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Tumor Target Prostate Specific Membrane Antigen (PSMA) and its Regulation in Prostate Cancer

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Abstract Prostate specific membrane antigen (PSMA), is a unique membrane bound glycoprotein, which is overexpressed manifold on prostate cancer as well as neovasculature of most of the solid tumors, but not in the vasculature of the normal tissues. This unique expression of PSMA makes it an important marker as well as a large extracellular target of imaging agents. PSMA can serve as target for delivery of therapeutic agents such as cytotoxins or radionuclides. PSMA has two unique enzymatic functions, folate hydrolase and NAALADase and found to be recycled like other membrane bound receptors through clathrin coated pits. The internalization property of PSMA leads one to consider the potential existence of a natural ligand for PSMA. In this review we have discussed the regulation of PSMA expression within the cells, and significance of its expression in prostate cancer and metastasis. J. Cell. Biochem. 91: 528–539, 2004. © 2003 Wiley-Liss, Inc.

Key words: prostate specific membrane antigen; carboxypeptidase; folate hydrolase; folylpoly-γ-glutamate; dileucine motif; internalization

Prostate cancer represents an excellent target, especially for monoclonal antibody therapy for a number of reasons that include [Ma et al., 2003].

1. The prostate is a non-essential organ and its destruction will not harm the host and the identification of tissue specific antigens for antibody development is easier than elusive tumor specific antigens.
2. The sites of prostate metastasis being lymph nodes and bone are sites that receive high levels of circulating antibodies.
3. Metastases are typically of small volume allowing ready access to therapy and are identified early following primary therapy by elevation in serum PSA.
4. The PSA serum marker provides a means to monitor therapeutic response.
5. Patients at high risk for subsequent failure from primary therapy can be readily predicted enabling initiation of therapy while the tumor burden is minimal.

PROSTATE SPECIFIC MEMBRANE ANTIGEN

The tissue specific protein, prostate specific membrane antigen (PSMA) is an excellent target for imaging and therapy because, it is a cell surface protein which presents a large extracellular target, and it is expressed at levels that are about a thousand-fold greater than the minimal expression seen in other tissues such as kidney, proximal small intestine, salivary gland. Some minimal expression is also observed in the brain, but most agents and especially antibodies do not penetrate the brain because of the blood–brain barrier. While the initial antibody used for imaging PSMA, capromab pentetide, is the only prostate cancer imaging agent approved by the FDA, it has had problems in terms of specificity and sensitivity. The poor quality of the initial antibody was felt to be due to the fact that it recognizes an internal epitope of the protein and is binding to areas of tumor necrosis, and necrosis is less likely in areas at metastatic sites in the

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bone. Second generation antibodies such as the humanized version of J591 developed by Dr. Bander at Cornell Weil School of Medicine in New York has been in clinical trials and has demonstrated an ability to image all sites of metastasis, especially bone with nearly 100% specificity and sensitivity. Other non-prostate sites of minimal expression are not imaged. Other companies are also developing second-generation antibodies such as the cytogen/progenics joint venture company developing fully human antibodies to the external domain of PSMA. The results of these different groups using these second generation antibodies are very encouraging and are demonstrating therapeutic activity in delivering radionuclides and cytotoxic agents resulting in therapeutic responses in both preclinical and early clinical trials [Bander et al., 2003]. An unanticipated site of PSMA protein expression is that it is found to be strongly expressed in the neovasculature of all solid tumors but not normal vasculature and as such will serve as an imaging and therapeutic target for all solid tumors.

PSMA has an important role in prostate carcinogenesis and progression, glutamatergic neurotransmission, and folate absorption [O'Keefe et al., 2001]. Each of these different areas of research activity leads to different names being given to PSMA. Because of its strong expression in the prostate (where its function is unknown), it is named as PSMA; in central nervous system, where it metabolizes the brain neurotransmitter, *N*-acetyl-aspartyl-glutamate, it is named NAALADase; in the proximal small intestine its role is removing gamma-linked glutamates from poly-γ-glutamated folate, folate hydrolase FOLH1, and as a carboxypeptidase, glutamate carboxypeptidase II, GCPII. Our focus in this review will be on its biology and role in the prostate and prostate cancer. PSMA is up-regulated many fold in prostate cancers (PCA), metastatic disease, and hormone-refractory PCAs. PSMA expression is modulated inversely by androgen levels [Israeli et al., 1993; Wright et al., 1996]. Most interestingly, PSMA expression has been found in the neovasculature of most of the solid tumors (not in vasculature of the normal tissues) [Silver et al., 1997]. The exact significance of this is not known. However, due to it's intriguing yet unexplained distribution, PSMA can serve as a detecting agent for metastatic foci of primary cancer. PSMA can be used as a target of imaging agent to detect metastatic tumor site, or PSMA can also be used to detect prostate cells in the circulation or lymphatics. PSMA serves as a target for the delivery of therapeutic agents such as cytotoxins or radionuclides.

PSMA Discovery and Mapping

PSMA is a type II membrane glycoprotein, Mr∼ 100,000 dalton with an intracellular segment (amino acids 1–18), a transmembrane domain (amino acids 19–43), and an extensive extracellular domain (amino acids 44–750) (Fig. 1A). Human *PSMA* gene was first cloned in Dr. Heston's laboratory from LNCaP cells [Israeli et al., 1993] and was found to be located in chromosome 11p11-12, which encodes for PSMA transcript expression in prostate [Leek et al., 1995; Rinker-Schaeffer et al., 1995; O'Keefe et al., 1998]. Another gene, highly homologous to PSMA was found to be located at the loci 11q14.3 is called PSM-like. The PSM-like gene is expressed in different tissues, such as kidney and liver, but not in prostate [O'Keefe et al., 2001].

Variants of PSMA

PSMA is alternatively spliced to produce at least three variants (Fig. 1B), most important of which is PSM', the cDNA of which is identical to PSMA except for a 266-nucleotide region near the 5′ end of PSMA cDNA (nucleotides 114–380) which codes for the transmembrane region of the protein. PSM' is therefore located in the cytoplasm. Su et al. [1995] used RNAse protection assays to examine the expression of PSMA and PSM' in normal versus benign prostate hyoperplasia (BPH) versus prostate cancers. They found increasing expression of PSMA in tumors relative to normals and generated a tumor index based on PSMA: PSM' ratio which is 9–11 in LNCaP cells, 3–6 in prostatic carcinoma, 0.75–1.6 in BPH, and 0.075–0.45 in normal prostate. The other two variants of PSMA are PSM-C (with transcription start site same as PSMA, splice donor site same as PSM', different splice acceptor site located within intron 1, includes a novel exon 1b), which is identical to PSM' and PSM-D (same donor site as PSM', but acceptor site includes novel exon 1c), has a new translation start site followed by 42 novel amino acids with a motif of AAYACTG-CLA (found in growth factor cys-knot family of

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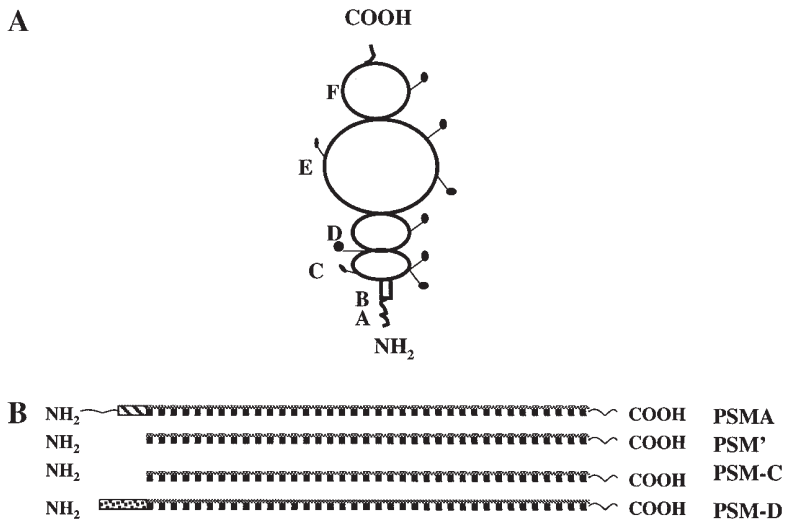

Fig. 1. **A**: Showing the globular nature of prostate specific membrane antigen (PSMA) protein with different domain. Domains A: Amino acids 1–19, intracellular, cytoplasmic; B: 20–39, transmembrane; C: 40–144; D: 173–248; E: 275–596; F: 597–756. There are two linker regions between domains C and D (amino acids 145–172) and domains D and E (amino acids 249–274). Domain C, D, E, and F are the extracellular domains. Domain E is the catalytic domain with the zinc binding and substrate binding sites. **B**: Schematic view of the different splice variant of PSMA detected in LNCaP cells. PSM′ lacks the intracellular cytoplasmic domain and transmembrane domain, the rest is identical to PSMA. PSM-C, although produced by alternative splicing, has a different splice acceptor site than PSM′, encodes a protein identical to PSM′. PSM-D, has a unique 42 amino acid long domain at N-terminus and rest is identical to PSM′.

proteins) and rest of the PSMA protein in frame [O'Keefe et al., 2001]. The implication of such alternative splice variants in prostate cancer cells is not known at present.

Unique Enzymatic Functions of PSMA

PSMA is a protein with two unique enzymatic functions, including NAALADase activity (cleaving terminal glutamate from the neurodipeptide, *N*-acetyl-aspartyl-glutamate, NAAG) and folate hydrolase activity, which cleaves the terminal glutamates from γ-linked poly glutamates. NAAG is concentrated in neuronal synapses while folylpoly-γ-glutamates are present in dietary components and PSMA protein of the surface of the brush border surface of small intestine enables the generation of folates and subsequent folate uptake. So the question that comes in one's mind is what is a protein like PSMA with such interesting activity profile doing on the surface of prostate cells and why is its expression level enhanced so many fold in prostate cancer cells? The answer is still unknown but there are several possible explanations, which we will discuss in this review.

The structural similarities between PSMA and other proteins are known. The *PSMA* gene is highly homologous to neuropeptidase, NAALADase, which releases neurotransmitter glutamate from neuropeptide NAAG; human glutamate hydrolase, which is capable of folyl-poly-γ-glutamate hydrolysis [Yao et al., 1996]; I100 (human dipeptidyl peptidase IV), associated with apical brush border of intestinal epithelial cells [Darmoul et al., 1992; Shneider et al., 1997]. Human prostate PSMA (FOLH1) and rat NAALADase is classified as GCPII, a member of M28 peptidase family of metalloproteases [Rawlings and Barrett, 1997], with the residues conserved for Zn^{2+} and substrate binding. Human PSMA has about 91% homology to mouse PSMA (folh1) [Bacich et al., 2001]. Human, mouse, rat, and porcine folylpoly-γ-glutamate carboxypeptidases have 10, 10, 9, and 12 putative glycosylation sites, respectively [Israeli et al., 1993; Bzdega et al., 1997; Halsted et al., 1998; Ghosh, 2003]. PSM' and PSM-like have nine and five potential glycosylation sites, respectively. Mouse, rat, and pig homologs have an additional glycosylation site, which is not present in human forms. Glycosylation of PSMA plays important role in its targeting the protein to the cell membrane, proper protein folding, and enzymatic activity. Removal of sugar residues partially or completely (enzymatically or by mutagenesis) abolishes the enzyme activity of the protein [Ghosh, 2003]. More interestingly, the glycosylation profile of PSMA

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obtained from different prostate cancer cell lines was found to be different, leading one to speculate that the different sugar epitopes may play a role in metastasis of prostate cancer. Further work has been carried out in the laboratory to address such question. Human PSMA has homology to human transferrin receptor (Tfr). Both are type II glycoproteins and PSMA shares about 54% homology to the Tfr and about 60% with transferrin receptor 2 (Tfr2) [Kawabata et al., 1999]. The Tfr exists in dimer form due to interstrand sulfhydryl links. PSMA exists in dimeric/monomeric form. Recombinant protein of the extracellular domain of PSMA also exists in readily interconvertible dimer–monomer forms. PSMA is expressed as non-covalent homodimer on the surface of LNCaP cells as well as on the 3T3 cells stably transfected with full-length PSMA [Schulke et al., 2001]. Like the Tfr, PSMA can undergo internalization. It is yet to be characterized whether the dimer or monomer internalize. It is not understood what induces dimer formation or cause dissociation to the monomer form. The difference is important because the dimer has enzymatic activity and the monomer does not have enzymatic activity.

Endocytic Function of PSMA

PSMA, like other cell surface receptors undergoes internalization constitutively and such spontaneous internalization is enhanced three-fold in a dose dependent manner by PSMA specific monoclonal antibody J591 [Liu et al., 1998]. It has been shown very clearly biochemically (by using biotinylated cell surface PSMA followed by internalization of the protein), by immuno-fluorescence analysis or immuno-electron microscopy, that PSMA or PSMA–antibody complex undergoes internalization through clathrin coated pits and closely resembles the internalization pathway of Tfr and finally ends up in the lysosomes. Such constitutive internalization of PSMA may reflect the recycling of a structural protein or may be mediated by binding of a ligand. A detailed characterization of antibody mediated PSMA internalization revealed the resemblance with EGFR receptor with its ligand [Haigler, 1983]. It is well known that many ligands and their transmembrane receptors are internalized through clathrin coated pits (receptor mediated endocytosis). Formation of antibody–antigen complexes on the cell surface often results in internalization through a pathway closely resembling the receptor mediated endocytosis of peptide hormones, growth factors, and natural ligands [Pastan and Willingham, 1981] (Fig. 2). It can be speculated from these findings that PSMA may have a transport function for an yet unidentified ligand. Monoclonal antibody such as J591 acts as surrogate ligand inducing internalization.

Targeting internalization of receptors through coated pits and their traffic through endocytic compartments are mediated through specific signals or motifs located on the cytoplasmic tail of the receptors. There are two major class of sorting signals which mediate internalization of membrane proteins from plasma membrane and sort these proteins to endosomes/lysosomes and finally to the compartment for peptide loading [Sandoval and Bakke, 1994; Marks et al., 1997; Nordeng et al., 1998]. These sorting signals are tyrosine based motifs NPXY and YXXΦ motifs [Marks et al., 1997] and dileucine motif [Sandoval and Bakke, 1994]. Tyrosine motifs are identified in a variety of receptor molecules like the Tfr, low density lipoprotein receptor, and asialoglycoprotein receptor [Trowbridge et al., 1993]. Dileucine (or leucine–isoleucine sequence) motif (Fig 3A) is important for internalization and lysosomal targeting were found in the γ-δ chain of T cell receptor, CD4, IFN γ [Shin et al., 1991; Letourneur and Klausner, 1992; Farrar and Schreiber, 1993]. Tyrosine based motifs interact with adaptor complexes AP1, AP2, and AP3 [Traub and Kornfeld, 1997; Hirst and Robinson, 1998] and dileucine based motifs bind to the β subunits of AP1 and AP2, μ chains of AP1 and AP2 have also been reported to bind to these signals. Apart from this, leucine based signals of lysosomal protein LIMPII and melanosomal membrane protein tyrosinase have been shown to bind to AP3 [Honing et al., 1998]. PSMA has a dileucine motif present at its cytoplasmic tail. Mutation of first leucine (Leu 4) did not change the internalization of mAb J591, in contrast, conversion of second leucine (Leu 5) resulted in complete lass of internalization indicating this leucine is important for the internalization [Rajasekaran et al., 2003; Ghosh and Heston, 2003b]. This implied that the dileucine motif is responsible for the internalization. However, the dileucine motif is associated with the basolateral targeting of protein and PSMA is found at the apical surface of the cell. In case of

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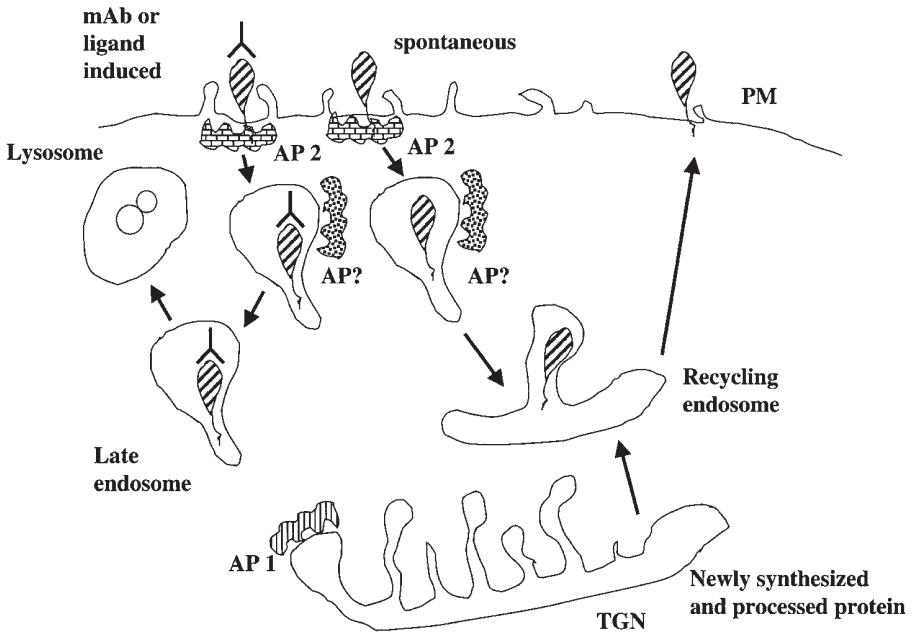

Fig. 2. Showing the spontaneous or antibody induced internalization of PSMA through clathrin coated pits. The proteins can either recycle through recycling endosomal compartment (REC) and go to the plasma membrane or they can go to the lysosomes through late endosomes. The cytoplasmic tail of PSMA contains an internalizaton signal, which enables it to internalize into endosomal vesicles.

PSMA, the first amino acid methionine located five amino acids up-stream of the crucial leucine is involved in the internalization, which makes this signal a unique internalization signal "MXXXL" in PSMA. Amino acid residues adjacent to such motifs has been shown to influence its function. Dileucine signal of CD4 is active when adjacent serine residues are phosphorylated [Pitcher et al., 1999]. Cytoplasmic tail of PSMA has consensus protein kinase C sequence (Thr-14) and has two other hydroxyl containing residues (Thr-8, Ser-10), that might serve as phosphorylation acceptor sites. It remains to be seen how mutation of such residue affect the internalization function of the protein. A detailed mutational analysis has been carried by Rajasekaran's group, which did not have any effect on internalization function of PSMA. It is known at least in EGFR [Kil et al., 1999], that the dileucine motif and its neighboring residues need to form an amphipathic helix with hydrophilic residues pointing towards one surface, and hydrophobic residues pointing towards the other for interaction with adaptor proteins needed for sorting. The predicted protein structure of PSMA N-terminal cytoplasmic tail showed that this region (residues N^3 to R^{19}) has a probability to take up a α helical structure and helical wheel projection of this region showed that this helix is an amphipathic α helix with hydrophobic residues projecting towards one surface and hydrophilic residues on the other (Fig. 3B).

A cytoplasmic leucine based motif has been shown to be involved in the lysosomal targeting of several membrane proteins [Letourneur and Klausner, 1992; Haft et al., 1994; Dittrich et al., 1996; Kil et al., 1999]. PSMA colocalizes with lysosomal marker Lamp1 in LNCaP cells (endogenously express PSMA) or Cos cells expressing transfected PSMA [Rajasekaran et al., 2003] or PC3 cells ectopically expressing PSMA [Ghosh and Heston, 2003b] indicating PSMA is being localized within lysosome. Furthermore swapping the MWNLL (the first five amino acid of PSMA sequence) and MWNLA (fifth leucine has been changed to alanine) to Tac antigen, this group has shown that wild type motif could transport the Tac antigen (which is not a lysosome resident) to the lysosome, whereas mutant motif could not, indicating that MXXXL signal in PSMA is indeed a lysosomal signal. Knowledge of PSMA's internalization function and regulation can be exploited in cancer therapeutics. A detailed analysis is required to define the putative natural ligand for internalization,

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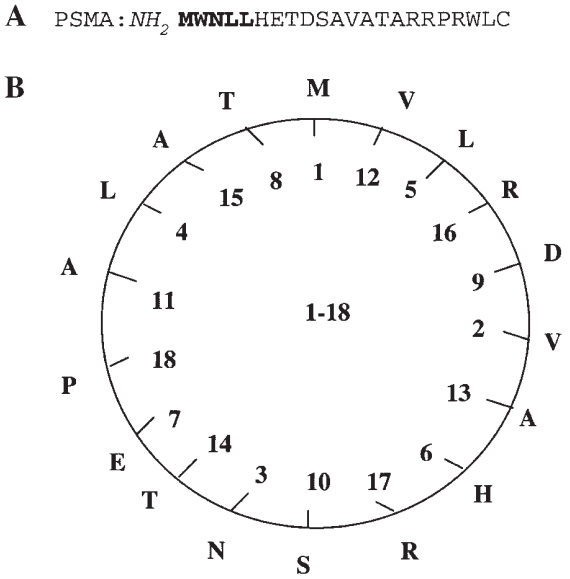

Fig. 3. PSMA has a dileucine motif at its C-terminal tail. Dileucine motifs serve as lysosomal targeting signal and usually reside within the cytoplasmic tail of the protein (either N- or C-terminal). **A**: Showing the peptide sequence N-terminal cytoplasmic sequence here with putative internalization motif shown in bold. The actual internalization signal of PSMA is the first five amino acids MXXXL. **B**: The helical wheel projection of the C-terminal tail region (N-terminal 19 amino acid). By using the predict protein program (http://cubic.bioc.columbia.edu/predict-protein), it was found that the N-terminal region contains a α-helical region. Using this sequence, we could make a helical wheel projection by using the site http://www.site.uottawa.ca/~turcotte/resources/helixwheel and found that the one face of the helix contains the hydrophobic residues and the other face of the helix contains the hydrophilic residue, indicating that this region is important for protein–protein interaction.

and what role it plays on the biological function of PSMA. Our lab has shown that substrates and antagonists of the carboxypeptidase function do not alter the rate of internalization, which shows that internalization and enzymatic function are two independent processes. Furthermore, it will be interesting to find out if this natural ligand for internalization could substitute for the mAb in a targeted therapy approach.

REGULATION OF PSMA EXPRESSION AND ITS IMPLICATION IN PROSTATE CANCER

PSMA–Filamin Interaction

Recently it has been shown that cytoplasmic tail of PSMA interacts with actin binding protein Filamin a (FLNa) [Anilkumar et al., 2003]. PSMA's association with FLNa is necessary for its localization to recycling endosomal compartment (REC). In filamin negative cells, the internalized PSMA accumulates in diffused vesicles throughout the cytoplasm. This distribution can be altered by introducing FLNa in this cell and the proteins localize into REC. PSMA–FLNa interaction decreased its rate of internalization by 50%. It could be that linking of PSMA with actin cytoskeleton by FLNa keeps the proteins attached to cell membrane, and eliminates their ability to bind adaptor proteins, hence reduction in internalization rate. A dissociation from FLNa might help in binding of PSMA cytoplasmic tail with adaptor proteins, which leads to endocytosis. Therefore, FLNa and adaptor proteins could be competing for binding at the same site of PSMA. Internalization motif mutants that could not undergo endocytosis, but could bind very srongly to PSMA support this theory. Figure 4 gives a summary of this event. The importance of phosphorylation of PSMA protein at certain putative phosphorylation sites and its implication on its binding with FLNa remains to be solved.

Regulation of PSMA Expression by PSMA Enhancer (PSME)

PSMA has been shown to be severalfold increased expression in prostate cancer; its expression is suppressed by androgen. Currently, two regulatory elements controlling PSMA expression have been characterized. The proximal 1.2 kb PSMA promoter and PSME [O'Keefe et al., 1998], located within the third intron of FOLH1, rendering prostate specific expression of PSMA. PSME is activated in prostate specific manner, negatively regulated by androgen receptor (AR) and its expression is up-regulated in prostate cancer. Detailed study showed that proximal 90 bp of PSME contained enhancer element with an AP3 site responsible for elevation of promoter activity of PSME beyond the basal level [Lee et al., 2002]. Furthermore, recent work by this group has shown that Ca^{2+}-dependent activation PSME transcription factor NFATc1 isoform binds to AP1. In presence of Ca^{2+}, NFATc1, protein gets dephosphorylated through Ca^{2+}-dependent calcineurin, which drives the translocation of NFAT protein to nucleus and activates transcription of PSMA.

Direct repeat regions of PSME harbors nine copies of SRY/SOX sites. SRY, SOX 7, and SOX

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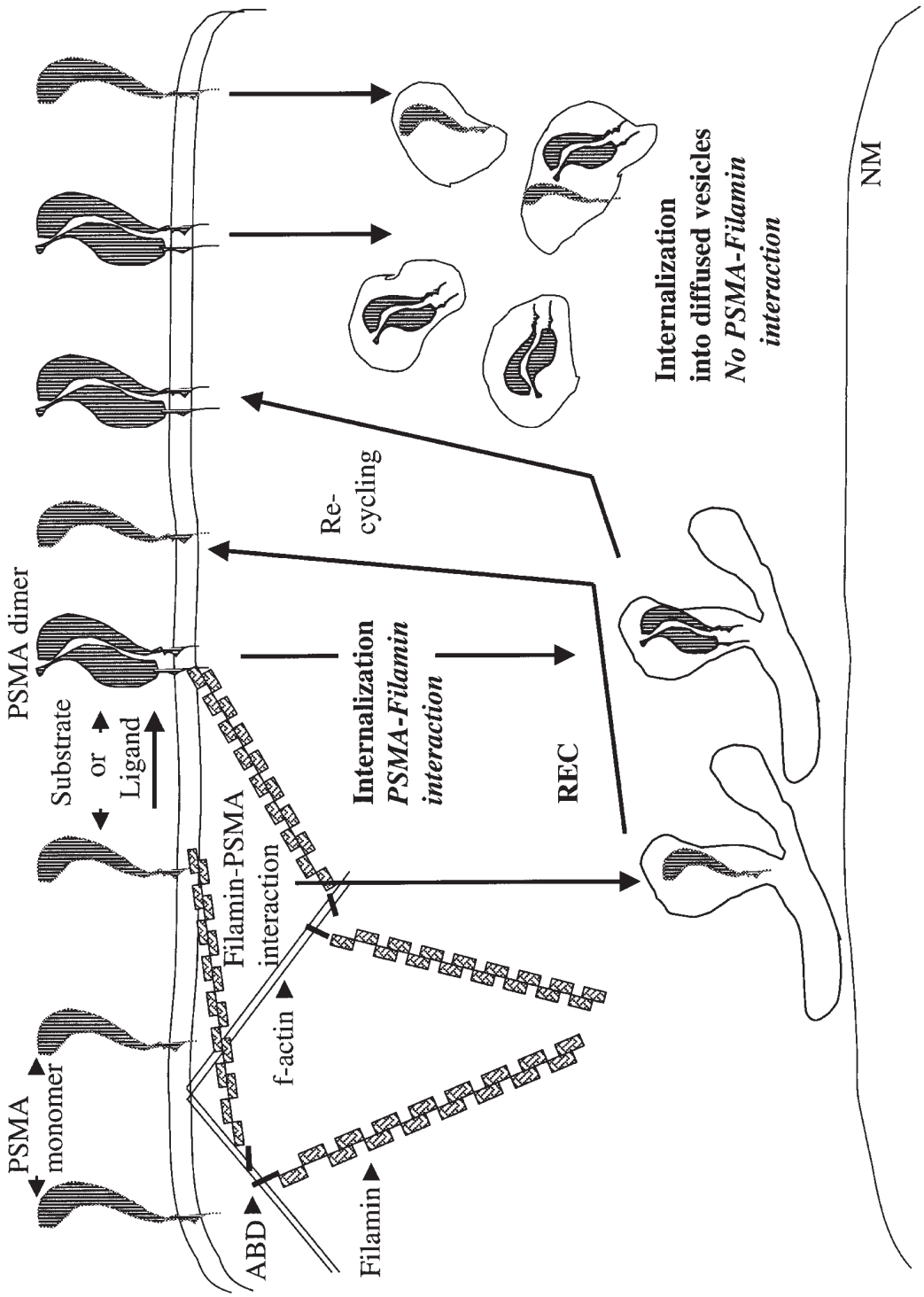

Fig. 4. A schematic diagram showing the PSMA–Filamin a (FLNa) interaction within the cells and how such interaction modulates PSMA function. Which form of PSMA undergoes internalization (monomer or dimer form) is not yet known clearly. In presence of FLNa, PSMA undergoes internalization to the REC located at the perinuclear region. In absence of FLNa, PSMA is distributed through the diffused vesicle throughout the cytoplasm.

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18 are reportedly expressed prostate cancer and prostate epithelial cells [Takash et al., 2001]. SRY or SOX may interact with androgen receptor DNA binding domain (ARDBD), and as a result AR sequesters these tissue specific proteins, causing repression of PSME, which could partially explain AR mediated repression of PSME.

Ca^{2+} ions positively regulate PSMA expression. Ca^{2+} influx probably takes place through CaT-like calcium channel, which is strikingly correlated with the malignancy of prostate cancer as well as PSMA expression [Wissenbach et al., 2001]. But how could that start? The possible explanation may involve glutamate receptors. We have observed metabotropic glutamate receptors by gene array analysis of LNCaP cells (Heston et al., unpublished observation). In prostate cancer cells, such receptors could get activated constitutively by free glutamates (an agonist to such receptor) released as a by-product of folate hydrolase/NAALADase action of PSMA expressed on the cell surface (PSMA level is up-regulated many fold in prostate cancers) and can modulate the function of potassium and calcium channels, which might cause change in resting membrane potential. Such change in membrane potential could cause oxidative damage to the cells, causes release of Cl^{-} ions [Shuba et al., 2000] and continuous influx of Ca^{2+} ions through calcium channels (to compensate for such change in membrane potential).

Interaction of AR–FLN a

AR, is a member of steroid/ nuclear receptor superfamily, mediates male morphogenesis in utero, gametogenesis, and prostate growth in older man. It has four principle domains, large N-terminal transactivation domain (ARTAD), ARDBD, a hinge region, and a C-terminal ligand binding domain (ARLBD). In absence of androgen, AR is cytoplasmic. Androgen binds specifically to a ligand binding pocket in the lower half of LBD, causing conformational change. Ligand-AR goes to nucleus, where ARDBD interacts with specific response elements on the promoter of target genes. There are several repressors of AR activity, most notable of which are calreticulin, cyclins, HBO1, Smad3, and TSG101, and p53 are all nuclear proteins. Cytoplasmic AR in absence of ligand, is tethered to the C-terminal end of FLNa through its hinge domain and LBD. Full length FLNa, cleaved at calpain cleavage site between repeats 15 and 16, releases FLNa (16–24), which colocalizes liganded AR to the nucleus. In the nucleus, FLNa disrupts the interaction between the N and C termini of AR and interferes with the binding of the co activator TIF2, causing repression of AR-transactivation. Mutation or deletion of hinge region disrupts AR–FLNa interaction, causing AR to bind to TIF2 and facilitates AR-mediated transactivation. Several mutations in the hinge region have been implicated in the androgen-driven prostate cancer.

SRY or SOX may interact with ARDBD, and as a result AR sequesters these tissue specific proteins, causing repression of PSME. Which could partially explain AR-mediated repression of PSME. Furthermore PSME also has eight AP1 at the repeat region. NFAT proteins by binding with AP3 interacts with AP1 binding proteins for optimal activity and AR negatively regulates PSME activity by interacting with AP1 protein as well. Therefore in the situation of Ca^{2+} ion influx, activates the function Ca^{2+} requiring proteins, such as calpain, calreticulin, causing repression of AR and activation of PSME, or calcineurin dephosphorylate NFATc1, which activates the transcription of PSMA (Fig. 5). This is the possible explanation for the androgen mediated suppression of PSME. Therefore tissue specificity and up-regulation in absence of androgen make PSME the best candidate for expressing toxic genes in the prostate in an androgen dependent environment.

ROLE OF FOLATE HYDROLASE IN PROSTATE CANCER

Folic acid is integral to the various metabolic processes within the body involving one carbon transfers in DNA synthesis, DNA methylation, formation of methionine which when decarboxylated is used in polyamine synthesis [Eto and Krumdieck, 1986; Jennings, 1995]. Our initial thinking was that PSM' the alternaive spliced form of PSMA may put the prostate at risk of folate deficiency because as a folate hydrolase it would allow for deglutamation of the poly-γ-glutamated folates which are the intracellular storage form of folates. Given that PSM' is less glycosylated being an intracellular protein, we now consider it likely that PSM' having folate hydrolase activity is unlikely. We

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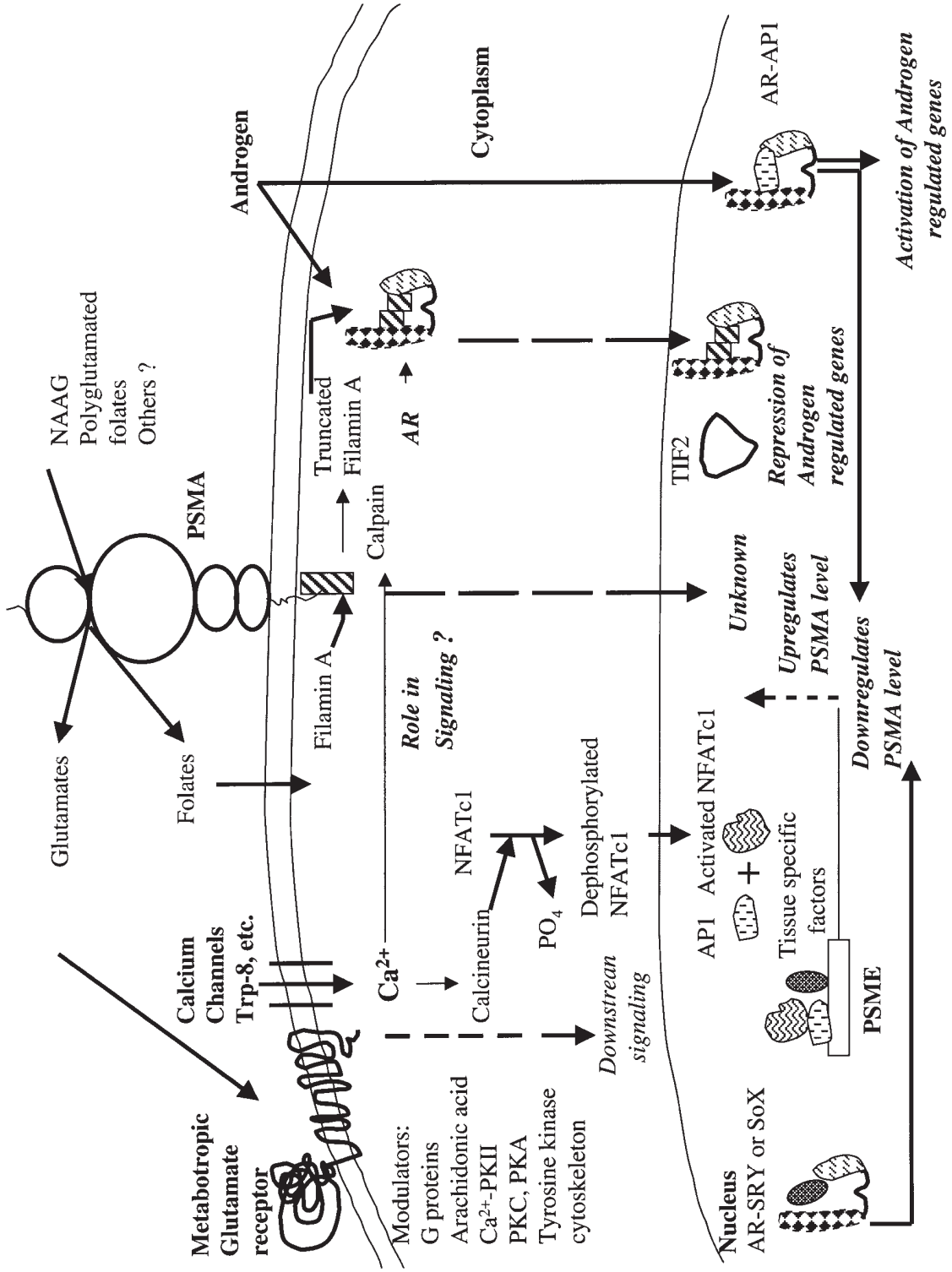

Fig. 5.

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at present have no idea what the intracellular form PSM' may be doing inside the cell. Outside the cell PSMA does bind and hydrolyze poly-γ-glutamated folate. But serum contains folate in a form that is not poly-γ-glutamated and is ready for transport into tissues. In the normal prostate, PSMA is at the apical surface, it is possible that it is exposed to a glutamated form of folate at this surface in the prostate cells. In cancers, it is not uncommon to have dead and dying cells in the tumor. As these cells would release their stored folate as poly-γ-glutamated folate, it is possible that PSMA enables cells to capture this folate by removing the γ-linked glutamates, thus freeing folate which then can be taken into the cell by folate binding proteins (FBP) or folate carrier systems. Given the interstitial pressure inside tumors because they lack lymphatics, and the cell death in hypoxic regions, that may provide a rational for the expression of PSMA on the tumor neovasculature, where it may be involved in helping capture the poly-γ-glutamated folates being released from dead and dying cells. Also because PSMA does internalize it may be that it has some transport property for poly-γ-glutamated folates that is as yet not understood.

SUMMARY

PSMA represents an excellent ideal cell surface protein for targeted therapy of prostate cancer and vasculotoxic therapy of non-prostate solid cancers. Clinical trials using antibodies that target the external domain of PSMA with imaging or toxic agents have been encouraging. PSMA is highly expressed in prostate cancer and is found to be strongly up-regulated in prostate cancer and it appears that calcium may be involved in the signaling for this increased expression. PSMA has to be glycosylated and to be in a dimer state to be enzymatically active. PSMA has activity as a carboxypeptidase with the preferred substrates NAAG and poly-γ-glutamated folate releasing glutamate upon hydrolysis of the substrate. The released glutamate may have a role in signaling. PSMA is internalized and exhibits a unique internalization motif, MXXXL. PSMA also binds to FLNa. FLNa is also associated with transducing extracellular stress to internal signaling, and PSMA may modify that signaling process.

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Fig. 5. Cartoon showing the regulation of PSMA in prostate cancer cells. Negative regulation by androgen receptor (AR) and positive regulation Ca^{2+} is shown here. Polyglutamated folates get enzymatically cleaved to deglutamated folates and glutamates. The folates can enter the cells through reduced folate carrier (RFC) or folate binding proteins (FBP). The glutamates produced by the PSMA expressing cells can activate metabotrophic glutamate receptors which can get activated and can alter resting membrane potential, which causes efflux of Cl^- ions and influx of Ca^{2+} ions to compensate for the damage of the cells. Ca^{2+} ions can modulate the expression level of PSMA in many ways. Increased Ca^{2+} concentration, can activate inactive transcription factor NFATc1 (which is a transcriptional activator of PSMA enhancer), or cause activation of calpain, which cleaves FLNa. Truncated FLNa binds to AR and localizes to nucleus and suppresses AR-mediated transactivation. Normally AR would sequester AP1 or tissue specific transcription factors (e.g., SRY or SOX), causing inhibition of PSMA enhancer (PSME).

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