘signaling rewiring’. In particular, this shift in signaling balance promotes cell survival, migration and angiogenic potential, which has been associated with more aggressive tumor phenotypes (16).

However, scaffold-mediated PSMA signaling is independent of its catalytic activity. Pharmacologic inhibition of GCPII enzymatic function does not abrogate PSMA-driven PI3K-AKT activation mediated through integrin-associated complexes, underscoring that this pathway operates separately from its role in substrate hydrolysis-dependent metabolic signaling. This distinction highlights PSMA’s dual identity as both an ectoenzyme and a non-enzymatic signaling adaptor.

In addition to integrin and growth factor receptor signaling, emerging proteomic studies have suggested that PSMA can associate with broader cytoskeletal and membrane-organizing proteins, potentially expanding its scaffold repertoire beyond

its currently characterized partners (19). Defining the full interactome of PSMA will be essential for understanding how its non-enzymatic scaffolding functions can contribute to therapy resistance, cellular plasticity and tumor progression.

Scaffold functions and unexplored protease activity. Collectively, PSMA functions as a dual-mode regulator of tumor biology by integrating catalytic metabolic inputs and non-enzymatic signaling rewiring through distinct but convergent mechanisms. Its enzymatic activities can generate glutamate and monoglutamyl folates, thereby indirectly activating oncogenic signaling pathways through metabolic reprogramming. In parallel, PSMA can also serve as a signaling scaffold to reorganize receptor-associated complexes at the plasma membrane, redirecting downstream signaling towards PI3K-AKT dominance independently of its substrate hydrolysis capabilities.

Although both axes tend to converge on PI3K-AKT signaling, their biological origins and regulatory contexts are fundamentally different. Metabolically-driven signaling depends on local substrate availability and enzymatic activity, whereas scaffold-mediated signaling is governed by membrane localization, protein-protein interactions and cytoskeletal organization. The relative contribution of these two mechanisms is therefore context-dependent, varying across cell types, disease stages and therapeutic pressures.

This dual functional framework provides a conceptual basis to understand several unresolved features of PSMA biology, including intratumoral heterogeneity, treatment-induced PSMA modulation and differential therapeutic responses. In particular, it offers an explanation into how PSMA can sustain oncogenic signaling even under conditions of enzymatic inhibition or metabolic stress.

In endothelial cells, PSMA-driven angiogenesis appears to rely predominantly on scaffold-mediated signaling rather than its metabolic enzyme activity, whereas in prostate cancer cells both axes may operate in parallel. Recognizing this functional duality is critical for the rational design of PSMA-targeted therapies and combination strategies.

3. Intracellular trafficking and endocytosis of PSMA

Clathrin-dependent endocytosis triggered by ligand binding. PSMA undergoes constitutive internalization, which is markedly accelerated upon ligand or antibody engagement. The short N-terminal cytoplasmic tail contains the MWNLL motif, both necessary and sufficient for clathrin- and adaptor protein-2-dependent endocytosis (12,20). Imaging and mutagenesis studies have reported its localization to clathrin-coated pits, whilst disruption of the MWNLL motif has been shown to abrogate its internalization and delays surface clearance (20). Ligand-triggered endocytosis has been observed with monoclonal antibody (such J591), Glu-urea-based inhibitor and ADC treatment, resulting in rapid trafficking to early endosomes. From there, PSMA can either recycle back to the plasma membrane or proceed to late endosomes and lysosomes for ligand degradation or payload release (1).

Filamin A and endocytic regulation. The cytoplasmic tail of PSMA also binds FLNa, linking it to the actin cytoskeleton.

FLNa binding restrains constitutive endocytosis and reduces NAALADase activity (12). Disruption of this interaction has been observed to enhance internalization, indicating that cytoskeletal context and extracellular cues can dynamically regulate PSMA trafficking (2).

Implications for targeted drug delivery. Ligand-induced internalization provides the mechanistic basis for using PSMA as a therapeutic gateway. ADCs exploit this process for lysosomal trafficking and payload release, resulting in potent cytotoxicity in PSMA-positive cells (1). Similarly, RLT benefits from intracellular trapping of radionuclides, such as ¹⁷⁷Lu and ²²⁵Ac, which increase the tumor absorbed dose (2). Beyond these, PSMA-targeted nanoparticle platforms, including supramolecular systems using cucurbit-[8]-uril host-guest chemistry, can achieve selective uptake and therapeutic efficacy in prostate cancer models (21). Taken together, these approaches highlight PSMA as a versatile entry point for the precise delivery of drugs, nucleic acids and imaging agents.

Clinical and translational implications. PSMA's trafficking behavior has direct clinical relevance. Its saturable, time-dependent uptake informs dosing strategies to maximize tumor targeting whilst avoiding receptor downregulation (12). Recycling of internalized PSMA to the cell surface allows for repeated ligand binding and payload delivery, a principle leveraged in fractionated RLT to enhance the cumulative tumor dose (1). However, resistance may arise from altered endosomal trafficking, lysosomal dysfunction or the transcriptional downregulation of PSMA, all of which reduce payload release. Co-targeting trafficking pathways or pharmacologically enhancing PSMA surface expression may therefore improve therapeutic efficacy and durability (17).

4. Regulation of PSMA expression and transcriptional control

Androgen receptor (AR) signaling and PSMA expression dynamics. FOLH1 expression is strongly regulated by AR signaling and generally shows an inverse correlation with AR activity. Under androgen stimulation, AR represses PSMA transcription, reducing PSMA protein expression at the cell surface (22). Conversely, androgen deprivation therapy (ADT) or potent AR antagonists, such as enzalutamide or apalutamide, induce a rapid 'PSMA flare', characterized by increased transcription and surface expression (23). Clinically, this enhances lesion detectability on PSMA PET/CT and may improve the uptake of PSMA-targeted radioligands (24).

Mechanistic studies have suggested that AR may directly repress FOLH1 through intronic androgen response elements and indirectly through MYC and homeobox B13 signaling (17). In castration-resistant prostate cancer (CRPC), PSMA expression typically remains high or even increases, reflecting altered AR dependency and partial escape from AR-mediated repression.

Transcriptional and epigenetic regulation. Beyond AR, FOLH1 expression is shaped by prostate-specific cis-regulatory elements and inflammatory pathways. Chronic NF- κ B activation is a known driver of prostate cancer

progression (25), where crosstalk between AR and NF- κ B represents a key axis influencing FOLH1 transcription (26). A well-characterized intragenic enhancer within intron 3 of FOLH1, known as the PSMA enhancer element, augments promoter activity in PSMA-expressing cells (27,28). Although direct NF- κ B-driven activation of the FOLH1 promoter has not been conclusively shown, PSMA can itself activate NF- κ B through integrin-dependent complexes, linking it to angiogenesis and survival pathways (29,30).

Splice variants and functional implications. PSMA undergoes alternative splicing, generating isoforms with distinct subcellular localization and functional properties.

However, the canonical full-length protein is membrane-anchored and enzymatically active, enabling ligand binding, internalization and downstream signaling. The splice variant lacking exon 13 (PSMA Δ 13) is predominantly retained in the cytoplasm and fails to localize to the cell surface, resulting in reduced availability of functional PSMA at the plasma membrane (31).

Functionally, PSMA Δ 13 has been proposed to act as a dominant-negative isoform by interfering with membrane trafficking or stability of the full-length protein, thereby decreasing effective surface PSMA levels. Such a reduction is expected to directly impair ligand binding, internalization and payload delivery, providing a mechanistic basis for reduced sensitivity to PSMA-targeted approaches, including RLT and antibody-drug conjugates (ADCs).

Experimental studies have previously demonstrated that PSMA protein expression alone is insufficient to confer its biological effects. In prostate cancer models, expression of catalytically inactive or structurally-altered PSMA mutants abolished PSMA-mediated functional effects despite preserved protein expression, indicating that intact enzymatic activity and proper membrane localization are critical for PSMA function (31). These findings support the notion that PSMA Δ 13 represents a functionally deficient PSMA state rather than a simple quantitative reduction in expression.

Additional truncated PSMA isoforms have also been reported, including variants that may be secreted and detectable in circulation (1). Whilst the biological and clinical significance of PSMA Δ 13 and related truncated isoforms remains to be fully elucidated, further studies are required to define their prevalence, regulation and direct impact on PSMA-targeted diagnostics and therapies.

Upregulation during progression and spatial heterogeneity. PSMA expression generally increases with disease progression, showing higher expression levels in tumors with higher Gleason scores, advanced stage, and CRPC (32). However, marked spatial heterogeneity complicates its clinical application. Studies have shown site-specific variation in PSMA expression across metastases, with major therapeutic implications (33).

Liver metastases frequently display PSMA-low or PSMA-negative phenotypes, making them difficult to detect on PSMA-PET (34,35). This 'cold' phenotype is associated with the reduced uptake of RLT and inferior responses, possibly reflecting lineage plasticity, neuroendocrine differentiation or the epigenetic silencing of FOLH1 (36). Importantly,

PSMA-low or PSMA-negative phenotypes do not necessarily indicate the complete loss of FOLH1 transcription, since alternative splicing, impaired membrane trafficking or intracellular retention can all result in functionally inactive PSMA despite preserved gene expression.

By contrast, bone and nodal metastases typically maintain high expression levels, at time exceeding that of primary tumors. Hope *et al* (37) reported the high specificity of ^{68}Ga -PSMA-11 PET/CT for nodal disease, although sensitivity was reduced for lesions <5 mm. These findings highlight the need to integrate PSMA-PET with histopathology and complementary diagnostics, such as dual-tracer imaging, image-guided biopsy or circulating tumor DNA profiling. Elucidating the molecular drivers of PSMA-low states will be essential to optimize patient selection and develop strategies to restore expression before therapy.

5. Tumor angiogenesis and TME

PSMA expression in the tumor endothelium across cancer types. PSMA expression is not restricted to prostate epithelium but is also strongly expressed in the endothelium of the tumor-associated neovasculature across a number of other solid tumors. Haffner *et al* (3) reported vascular PSMA positivity in 66% of gastric and 85% of colorectal adenocarcinomas, as well as in most metastases derived from these primary tumors. Subsequent studies confirmed vascular PSMA expression in renal cell carcinoma, glioblastoma, non-small-cell lung cancer, pancreatic, breast and thyroid cancers (38-44). Representative immunohistochemistry images from three genitourinary cancer cases analyzed in the present study (Fig. 2) demonstrated that PSMA expression was consistently restricted to the endothelium of tumor-associated neovessels across renal cell carcinoma, upper tract urothelial carcinoma and bladder cancer. As summarized in Table I, vascular PSMA expression has also been reported across a wide range of solid tumors, with substantial variability in reported frequencies (n/N) depending on cancer type and study. However, in other urological malignancies, such as testicular germ cell tumors or penile carcinoma, PSMA expression has not been detected in tumor-associated vasculature, indicating that endothelial PSMA expression is not universally present across all cancer types.

It should however be noted that vascular PSMA expression is not uniformly observed across all tumor types, where reported positivity rates vary substantially among studies and malignancies. These observations underscore the need for further stringent tumor-type-specific validation rather than assuming the universal applicability of vascular PSMA-targeted imaging or therapeutic strategies.

Functional models also support a causal role of PSMA: in mice with transgenic adenocarcinoma of prostate, PSMA knockout (Folh1 $^{-/-}$) reduced microvessel density, increased hypoxia and apoptosis, impairing tumor growth (16). Altogether, all of the aforementioned findings proposed PSMA as an active contributor to pathological angiogenesis and a robust, pan-cancer vascular marker with translational potential for imaging and therapy.

Paracrine and epigenetic regulation of endothelial PSMA. Endothelial PSMA is actively regulated rather than passively

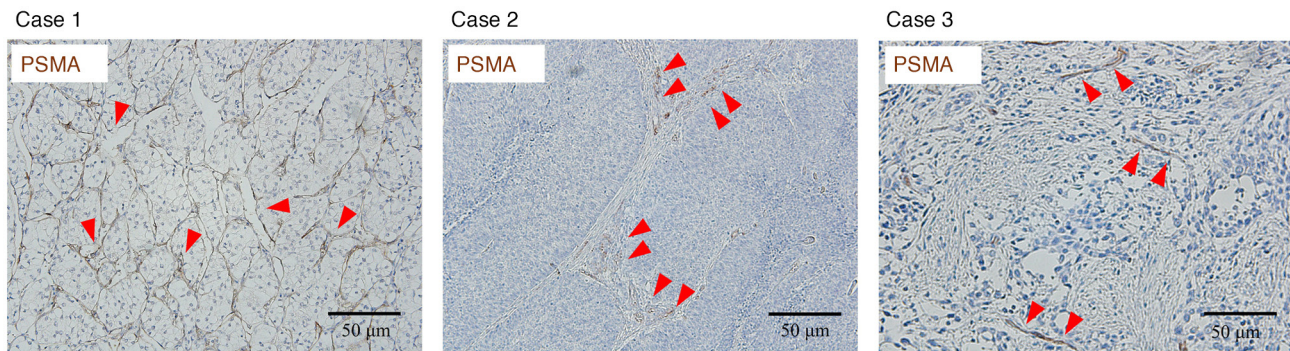


Figure 2. Immunohistochemical detection of PSMA in the tumor-associated vasculature of genitourinary malignancies. Representative immunohistochemical images from 3 genitourinary cancer cases using surgically resected specimens obtained during routine clinical care as part of the present study: Case 1, renal cell carcinoma; case 2, upper tract urothelial carcinoma; and case 3, bladder cancer. All cases exhibited PSMA expression in tumor-associated blood vessels. PSMA-positive endothelial cells are highlighted by red arrowheads and were predominantly localized in the tumor-associated vasculature surrounding tumor nests, whereas adjacent normal vessels remained negative. Formalin-fixed, paraffin-embedded sections (3-5 μm) were stained with a mouse monoclonal anti-PSMA antibody (M3620; Dako; Agilent Technologies, Inc.). Endothelial PSMA positivity was defined as distinct membranous and/or cytoplasmic staining in tumor-associated blood vessels. Magnification, x400; scale bar, 50 μm . PSMA, prostate-specific membrane antigen.

expressed. EVs shed from PSMA-positive tumor cells can induce *de novo* PSMA expression in HUVECs, promoting angiogenesis through NF- κ B signaling (5). Machado *et al* (45) showed that PSMA-positive particles can stimulate VEGF-A and angiogenin release with 4EBP1 phosphorylation, linking PSMA to mTOR-dependent translational control. Similar findings were observed with renal cell carcinoma-derived vesicles carrying growth differentiation factor 15 and myeloid-derived growth factor (6). Although these findings support a paracrine regulatory model, the precise molecular mechanisms and their relevance in human tumors remain to be fully elucidated.

These data support a paracrine model in which tumor-derived vesicles provide both inductive signals and pro-angiogenic cargo. Importantly, vascular PSMA expression persists even in metastases lacking epithelial PSMA (36). However, epigenetic regulation also contributes. Promoter hypermethylation or histone deacetylation can silence FOLH1, whereas histone deacetylase (HDAC) inhibition can restore endothelial PSMA (37). Therefore, endothelial PSMA is likely dynamically regulated through a paracrine-epigenetic feedback loop.

Downstream signaling pathways. PSMA can function not only as a peptidase but also as a signaling hub (Fig. 3). Conway *et al* (46) previously showed that PSMA can regulate laminin-specific β 1-integrin-p21-activated kinase 1 signaling, which is essential for endothelial invasion. Loss of PSMA was found to impair extracellular matrix (ECM) invasion and angiogenesis (46). Caromile *et al* (16) demonstrated that PSMA can redirect signaling from MAPK to PI3K-AKT-mTOR, enhancing endothelial survival, proliferation and sprouting. In another study, Grant *et al* (47) further showed that PSMA can regulate angiogenesis in experimental models, including *in vitro* endothelial assays and *in vivo* mouse neovascularization models, at least in part independently of canonical VEGF signaling pathways (5). Although the precise relationship between PSMA-driven angiogenic signaling and the canonical VEGF/VEGFR pathway remains incompletely understood, PSMA is currently hypothesized to regulate angiogenesis through mechanisms that are at least partially

independent of VEGF signaling. These studies position PSMA as a central integrator of ECM cues, cytoskeletal remodeling and pro-survival pathways, reinforcing its therapeutic relevance.

Emerging pan-cancer implications. Recognition of vascular PSMA across cancers broadens its biological and clinical significance. Immunohistochemistry and PET studies showed positivity in bladder cancer, upper tract urothelial carcinoma (UTUC), glioblastoma, thyroid, pancreatic, lung and breast tumors (38,39,47). In urothelial carcinoma, vascular PSMA expression has been shown to be positively correlated with increased microvessel density and poor prognosis (48,49). These findings suggest that prostate-derived therapies can be repurposed for vascular targeting across tumor types. Bladder and UTUC show heterogeneous but detectable vascular PSMA (4,50), where vascular expression persists even in tumors with low epithelial PSMA expression (such as in neuroendocrine variants) (33). Site-specific heterogeneity is likely critical. In prostate cancer, liver metastases frequently lack PSMA expression, causing false-negative PET and poor RLT responses (34,35), whereas bone and nodal metastases maintain robust expression and remain excellent therapeutic targets for exploiting PSMA. Knockout models have previously confirmed that the loss of PSMA can profoundly impair angiogenesis and tumor growth (16).

Collectively, these data suggest that vascular PSMA can serve as both a biomarker and therapeutic entry point. Future strategies may combine vascular-directed RLT, ADCs or epigenetic modulators (36). Integrating PSMA-PET with complementary imaging and liquid biopsy may more efficiently capture heterogeneity and refine patient selection.

6. Immunological roles of PSMA

PSMA expression and immunosuppressive TME. PSMA expression has been associated with an immunosuppressive TME. In prostate cancer and other solid tumors, PSMA-positive lesions frequently show infiltration by immunosuppressive myeloid populations and increased levels of

Table I. Tumor-associated vascular PSMA expression and clinical implications across cancer types.

Tumor type	Vascular PSMA expression, n/N (%) (ref.)	Detection method (as reported)	Clinical implications (ref.)
Prostate cancer	4/33 (12.1) (5)	IHC (PSMA clone 3E6; CD31)	High diagnostic performance of PSMA-PET for primary and metastatic disease, with reduced sensitivity for liver metastases (34,35,65); transient PSMA flare after ADT (70,71); efficacy of PSMA-targeted RLT in mCRPC (67-69).
Bladder UC	84/124 (67.7) (48); present (n/N not reported) (49)	IHC/IF (PSMA; CD31)	PSMA-PET signal largely vascular; vascular PSMA associated with prognosis and therapeutic targeting potential (48,49,66).
UTUC	Detectable (n/N not reported) (4)	IHC	Variable PSMA-PET uptake compared with FDG; vascular PSMA expression may enable targeting in selected cases (50,66).
Renal cell carcinoma	16/21 (76.2) (23); 24/30 (80.0) (24); 45/45 (100.0) (6)	IHC (various antibodies; CD31)	Non-prostate PSMA uptake predominantly reflects vascular PSMA expression; rationale for vasculature-directed strategies rather than tumor-cell imaging (39,53,66).
Non-small cell lung cancer	135/275 (49.1) (44)	IHC (various antibodies; CD31/CD34)	PSMA-PET uptake largely reflects vascular PSMA expression in tumor-associated vasculature; potential angiogenesis-related imaging/targeting (39,44,66).
Pancreatic cancer	Reported (n/N not reported) (4,38)	IHC (reported in reviews)	Generally weak PSMA-PET uptake; vascular PSMA expression suggests possible targeting in selected settings (39,66).
Breast cancer	Vascular PSMA expression reported (n/N not reported) (17,43)	IHC	PSMA-PET uptake may be observed, including in brain metastases; vascular PSMA expression represents a potential therapeutic target (39,66).
Thyroid cancer	Distant mets: 9/9 (100.0) (40); LN mets: 8/12 (66.7) (40); 120/267 (44.9) (42)	IHC (PSMA; CD31/CD34)	PSMA-PET uptake predominantly vascular; therapeutic relevance particularly in RAIR disease and metastases (39,40,42,66).
Glioblastoma	32/32 (100.0) (38); 5/5 (100.0) (41); 53/69 (76.8) (54)	IHC (3E6 or other clones; CD31/VWF)	PSMA-PET and therapeutic approaches primarily target vascular PSMA expression in tumor-associated vasculature; neuro-oncological vascular targeting rationale (38,39,63).
Gastric/colorectal cancer	Gastric: 79/119 (66.4) (3); CRC: 110/130 (84.6) (3)	IHC (PSMA clone 3E6; CD31)	Frequent vascular PSMA expression supports feasibility of vascular imaging/targeting; association with tumor grade in CRC (3,39,66).

Tumor-associated vascular PSMA expression is summarized by cancer type with reported n/N (%). n/N indicates the number of cases showing vascular PSMA expression (n) out of the total number of cases evaluated (N). Reference numbers supporting vascular PSMA expression are shown in the second column. The clinical implications column cites only clinical/translation studies explicitly discussed in the main text. When n/N was not reported, this is indicated. Differences in antibodies, staining protocols and scoring preclude cross-study quantitative comparisons. ADT, androgen deprivation therapy; CRC, colorectal cancer; FDG, fluorodeoxyglucose; IF, immunofluorescence; IHC, immunohistochemistry; LN, lymph node; mCRPC, metastatic castration-resistant prostate cancer; mets, metastasis; PET, positron emission tomography; PSMA, prostate-specific membrane antigen; RAIR, radioactive iodine-refractory; RLT, radioligand therapy; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma; VWF, von Willebrand factor.

inhibitory cytokines, such as IL-10 and TGF- β (51,52). These features suggest that PSMA-positive tumors create conditions favoring immune evasion, by attenuating cytotoxic T cell activity. Reviews further emphasized that PSMA targeting could reshape the TME by reducing immunosuppressive signaling and altering myeloid balance, although direct mechanistic evidence in patients remains limited (51,53). There is

also conceptual interest in whether the enzymatic activity of PSMA in folate and glutamate metabolism can influence T cell function, an area requiring further study.

Correlations with tumor-infiltrating lymphocytes (TILs), tumor-associated macrophages and immune checkpoints. Transcriptomic and immunohistochemical studies have

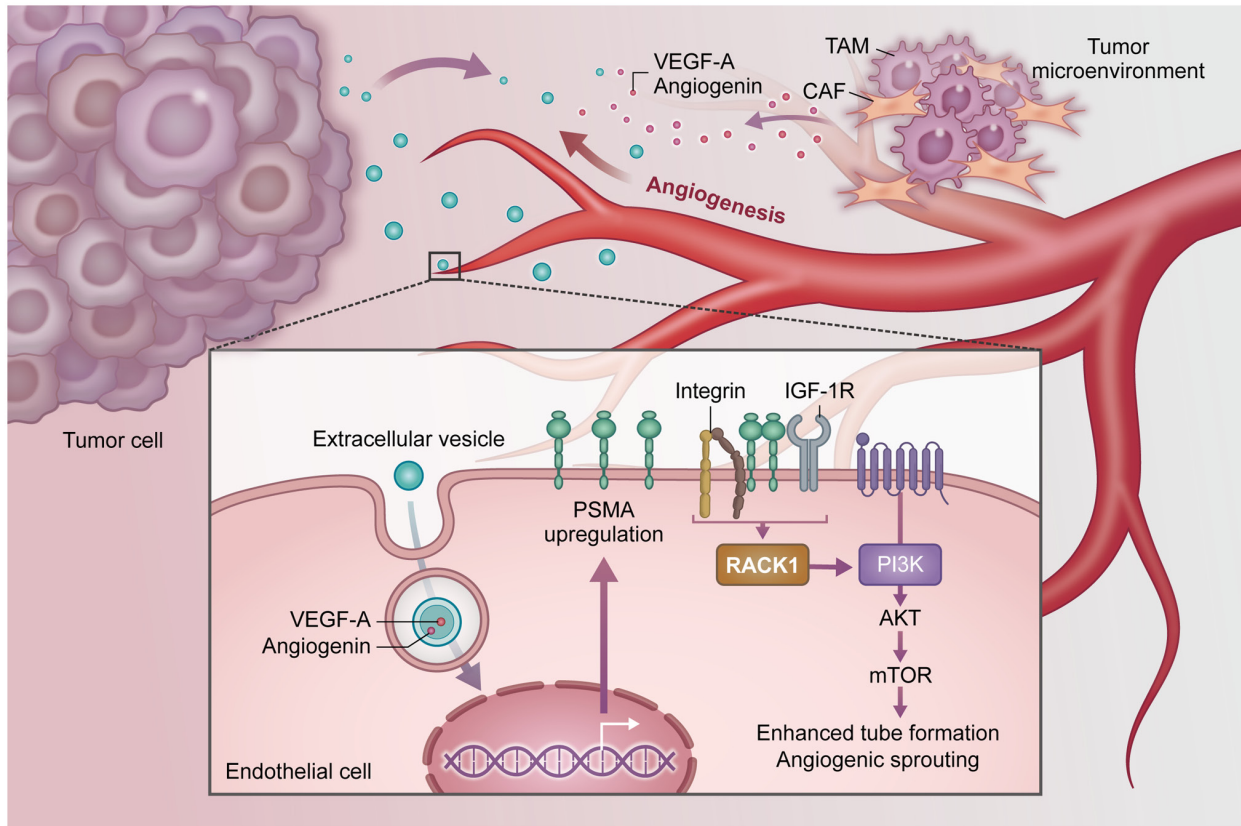


Figure 3. Proposed model of PSMA-associated angiogenic signaling in the tumor microenvironment. Schematic model illustrating how tumor-derived extracellular vesicles modulate the tumor microenvironment and induce PSMA upregulation in tumor-associated endothelial cells. PSMA-positive endothelial cells, together with other stromal components such as CAFs and TAMs, contribute to the release of pro-angiogenic factors and activation of scaffold-mediated signaling, thereby promoting endothelial activation and angiogenic sprouting. This schematic illustration was created with the support of Medical Fig (<https://medicalfig.medicaleducation.co.jp/>). CAF, cancer-associated fibroblast; IGF-1R, insulin-like growth factor 1 receptor; RACK1, receptor for activated C kinase 1; PSMA, prostate-specific membrane antigen; TAM, tumor-associated macrophage.

indicated that PSMA-positive tumors tend to harbor low levels of functional CD8⁺ TILs and an enrichment of exhausted or suppressive phenotypes (51,52). In gliomas, endothelial PSMA correlated with aberrant vascular morphology (53). Such vessels exhibit reduced adhesion molecule expression and a disorganized structure, impairing T cell trafficking and contributing to ‘vascular immune exclusion’ (54). These observations support a model in which both epithelial and endothelial PSMA indirectly reinforce an immunologically cold phenotype by altering the immune composition and restricting lymphocyte access. However, direct causal links between PSMA activity and immune modulation in patients remain largely inferential and require further experimental validation.

Potential for vascular normalization and immune checkpoint inhibitor (ICI) combination therapy. Given its vascular localization, PSMA is currently being explored as a target for vascular normalization to improve perfusion and immune infiltration, analogous to VEGF/VEGFR blockade (52). Preclinical studies have suggested that PSMA inhibition may synergize with ICIs, by reversing immune exclusion and enhancing T cell recruitment (55,56). In addition, PSMA-targeted radiopharmaceutical therapy (RPT) can exert immunomodulatory effects beyond cytotoxicity, including induction of immunogenic cell death, antigen release and activation of innate pathways

(such as cyclic GMP-AMP synthase-stimulator of interferon genes) (55-57). These mechanisms provide the rationale for ongoing trials combining RPT with ICIs, as well as the development of PSMA-targeted bispecific T-cell engagers (BiTEs) and other immunotherapies that can convert PSMA-positive tumors from immunologically ‘cold’ to ‘hot’ states (56,57).

7. Novel and exploratory functions of PSMA

Metabolic reprogramming. The enzymatic products of PSMA, glutamate and monoglutamyl folate, connect it to central metabolic pathways. Hydrolysis of NAAG elevates pericellular glutamate levels, which fuels the TCA cycle through glutaminolysis and activates mGluR1-PI3K β -AKT signaling, driving downstream phosphorylation of mTOR effectors S6K and 4EBP1 (58,59). Simultaneous parallel folate hydrolase activity generates monoglutamyl folate for uptake by PCFT and RFC, augmenting one-carbon metabolism and nucleotide synthesis whilst supplying methyl donors required for epigenetic regulation (60).

Importantly, PSMA expression is highest in CRPC, a stage marked by metabolic plasticity, suggesting that PSMA functions as a metabolic gatekeeper aligning nutrient availability with proliferative demand (18). This dual contribution to energy and biosynthetic flux positions PSMA at the intersection of metabolism, epigenetics and oncogenic signaling.

Table II. Therapeutic strategies targeting PSMA: Modalities, mechanisms and clinical development.

Modality	Examples	Mechanism	Clinical status/notes
RLT	¹⁷⁷ Lu-PSMA-617, ²²⁵ Ac-PSMA-617	Internalization → delivery of β- or α-particles	VISION trial demonstrated OS and PFS benefit; ²²⁵ Ac-PSMA-617 was effective in disease refractory to ¹⁷⁷ Lu-PSMA-617 therapy but was associated with xerostomia and cytopenia.
ADC	MLN2704, novel cleavable-linker ADCs	PSMA-mediated endocytosis → cytotoxic payload release	Early-phase trials; toxicity (neuropathy and myelosuppression) improving with next-generation designs
BiTEs	Pasotuxizumab	T-cell recruitment to PSMA ⁺ cells	Early trials: PSA declines; CRS major AE
CAR-T cells	PSMA CAR-T	Engineered T cells targeting PSMA	Antitumor activity shown; on-target/off-tumor effects in salivary glands and kidney
Nanoparticles	PSMA-targeted liposomes, supra-molecular nanoparticles	Targeted drug/siRNA delivery	Preclinical and early translational studies
Combination strategies	RLT + ICI; RLT + PARPi; epigenetic priming	Synergistic effects mediated by vascular normalization, radiosensitization and PSMA upregulation	Trials ongoing

Overview of PSMA-targeted therapeutic modalities, including RLT, ADC, bispecific antibodies, CAR-T cells and nanoparticle-based approaches, including representative agents, mechanisms of action and clinical development status. ADC, antibody-drug conjugates; AE, adverse event; BiTEs, bispecific T-cell engagers; CAR-T cells, chimeric antigen receptor T cells; CRS, cytokine release syndrome; ICI, immune checkpoint inhibitor; OS, overall survival; PARPi, poly(ADP-ribose) polymerase inhibitor; PFS, progression-free survival; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; RLT, radioligand therapy; siRNA, small interfering RNA.

Crosstalk with cancer-associated fibroblasts (CAFs) and the ECM. Although direct evidence for PSMA-CAF interactions remains limited, the role of CAFs in ECM remodeling and tumor progression is well established (61,62). Within this context, PSMA expression has been correlated with enhanced MMP-2/9 activity and altered integrin signaling to facilitate ECM degradation and invasion (63,64). Furthermore, EVs carrying PSMA can be internalized by fibroblasts, triggering the secretion of VEGF-A and FGF2 to promote a CAF-like phenotype that is characterized by α-smooth muscle actin expression and contractility (19). These changes stiffen the stroma and generate invasive tracks, suggesting that PSMA-mediated stromal reprogramming contributes to both angiogenesis and metastatic potential.

Neuro-oncological significance. In the central nervous system (CNS), PSMA can regulate synaptic NAAG turnover, generating glutamate to activate mGluR and ionotropic GluR signaling to modulate neuronal excitability (8,15). Human biochemical studies further support the neurophysiological importance of PSMA (14). These functions explain the low PSMA-tracer uptake in normal brain but increased signals under neuroinflammatory or blood-brain barrier-compromised conditions.

Beyond physiological roles, PSMA expression can be detected in both glioma cells and the tumor vasculature (41). Elevated PSMA activity may increase extracellular glutamate levels, contributing to excitotoxic neuronal injury and seizures. PSMA-derived glutamate can also promote neuron-glioma synapse formation by α-amino-3-hydroxy-5-methyl-4-isoxaz

olepropionic acid and N-methyl-D-aspartate receptor activation, now recognized to be a driver of glioma progression (63). Collectively, these findings link PSMA-mediated neurotransmitter metabolism to neuro-oncological processes and suggest therapeutic potential for PSMA inhibition in brain tumors.

8. Clinical applications and therapeutic implications

The major PSMA-targeted therapeutic strategies, including radioligand therapy, antibody-drug conjugates, bispecific antibodies, CAR-T cells and combination approaches, are summarized in Table II.

PSMA-PET/CT: Current status and limitations. PSMA-PET/CT with tracers, such as ⁶⁸Ga-PSMA-11, ¹⁸F-DCFPyL and ¹⁸F-rhPSMA-7.3, have transformed prostate cancer staging and restaging. The proPSMA trial demonstrated superior sensitivity and accuracy over conventional imaging, detecting lesions even at low PSA levels (65). This has enabled metastasis-directed treatment and improved systemic therapy selection. In this section, we focus on the current clinical performance and limitations of PSMA-PET/CT.

Key limitations of PSMA tracker include heterogeneous PSMA expression, particularly in liver metastases, physiologic uptake in salivary glands, kidneys and ganglia and restricted accessibility due to cost. Dual-tracer PET (PSMA + fludeoxyglucose F-18) and quantitative PET metrics are currently being explored to overcome these challenges and provide prognostic information.

PSMA-PET uptake in non-prostate malignancies and the role of vascular PSMA. PSMA uptake has been observed in multiple non-prostate cancers, including renal, liver, brain and thyroid malignancies (66). While initially attributed to tumor-cell expression, immunohistochemistry showed widespread PSMA in the tumor-associated vasculature (3). Epigenetic regulation and tumor-derived EVs also contribute to endothelial induction (36,5). Therefore, a substantial fraction of PSMA-PET signals in non-prostate cancers likely reflects vascular, rather than epithelial, expression. This reframes PSMA-PET as a tool for imaging angiogenesis and supports its use in selecting patients for vascular-targeted therapies.

RLT: Efficacy and resistance. ¹⁷⁷Lu-PSMA-617 (Pluvicto™) has been approved for metastatic CRPC following AR-pathway inhibition and taxanes, based on results from the VISION trial, which showed survival and progression-free survival benefits (67). The TheraP trial demonstrated higher prostate-specific antigen (PSA) response rates and lower toxicity compared with cabazitaxel (68). α -emitter ²²⁵Ac-PSMA-617 has shown efficacy in ¹⁷⁷Lu-refractory patients, with high PSA90 responses (69), though xerostomia, cytopenias and nephrotoxicity remain limiting. This toxicity profile reflects physiological PSMA expression in non-malignant tissues, most prominently in salivary and lacrimal glands, in addition to in renal proximal tubules, where off-target uptake can contribute to salivary gland dysfunction and renal toxicity.

Resistance arises from PSMA downregulation, altered trafficking or lineage plasticity. Combination strategies with poly (ADP-ribose) polymerase inhibitors, AR blockade, DNA repair modulators and radiosensitizers are currently being investigated to overcome resistance.

Next-generation immuno- and cell-based therapeutics. PSMA is an appealing target for ADCs due to its tumor selectivity and internalization. First-generation constructs, such as MLN2704, were however limited by toxicity, but newer designs with optimized linkers and payloads are in development (1). BiTEs, such as pasotuzumab, have achieved durable PSA declines in early trials, though cytokine release syndrome remains a challenge (69). Chimeric antigen receptor (CAR) T-cell therapy cells targeting PSMA showed antitumor activity but raised concerns of on-target/off-tumor effects in salivary glands and kidneys. Logic-gated or transient CAR approaches are also currently being explored to mitigate risk.

Vascular PSMA as a therapeutic target. Consistent vascular expression of PSMA enables anti-angiogenic therapy across various tumor types. Unlike VEGF/VEGFR inhibitors, which frequently face obstructions of adaptive resistance, PSMA-targeted approaches can ablate PSMA-positive vessels or deliver drugs directly to the vasculature. Preclinical studies with PSMA-targeted liposomal doxorubicin and vascular-directed RLT have revealed their abilities of vessel ablation and tumor necrosis (45). Combinations with ICIs, VEGF blockade or CAR-T therapies may further normalize vasculature, enhance immune infiltration and improve tumor control.

Linking molecular function to patient selection and therapy design. PSMA expression is dynamic and can be pharmacologically modulated. Short-term ADT induces a transient ‘PSMA flare’, enhancing PET detectability and radioligand uptake (70,71). Epigenetic interventions, such as HDAC inhibition, can restore PSMA expression in low-expressing tumors (36). These approaches expand patient eligibility and support personalized strategies in which PSMA expression is actively enhanced before imaging or therapy.

Toward a universal anti-angiogenic strategy. Because vascular PSMA expression is pan-cancer, it represents a potential universal target for angiogenesis-directed therapy. Selective ablation of abnormal PSMA-positive vessels could reduce hypoxia-driven aggressiveness whilst providing a route for vascular-specific drug delivery. In combination with immunotherapy, PSMA vascular targeting may normalize the vasculature, promote immune infiltration and produce durable antitumor responses, positioning PSMA as a candidate for broad-spectrum anti-angiogenic strategies.

9. Conclusions

In conclusion, this review revisits PSMA through a unified conceptual framework in which its metabolic enzyme activity, non-enzymatic scaffold signaling, tumor-associated angiogenesis and immunological consequences are functionally interconnected, providing a coherent biological basis for its diverse clinical manifestations and therapeutic potential.

PSMA has progressed from being considered only as a biomarker of prostate cancer to being recognized as an active driver of tumor biology. Its dual role as a glutamate/folate-processing ectoenzyme and as a signaling scaffold situates PSMA at the crossroads of tumor metabolism, angiogenesis, immune modulation and therapeutic response. Evidence across preclinical and clinical studies shows that PSMA can contribute to remodeling the TME, enhancing neovascularization, supporting stromal activation and shaping immune cell infiltration, thereby exerting a functional influence beyond its diagnostic utility.

Looking forward, several priorities emerge for advancing PSMA biology toward clinical translation. First, a deeper mechanistic understanding of PSMA heterogeneity, including alternative splicing, functional loss of surface expression and context-dependent regulation, will be essential to explain discordant imaging findings and variable therapeutic responses. Second, clarifying how PSMA-driven angiogenic signaling interfaces with canonical VEGF/VEGFR pathways will inform rational combinations of PSMA-targeted and anti-angiogenic therapies. Third, strategies to mitigate off-target toxicities, particularly salivary gland and renal uptake in RLT, remain critical for improving treatment durability and patient quality of life.

At the same time, advances in precision medicine are reshaping the therapeutic landscape of prostate cancer. PSMA-targeted RLT now joins PARP inhibition, immune checkpoint blockade and AKT inhibitors as components of biomarker-driven treatment, whilst novel strategies for molecular subsets, such as CDK12-altered or neuroendocrine tumors are emerging. The integration of liquid biopsy,

multi-omics approaches and AI-assisted imaging-genomics fusion promises to refine patient selection and expand the reach of personalized medicine.

Taken together, PSMA should be viewed not only as a diagnostic tracer target, but as a versatile biological switch and therapeutic entry point capable of influencing multiple hallmarks of cancer. The convergence of PSMA-targeted modalities with systemic therapies and precision medicine strategies offers a realistic path to improve outcomes in prostate cancer and potentially other solid tumors. Whether PSMA ultimately fulfills its promise as a pan-cancer anti-angiogenic and immunomodulatory target will depend on sustained translational efforts in the coming decade, but its central role in tumor biology is now firmly established.

Acknowledgements

Not applicable.

Funding

This work was supported by JSPS KAKENHI (grant nos. 22K09449 and 22KK0135), Nippon Shinyaku Research Grant, and Sumitomo Foundation Grant for Basic Science Research Projects.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

RW wrote the manuscript. NM and TK revised the manuscript. ToS and TaS contributed to the conceptualization of the study and critically revised the manuscript. RW and NM confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Ehime University Hospital (IRB no. 2201011; Toon, Japan). Informed consent was obtained from all participants using an opt-out approach in accordance with institutional guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Maes J, Gesquière S, De Spiegeleer A, Maes A and Van de Wiele C: Prostate-specific membrane antigen biology and pathophysiology in prostate carcinoma, an update: Potential implications for targeted imaging and therapy. *Int J Mol Sci* 25: 9755, 2024.
2. Hyväkkä A, Virtanen V, Kempainen J, Grönroos TJ, Minn H and Sundvall M: More than meets the eye: Scientific rationale behind molecular imaging and therapeutic targeting of prostate-specific membrane antigen (PSMA) in metastatic prostate cancer and beyond. *Cancers (Basel)* 13: 2244, 2021.
3. Haffner MC, Kronberger IE, Ross JS, Sheehan CE, Zitt M, Mühlmann G, Ofner D, Zelger B, Ensinger C, Yang XJ, *et al*: Prostate-specific membrane antigen expression in the neovascularization of gastric and colorectal cancers. *Hum Pathol* 40: 1754-1761, 2009.
4. Van de Wiele C, Sathekge M, de Spiegeleer B, De Jonghe PJ, Debryne PR, Borms M, Beels L and Maes A: PSMA expression on neovascularization of solid tumors. *Histol Histopathol* 35: 919-927, 2020.
5. Watanabe R, Maekawa M, Kiyoi T, Kurata M, Miura N, Kikugawa T, Higashiyama S and Saika T: PSMA-positive membranes secreted from prostate cancer cells have potency to transform vascular endothelial cells into an angiogenic state. *Prostate* 81: 1390-1401, 2021.
6. Watanabe R, Kagimoto K, Chosei M, Sakaue T, Kurata M, Miura N, Kitazawa R, Kikugawa T, Higashiyama S and Saika T: Vesicles secreted by renal cell carcinoma cells cause vascular endothelial cells to express PSMA and drive tumor progression. *Cells* 14: 165, 2025.
7. Israeli RS, Powell CT, Fair WR and Heston WD: Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. *Cancer Res* 53: 227-230, 1993.
8. Bzduga T, Turi T, Wroblewska B, She D, Chung HS, Kim H and Neale JH: Molecular cloning of a peptidase against N-acetylaspartylglutamate from a rat hippocampal cDNA library. *J Neurochem* 69: 2270-2277, 1997.
9. Pinto JT, Suffoletto BP, Berzin TM, Qiao CH, Lin S, Tong WP, May F, Mukherjee B and Heston WD: Prostate-specific membrane antigen: A novel folate hydrolase in human prostatic carcinoma cells. *Clin Cancer Res* 2: 1445-1451, 1996.
10. Davis MI, Bennett MJ, Thomas LM and Bjorkman PJ: Crystal structure of prostate-specific membrane antigen, a tumor marker and peptidase. *Proc Natl Acad Sci USA* 102: 5981-5986, 2005.
11. Barinka C, Rovenská M, Mlcochová P, Hlouchová K, Plechanovová A, Majer P, Tsukamoto T, Slusher BS, Konvalinka J and Lubkowski J: Structural insight into the pharmacophore pocket of human glutamate carboxypeptidase II. *J Med Chem* 50: 3267-3273, 2007.
12. Liu H, Rajasekaran AK, Moy P, Xia Y, Kim S, Navarro V, Rahmati R and Bander NH: Constitutive and antibody-induced internalization of prostate-specific membrane antigen. *Cancer Res* 58: 4055-4060, 1998.
13. Anilkumar G, Rajasekaran SA, Wang S, Hankinson O, Bander NH and Rajasekaran AK: Prostate-specific membrane antigen association with filamin A modulates its internalization and NAALADase activity. *Cancer Res* 63: 2645-2648, 2003.
14. Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE and Coyle JT: Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch Gen Psychiatry* 52: 829-836, 1995.
15. Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Harukuni I, Bhardwaj A, Traystman RJ, Robinson MB, Britton P, Lu XC, *et al*: Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. *Nat Med* 5: 1396-1402, 1999.
16. Caromile LA, Dortche K, Rahman MM, Grant CL, Stoddard C, Ferrer FA and Shapiro LH: PSMA redirects cell survival signaling from the MAPK to the PI3K-AKT pathways to promote the progression of prostate cancer. *Sci Signal* 10: eaag3326, 2017.
17. Boixareu C, Taha T, Venkadakrishnan VB, de Bono J and Beltran H: Targeting the tumour cell surface in advanced prostate cancer. *Nat Rev Urol* 22: 569-589, 2025.
18. Sedlák F, Kvasnička A, Marešová B, Brumarová R, Dobešová D, Dostálová K, Šrámková K, Pehr M, Šácha P, Friedecký D and Konvalinka J: Parallel metabolomics and lipidomics of a PSMA/GCPII deficient mouse model reveal alteration of NAAG levels and brain lipid composition. *ACS Chem Neurosci* 15: 1342-1355, 2024.
19. Pellegrino S and Fonti R: A look into the future: The role of PSMA beyond prostate cancer. *Eur J Nucl Med Mol Imaging* 51: 278-280, 2023.
20. Rajasekaran SA, Anilkumar G, Oshima E, Bowie JU, Liu H, Heston W, Bander NH and Rajasekaran AK: A novel cytoplasmic tail MXXXL motif mediates the internalization of prostate-specific membrane antigen. *Mol Biol Cell* 14: 4835-4845, 2003.

21. Zhang X, Qi S, Liu D, Du J and Jin J: PSMA-Targeted supra-molecular nanoparticles prepared from cucurbit[8]uril-based ternary host-guest recognition for prostate cancer therapy. *Front Chem* 10: 847523, 2022.
22. Minner S, Wittmer C, Graefen M, Salomon G, Steuber T, Haese A, Huland H, Bokemeyer C, Yekebas E, Dierlamm J, *et al*: High level PSMA expression is associated with early PSA recurrence in surgically treated prostate cancer. *Prostate* 71: 281-288, 2011.
23. Meller B, Bremmer F, Sahlmann CO, Hijazi S, Bouter C, Trojan L, Meller J and Thelen P: Alterations in androgen deprivation enhanced prostate-specific membrane antigen (PSMA) expression in prostate cancer cells as a target for diagnostics and therapy. *EJNMMI Res* 5: 66, 2015.
24. Calais J, Kishan AU, Cao M, Fendler WP, Eiber M, Herrmann K, Ceci F, Reiter RE, Rettig MB, Hegde JV, *et al*: Potential impact of 68Ga-PSMA-11 PET/CT on the planning of definitive radiation therapy for prostate cancer. *J Nucl Med* 59: 1714-1721, 2018.
25. Staal J and Beyaert R: Inflammation and NF- κ B signaling in prostate cancer: Mechanisms and clinical implications. *Cells* 7: 122, 2018.
26. Basílio J, Hochreiter B, Hoesel B, Sheshori E, Mussbacher M, Hanel R and Schmid JA: Antagonistic functions of androgen receptor and NF- κ B in prostate cancer-experimental and computational analyses. *Cancers (Basel)* 14: 6164, 2022.
27. Watt F, Martorana A, Brookes DE, Ho T, Kingsley E, O'Keefe DS, Russell PJ, Heston WD and Molloy PL: A tissue-specific enhancer of the prostate-specific membrane antigen gene, FOLH1. *Genomics* 73: 243-254, 2001.
28. Noss KR, Wolfe SA and Grimes SR: Upregulation of prostate specific membrane antigen/folate hydrolase transcription by an enhancer. *Gene* 285: 247-256, 2002.
29. Gao Y, Zheng H, Li L, Feng M, Chen X, Hao B, Lv Z, Zhou X and Cao Y: Prostate-specific membrane antigen (PSMA) promotes angiogenesis of glioblastoma through interacting with ITGB4 and regulating NF- κ B signaling pathway. *Front Cell Dev Biol* 9: 598377, 2021.
30. Perico ME, Grasso S, Brunelli M, Martignoni G, Munari E, Moiso E, Fracasso G, Cestari T, Naim HY, Bronte V, *et al*: Prostate-specific membrane antigen (PSMA) assembles a macromolecular complex regulating growth and survival of prostate cancer cells 'in vitro' and correlating with progression 'in vivo'. *Oncotarget* 7: 74189-74202, 2016.
31. Ghosh A, Wang X, Klein E and Heston WD: Novel role of prostate-specific membrane antigen in suppressing prostate cancer invasiveness. *Cancer Res* 65: 727-731, 2005.
32. Silver DA, Pellicer I, Fair WR, Heston WD and Cordon-Cardo C: Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res* 3: 81-85, 1997.
33. Bakht MK, Yamada Y, Ku SY, Venkadakrishnan VB, Korsen JA, Kalidindi TM, Mizuno K, Ahn SH, Seo JH, Garcia MM, *et al*: Landscape of prostate-specific membrane antigen heterogeneity and regulation in AR-positive and AR-negative metastatic prostate cancer. *Nat Cancer* 4: 699-715, 2023.
34. Damjanovic J, Janssen JC, Prasad V, Diederichs G, Walter T, Brenner W and Makowski MR: 68Ga-PSMA-PET/CT for the evaluation of liver metastases in patients with prostate cancer. *Cancer Imaging* 19: 37, 2019.
35. Mattoni S, Farolfi A, Formaggio F, Bruno G, Caroli P, Cerci JJ, Eiber M, Fendler WP, Golfieri R, Herrmann K, *et al*: PSMA PET for the evaluation of liver metastases in castration-resistant prostate cancer patients: A multicenter retrospective study. *Cancers (Basel)* 14: 5680, 2022.
36. Sayar E, Patel RA, Coleman IM, Roudier MP, Zhang A, Mustafi P, Low JY, Hanratty B, Ang LS, Bhatia V, *et al*: Reversible epigenetic alterations mediate PSMA expression heterogeneity in advanced metastatic prostate cancer. *JCI Insight* 8: e162907, 2023.
37. Hope TA, Eiber M, Armstrong WR, Juarez R, Murthy V, Lawhn-Heath C, Behr SC, Zhang L, Barbato F, Ceci F, *et al*: Diagnostic accuracy of 68Ga-PSMA-11 PET for pelvic nodal metastasis detection prior to radical prostatectomy and pelvic lymph node dissection: A multicenter prospective phase 3 imaging trial. *JAMA Oncol* 7: 1635-1642, 2021.
38. Wernicke AG, Edgar MA, Lavi E, Liu H, Salerno P, Bander NH and Gutin PH: Prostate-specific membrane antigen as a potential novel vascular target for treatment of glioblastoma multiforme. *Arch Pathol Lab Med* 135: 1486-1489, 2011.
39. Fragomeni RA, Amir T, Sheikhabaei S, Harvey SC, Javadi MS, Solnes LB, Kiess AP, Allaf ME, Pomper MG, Gorin MA and Rowe SP: Imaging of nonprostate cancers using PSMA-targeted radiotracers: Rationale, current state of the field, and a call to arms. *J Nucl Med* 59: 871-877, 2018.
40. Moore M, Panjwani S, Mathew R, Crowley M, Liu YF, Aronova A, Finnerty B, Zarnegar R, Fahey TJ III and Scognamiglio T: Well-differentiated thyroid cancer neovasculature expresses prostate-specific membrane antigen-a possible novel therapeutic target. *Endocr Pathol* 28: 339-344, 2017.
41. Nomura N, Pastorino S, Jiang P, Lambert G, Crawford JR, Gymnopoulos M, Piccioni D, Juarez T, Pingle SC, Makale M and Kesari S: Prostate specific membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. *Cancer Cell Int* 14: 26, 2014.
42. Bychkov A, Vutrapongwatana U, Tepmongkol S and Keelawat S: PSMA expression by microvasculature of thyroid tumors-Potential implications for PSMA theranostics. *Sci Rep* 7: 5202, 2017.
43. Kasoha M, Unger C, Solomayer EF, Bohle RM, Zaharia C, Khreich F, Wagenpfeil S and Juhasz-Böss I: Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases. *Clin Exp Metastasis* 34: 479-490, 2017.
44. Schmidt LH, Heitkötter B, Schulze AB, Schliemann C, Steinestel K, Trautmann M, Marra A, Hillejan L, Mohr M, Evers G, *et al*: Prostate specific membrane antigen (PSMA) expression in non-small cell lung cancer. *PLoS One* 12: e0186280, 2017.
45. Machado CML, Skubal M, Haedicke K, Silva FP, Stater EP, Silva TLAO, Costa ET, Masotti C, Otake AH, Andrade LNS, *et al*: Membrane-derived particles shed by PSMA-positive cells function as pro-angiogenic stimuli in tumors. *J Control Release* 364: 312-325, 2023.
46. Conway RE, Petrovic N, Li Z, Heston W, Wu D and Shapiro LH: Prostate-specific membrane antigen regulates angiogenesis by modulating integrin signal transduction. *Mol Cell Biol* 26: 5310-5324, 2006.
47. Grant CL, Caromile LA, Ho V, Durrani K, Rahman MM, Claffey KP, Fong GH and Shapiro LH: Prostate specific membrane antigen (PSMA) regulates angiogenesis independently of VEGF during ocular neovascularization. *PLoS One* 7: e41285, 2012.
48. Li Y, Zhang K, Yang F, Jiao D, Li M, Zhao X, Xu C, Liu S, Li H, Shi S, *et al*: Prognostic value of vascular-expressed PSMA and CD248 in urothelial carcinoma of the bladder. *Front Oncol* 11: 771036, 2021.
49. Schreiber H, Hänze J, Nimphius W, Verburg FA, Luster M, Hofmann R and Hegele A: Prostate specific membrane antigen (PSMA) in urothelial cell carcinoma (UCC) is associated with tumor grading and staging. *J Cancer Res Clin Oncol* 146: 305-313, 2020.
50. Lin BH, Chen SH, Chen SM, Qiu QR, Gao RC, Wei Y, Zheng QS, Miao WB and Xu N: Head-to-head comparisons of 68Ga-PSMA-11 and 18F-FDG PET/CT in evaluating patients with upper tract urothelial carcinoma: A prospective pilot study. *Int Urol Nephrol* 55: 2753-2764, 2023.
51. Liu D, Wang L and Guo Y: Advances in and prospects of immunotherapy for prostate cancer. *Cancer Lett* 601: 217155, 2024.
52. Ge R, Wang Z and Cheng L: Tumor microenvironment heterogeneity an important mediator of prostate cancer progression and therapeutic resistance. *NPJ Precis Oncol* 6: 31, 2022.
53. Puik JR, Le C, Kazemier G, Oprea-Lager DE, Swijnenburg RJ, Giovannetti E, Griffioen AW and Huijbers EJ: Prostate-specific membrane antigen as target for vasculature-directed therapeutic strategies in solid tumors. *Crit Rev Oncol Hematol* 205: 104556, 2025.
54. Maguid MS, Saad El Dine MG, Gabal SM and Fandoud SM: Prostate-specific membrane antigen (PSMA) expression in the neovasculature of high grade gliomas (Histopathological and Immunohistochemical Study). *Asian Pac J Cancer Prev* 24: 1797-1808, 2023.
55. Bellavia MC, Patel RB and Anderson CJ: Combined targeted radiopharmaceutical therapy and immune checkpoint blockade: From preclinical advances to the clinic. *J Nucl Med* 63: 1636-1641, 2022.
56. Altunay B, Schäfer L, Morgenroth A, Peña Q, Lammers T, Saar M, Mottaghy FM and Lütje S: Combining PSMA-targeted radiopharmaceutical therapy with immunotherapy. *J Nucl Med* 28: 1522-1527, 2025.
57. Jiao R and Dadachova E: Combination of radioligand therapy and immunotherapy: How to make it work in clinic? *Immunotargets Ther* 14: 755-759, 2025.
58. Sheehan B, Guo C, Neeb A, Paschalis A, Sandhu S and de Bono JS: Prostate-specific membrane antigen biology in lethal prostate cancer and its therapeutic implications. *Eur Urol Focus* 8: 1157-1168, 2022.

59. Palamiuc L and Emerling BM: PSMA brings new flavors to PI3K signaling: A role for glutamate in prostate cancer. *J Exp Med* 215: 17-19, 2018.
60. O'Keefe DS, Bacich DJ, Huang SS and Heston WDW: A perspective on the evolving story of PSMA biology, PSMA-based imaging, and endoradiotherapeutic strategies. *J Nucl Med* 59: 1007-1013, 2018.
61. Wright K, Ly T, Kriet M, Czirok A and Thomas SM: Cancer-associated fibroblasts: Master tumor microenvironment modifiers. *Cancers (Basel)* 15: 1899, 2023.
62. Bonollo F, Thalmann GN, Kruithof-de Julio M and Karkampouna S: The role of cancer-associated fibroblasts in prostate cancer tumorigenesis. *Cancers (Basel)* 12: 1887, 2020.
63. McBriar JD, Shafian N, Scharf S, Boockvar JA and Wernicke AG: Prostate-specific membrane antigen use in glioma management: Past, present, and future. *Clin Nucl Med* 49: 806-816, 2024.
64. Hameed MY, Gul M, Chaudhry A, Muzaffar H, Sheikh M, Chee W, Ayyash S, Ayyash J, Al-Hindi M, Shahare H and Chaudhry A: From oncogenesis to theranostics: The transformative role of PSMA in prostate cancer. *Cancers (Basel)* 16: 3039, 2024.
65. Hofman MS, Lawrentschuk N, Francis RJ, Tang C, Vela I, Thomas P, Rutherford N, Martin JM, Frydenberg M, Shakher R, *et al*: Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): A prospective, randomised, multicentre study. *Lancet* 395: 1208-1216, 2020.
66. de Galiza Barbosa F, Queiroz MA, Nunes RF, Costa LB, Zaniboni EC, Marin JFG, Cerri GG and Buchpiguel CA: Nonprostatic diseases on PSMA PET imaging: A spectrum of benign and malignant findings. *Cancer Imaging* 20: 23, 2020.
67. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, Tagawa ST, Nordquist LT, Vaishampayan N, El-Haddad G, *et al*: Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. *N Engl J Med* 385: 1091-1103, 2021.
68. Kratochwil C, Bruchertseifer F, Rathke H, Bronzel M, Apostolidis C, Weichert W, Haberkorn U, Giesel FL and Morgenstern A: Targeted α -therapy of metastatic castration-resistant prostate cancer with ^{225}Ac -PSMA-617: Dosimetry estimate and empiric dose finding. *J Nucl Med* 58: 1624-1631, 2017.
69. Sridaran D, Bradshaw E, DeSelm C, Pachynski R, Mahajan K and Mahajan NP: Prostate cancer immunotherapy: Improving clinical outcomes with a multi-pronged approach. *Cell Rep Med* 4: 101199, 2023.
70. Ettala O, Malaspina S, Tuokkola T, Luoto P, Löyttyniemi E, Boström PJ and Kemppainen J: Prospective study on the effect of short-term androgen deprivation therapy on PSMA uptake evaluated with ^{68}Ga -PSMA-11 PET/MRI in men with treatment-naïve prostate cancer. *Eur J Nucl Med Mol Imaging* 47: 665-673, 2020.
71. Vaz S, Hadaschik B, Gabriel M, Herrmann K, Eiber M and Costa D: Influence of androgen deprivation therapy on PSMA expression and PSMA-ligand PET imaging of prostate cancer patients. *Eur J Nucl Med Mol Imaging* 47: 9-15, 2020.



Copyright © 2026 Watanabe et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.