Is prostate-specific membrane antigen a multifunctional protein?

Ayyappan K. Rajasekaran, Gopalakrishnapillai Anilkumar, and Jason J. Christiansen

Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, California

Prostate-specific membrane antigen (PSMA) was originally defined by the monoclonal antibody (MAb) 7E11 derived from immunization with a partially purified membrane preparation from the lymph node prostatic adenocarcinoma (LNCaP) cell line (26). A 2.65-kb cDNA fragment encoding the PSMA protein was cloned and subsequently mapped to chromosome 11p11.2 (28, 43).

Initial analysis of PSMA demonstrated widespread expression within the cells of the prostatic secretory epithelium. Immunohistochemical staining demonstrated that PSMA was absent to moderately expressed in hyperplastic and benign tissues, while malignant tissues stained with the greatest intensity (26). Subsequent investigations have recapitulated these results and evinced PSMA expression as a universal feature in practically every prostatic tissue examined to date. These results further demonstrate that expression of PSMA increases precipitously proportional to tumor aggressiveness (9, 13, 14, 30, 34, 50, 55, 64, 66, 71).

Consistent with the correlation between PSMA expression and tumor stage, increased levels of PSMA are associated with androgen-independent prostate cancer (PCa). Analysis of tissue samples from patients with prostate cancer has demonstrated elevated PSMA levels after physical castration or androgen-deprivation therapy. Unlike expression of prostate-specific antigen, which is downregulated after androgen ablation, PSMA expression is significantly increased in both primary and metastatic tumor specimens (30, 71). Consistent with the elevated expression in androgen-independent tumors, PSMA transcription is also known to be downregulated by steroids, and administration of testosterone mediates a dramatic reduction in PSMA protein and mRNA levels (27, 71). PSMA is also highly expressed in secondary prostatic tumors and occult metastatic disease. Immunohistochemical analysis has revealed relatively intense and homogeneous expression of PSMA within metastatic lesions localized to lymph nodes, bone, soft tissue, and lungs compared with benign prostatic tissues (14, 40, 64).

Some reports have also indicated limited PSMA expression in extraprostatic tissues, including a subset of renal proximal tubules, some cells of the intestinal brush-border membrane, and rare cells in the colonic crypts (13, 26, 27, 36, 66). However, the levels of PSMA in these tissues are generally two to three orders of magnitude less than those observed in the prostate (58). PSMA is also expressed in the tumor-associated neovasculature of most solid cancers examined yet is absent in the normal vascular endothelium (13, 34, 55). Although the significance of PSMA expression within the vasculature is unknown, the specificity for tumor-associated endothelium makes PSMA an intriguing potential target for the treatment of many forms of malignancy.

The highly restricted expression of PSMA and its upregulation in advanced carcinoma and metastatic disease portend a promising role for PSMA as a clinical biomarker for the diagnosis, detection, and management of prostate cancer. Furthermore, as an integral membrane protein, PSMA can be exploited as an antigenic target for a variety of clinical applications (19).

Immunoscintigraphic scanning using an $^{111}$In-labeled form of MAB 7E11 has shown promise for the detection and in vivo imaging of PSMA-expressing tumor cells. This antibody has received approval from the U.S. Food and Drug Administration for the detection and imaging of metastatic prostate cancer in soft tissues and is currently marketed under the brand name ProstaScint (Cytogen, Philadelphia, PA) (24, 36, 49). However, positive signals detected using this technology are ascribed as likely being due to the immunoreactivity of this antibody in dead or dying cells within a tumor mass because MAB 7E11 recognizes an intracellular epitope and is incapable of binding to viable cells (67). This observation provides a
rationale to explain why ProstaScint is more effective at identifying metastases in well-vascularized soft tissues than in bone, in which metastatic lesions tend to be relatively small and do not characteristically have a high percentage of necrotic or apoptotic cells. Development and application of antibodies that recognize epitopes encoded within the extracellular domain of PSMA substantially enhanced the sensitivity, and should improve the usefulness, of PSMA-based in vivo imaging techniques (24, 34).

In addition to in vivo imaging strategies, the use of PSMA-specific MAbs is also being assessed for therapeutic purposes. PSMA-specific MAbs have been conjugated to radionuclides and cytotoxic drugs (5, 6, 17, 38, 41). Such MAbs can be exploited as a vehicle with which to deliver concentrated doses of therapeutic agents directly to the site of prostate tumor cells while sparing damage to normal tissues. These antibodies can induce cell death specifically in PSMA-expressing cells and can reduce the size of LNCaP spheroids in vitro (57). Furthermore, administration of a single dose of radioactively labeled PSMA-specific MAbs was able to achieve a 15–90% reduction in mean tumor volume in xenograft-bearing mice, concomitant with a two- to threefold increase in median survival time relative to untreated control mice (69).

Despite the potential use of PSMA for immunotherapy of PCa, a paucity of information exists regarding the physiological functions of this protein. The purpose of this review is to highlight the possible functions of PSMA on the basis of its structural and enzymatic properties, cellular localization, trafficking route, interacting partners, and information gathered from related proteins.

STRUCTURE OF PSMA

The PSMA gene consists of 19 exons that span ~60 kb of genomic DNA. This gene encodes a type II transmembrane protein with a short NH2-terminal cytoplasmic tail (19 amino acids), a single hydrophobic transmembrane domain (24 amino acids), and a large extracellular domain (707 amino acids) at the COOH terminus (Fig. 1) (28, 43).

The extracellular domain of PSMA is highly glycosylated, with linked oligosaccharides accounting for up to 25% of the molecular weight of the native protein (25). Regions within this domain share modest degrees of homology with the transferrin receptor (TIR) (28) and with members of the M28 family of cocatalytic aminopeptidases (47). Although the TIR has only a vestigial catalytic site, PSMA is known to possess both N-acetylated, α-linked acidic dipeptidase (NAALADase) and folate hydrolase (FOLH) activities (11, 45). These two related peptidase activities hydrolyze γ-peptide bonds between N-acetylaspartate and glutamate in the abundant neuropeptide N-acetylaspartylglutamate (NAAG) and the γ-glutamyl linkages in pteroylpolyglutamate, respectively. Thus this enzyme has been referred to alternatively as glutamate carboxypeptidase II (GCP-II) and folate hydrolase 1 (FOLH1). The enzymatic activity of PSMA is largely inhibited phosphate, even at millimolar concentrations (56), and is dependent on glycosylation and dimerization for proper function (18, 53). In contrast to the large extracellular domain, the cytoplasmic tail of PSMA consists of just 19 amino acids. In spite of its diminutive stature, the cytoplasmic domain interacts with a number of proteins and has a major impact on the localization and molecular properties of PSMA (2, 46).

Evidence obtained using RT-PCR has suggested the existence of alternative PSMA isoforms, including PSM and recently described PSM-B and PSM-C. In contrast to the integral transmembrane orientation of full-length PSMA, these variants are thought to exist within the cytosol and to be the consequence of alternative splicing of the PSMA gene (52, 60). Although reports have suggested that the ratio of transmembrane to cytosolic PSMA transcript increases proportionally with advancing cancer grade, little is known regarding the significance of alternatively spliced PSMA mRNA.

A murine homolog to PSMA also has been identified and is referred to as either GCP-II or Folh1. This protein shares >80% amino acid identity with human PSMA within the extracellular domain and possesses the same enzymatic peptidase activities as human PSMA (3). Interestingly, while Tsai et al. (68) reported that ablation of Folh1/GCP-II resulted in embryonic lethality, Bacich et al. (4) reported that Folh1/GCP-II-knockout mice experienced no detectable detriment. The reasons for this discrepancy are not completely clear, highlighting the need for caution in attempting to generalize data...
from mouse models to human PSMA. In addition, these two homologs display disparate profiles of tissue expression (3) and deletion of Folh1/GCP-II gene expression did not result in loss of NAALADase activity, suggesting the functional redundancy of this enzyme in murine cells (4). Furthermore, while PSMA and Folh1/GCP-II share significant homology within their respective extracellular domains, these proteins have minimal conservation of sequence homology within their cytoplasmic domains, including domains involved in mediating PSMA endocytic traffic and binding of interacting partners such as filamin A (FLNa) (2, 3, 46).

**DIMERIZATION OF PSMA**

Homodimerization is a fundamental feature of many transmembrane receptors. Induction of homodimer formation is often induced by ligand binding, which is in turn necessary for mediating the cellular response of the receptor (51). The TIR is an archetypal example of one such receptor. This type II transmembrane protein is involved in regulating cellular iron homeostasis through binding and internalization of iron-laden transferrin (1).

PSMA shares homology with the TIR at the levels of both amino acid identity and domain organization (37). Like the TIR, PSMA is expressed as a noncovalently linked homodimer on the cell surface (33, 53). This dimerization is apparently mediated by epitopes within the large extracellular domain, because truncated versions of PSMA lacking the cytoplasmic and transmembrane domains are still capable of interacting. PSMA dimerization is critical to maintaining the conformation and enzymatic activity of PSMA (53). Although the possibility has yet to be addressed fully, the similarity between PSMA and TIR at the amino acid and structural levels, combined with the common dimerization requirement, may suggest that these proteins share similar receptor and ligand transport functions.

**RESEMBLANCE OF PSMA CELLULAR TRAFFICKING WITH MEMBRANE RECEPTORS**

A variety of transmembrane receptors and membrane components are internalized from the plasma membrane and trafficked through the endocytic system. This endocytic trafficking allows cells to maintain homeostasis and internalize vital nutrients, lipids, and proteins. For example, binding of iron-bound transferrin to the TIR results in an induction of receptor internalization and iron transport into the cell (31). In addition, endocytosis of membrane receptors is also an established mechanism to downregulate signal transduction cascades. One classic example is the regulation of epidermal growth factor receptor (EGFR) signaling. Binding of epidermal growth factor (EGF) induces EGFR endocytosis and signal attenuation (12).

Like the transferrin and EGF receptors, PSMA undergoes endocytosis from the plasma membrane. This endocytosis occurs through clathrin-coated pits and involves the first five NH$_2$-terminal amino acids of the cytoplasmic tail. This MWWLL motif appears to constitute a novel endocytic targeting signal and likely interacts with the activator protein-2 (AP-2) adaptor protein complex (46). Although PSMA is constitutively internalized from the cell surface, binding of antibodies or related antibody fragments to the extracellular domain increases the rate of PSMA internalization (35). These antibodies may act like a natural ligand, indicating that, like the TIR, PSMA may have a receptor function involved in endocytosis of a putative unknown ligand.

Interestingly, the NAALADase activity of PSMA is inhibited by the millimolar concentration of phosphate present in culture medium (65). Therefore, because internalization assays are performed under normal culture conditions, it appears that NAALADase activity is not required for the internalization function of PSMA. In addition, NAAG, a well-known substrate of PSMA, did not increase the rate of PSMA internalization in PCa cells (unpublished data).

After endocytosis, a number of receptors are recycled back to the plasma membrane surface. While some proteins are recycled directly from early endosomes, other receptors are first targeted to a tubulovesicular membrane structure proximal to the centrosomes, a structure that is referred to as the recycling endosomal compartment (REC) (39, 48). The TIR is one of the best studied markers of the REC. After internalization, PSMA is targeted to the REC with kinetics similar to those of the TIR (2, 46).

The antibody-induced, clathrin-mediated internalization of PSMA and the accumulation in the REC support the hypothesis that PSMA might function as a receptor internalizing a putative ligand. Whether PSMA acts in a manner analogous to the TIR in the transport or metabolism of specific elements or similarly to the EGFR in the regulation of signal transduction has yet to be addressed. However, future studies to identify the ligand of PSMA are crucial to answering this question.

**ASSOCIATION OF PSMA WITH FLNA, AN ACTIN FILAMENT CROSS-LINKING PROTEIN**

FLNa is a dimeric actin cross-linking phosphoprotein that plays a vital role in the stabilization of many receptors at the plasma membrane (59). It is known that many membrane receptors, such as the metabotropic glutamate receptor, dopamine receptor, calcitonin receptor, tumor necrosis factor receptor, and insulin receptor, interact with FLNa. The interaction between FLNa and these receptors plays a crucial role in modulating receptor function (16, 23, 54).

With the 19 NH$_2$-terminal amino acids used as bait, the cytoplasmic domain was shown to interact with the 23rd to 24th repeat of FLNa in a yeast two-hybrid assay. When expressed in a filamin-negative cell line, PSMA was rapidly internalized from the cell surface; however, ectopic expression of FLNa in these cells resulted in a 50% reduction in the rate of PSMA internalization. These data suggest that FLNa may stabilize PSMA at the cell surface by tethering it to the actin cytoskeleton, likely preventing AP-2 from binding. Interestingly, expression of FLNa also reduced the NAALADase activity of PSMA at the cell surface, perhaps by inducing a conformational change in the extracellular domain (2). These data suggest that competitive binding of AP-2 and FLNa to the PSMA cytoplasmic tail regulates endocytosis, recycling, and the enzymatic activities of PSMA. Furthermore, the fact that glutamate receptor, a protein that transports glutamate, and PSMA, an enzyme that releases glutamate, both bind to FLNa raises the intriguing possibility that PSMA and glutamate receptor might exist as a multiprotein complex on the plasma membrane. This interaction would potentially facilitate the generation and transport of glutamate into the cell.
FLNa is also known to play a role in cell adhesion and motility. Calderwood et al. (10) found evidence of an interaction between β-integrin and FLNa and further demonstrated that this interaction inhibits cell migration. Another binding partner for FLNa is RalA, a small GTP-binding protein known to play a role in filopodia formation (42). We have observed an accumulation of PSMA in filopodial structures (unpublished data), and it is not known whether this indicates a role for PSMA in cell migration.

RESEMBLANCE OF PSMA TO MULTIFUNCTIONAL PEPTIDASES

Numerous examples suggest a role for enzymatic peptidases in mediating cell migration by affecting signaling cascades. For example, the interaction of the neutral endopeptidase (NEP) cytoplasmic tail with Lyn kinases blocks the activation of phosphatidylinositol 3-kinase (PI3-kinase). This inhibition of PI3-kinase prevents FAK phosphorylation-mediated cell migration (62). NEP is also known to inhibit the proliferation of prostate epithelial cells by its direct association with phosphatase and tensin homolog (PTEN) (61). PTEN is a lipid and protein phosphatase that inhibits PI3-kinase-mediated activation of Akt, a kinase involved in cell survival. A catalytic mutant of NEP also was able to block cell proliferation and migration, suggesting that the enzymatic activity of NEP was not necessarily required. Mutational analysis of the cytoplasmic tail of NEP identified a basic amino acid-rich motif containing five lysine and arginine residues proximal to the transmembrane domain that mediates the interaction between NEP and PTEN (61). The cytoplasmic tail of PSMA also has a stretch of three basic arginine residues proximal to the membrane-spanning domain, thus raising a possibility that PSMA may also interact with PTEN.

CD26 is another interesting example of a multifunctional type II cell surface glycoprotein with important roles in cell signaling. This molecule is expressed in a wide variety of cells and possesses dipeptidylpeptidase IV (DPIV) activity (7, 8, 21). CD26 has been shown to regulate cell migration and proliferation independent of its enzymatic activity (20). CD26 is known to bind adenosine deaminase, an enzyme involved in irreversible deamination of adenosine, and this association has been shown to be essential for the promotion of cell proliferation and cytokine production (29). CD26 also has been shown to affect the migratory behavior of T cells through interactions with extracellular matrix proteins such as collagen and fibronectin (22, 44). These examples clearly indicate that although PSMA is a peptidase, it could have multiple roles not only as an enzyme but also as a protein with cell survival and migratory functions.

POTENTIAL ROLE OF PSMA ENZYME ACTIVITY IN PCA

Increased PSMA enzymatic peptidase activity is associated with metastatic PCa (32). However, the significance of these enzymatic activities in the context of benign and malignant prostatic cells remains to be elucidated.
The prostate gland is composed mainly of stromal, epithelial, and neuroendocrine cells. The dynamic balance of cell proliferation, differentiation, and apoptosis in general maintains the cellular and tissue homeostasis. This balance is generated by the continuous cross talk among these cell populations (63). For this purpose, epithelial and stromal cells secrete various types of growth factors, chemokines, and neuropeptides (70). Deregulation in this paracrine communication can result in derangement of the prostate gland, such as benign prostate hyperplasia and prostate carcinoma (15). For example, the peptidase NEP normally acts to inhibit the migratory properties of prostate epithelial cells. NEP achieves the inhibition of prostatic epithelial cell migration by cleaving critical neuropeptides such as bombesin and endothelin and thereby prevents the relay of signal transduction mediated by G protein-coupled receptors (62). CD26 is also involved in the regulation of paracrine signaling by promoting cleavage of growth factors, chemokines, neuropeptides, and hormones and thus contributes to the regulation of T-cell and monocyte migration (20).

Like NEP and CD26, PSMA is also a type II transmembrane glycoprotein with catalytic metallopeptidase activity. The increased expression of PSMA in prostatic adenocarcinoma may indicate a role in the cleavage of signaling molecules involved in maintaining prostate gland architecture and function. Although neuropeptide and growth factor substrates that influence signaling mechanisms have yet to be identified, the overexpression of PSMA could potentially disturb the growth balance of the prostate gland. Future investigation along these lines should provide important insights into the role of PSMA in prostate cancer.

CONCLUSIONS

In conclusion, we suggest that PSMA could be a multifunctional protein. We summarize the potential multifunctional nature of PSMA in Fig. 2. Dimerization, structural similarity to TIR, and localization to REC indicate a potential receptor function for PSMA. NAALADase and folate hydrolase activities of PSMA are consistent with a role in enzyme activities involved in nutrient uptake. Interaction of PSMA with FLNa and its localization to filopodia indicate a possible role in cell migration. Finally, PSMA as a peptidase might also activate signaling cascades involved in cell survival, cell proliferation, and cell migration. Future investigations into these areas should provide valuable information regarding the possible functions of this clinically important molecule. Improved understanding of PSMA function should allow the fulfillment its therapeutic and diagnostic potential.

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