

Emerging Roles of Human Prostatic Acid Phosphatase

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Abstract

Prostate cancer is one of the most prevalent non-skin related cancers. It is the second leading cause of cancer deaths among males in most Western countries. If prostate cancer is diagnosed in its early stages, there is a higher probability that it will be completely cured. Prostatic acid phosphatase (PAP) is a non-specific phosphomonoesterase synthesized in prostate epithelial cells and its level proportionally increases with prostate cancer progression. PAP was the biochemical diagnostic mainstay for prostate cancer until the introduction of prostate-specific antigen (PSA) which improved the detection of early-stage prostate cancer and largely displaced PAP. Recently, however, there is a renewed interest in PAP because of its usefulness in prognosticating intermediate to high-risk prostate cancers and its success in the immunotherapy of prostate cancer. Although PAP is believed to be a key regulator of prostate cell growth, its exact role in normal prostate as well as detailed molecular mechanism of PAP regulation is still unclear. Here, many different aspects of PAP in prostate cancer are revisited and its emerging roles in other environment are discussed.

Key Words: Prostatic acid phosphatase (PAP), Prostate cancer, Biomarker, Prognosis, Diagnosis, Immunotherapy

INTRODUCTION

Prostate cancer is one of the most prevalent non-skin cancers in men. In America, prostate cancer related death is ranked second out of all cancer related deaths in men, but its etiology has not been clearly identified yet (Hsing and Chokalingam, 2006). However, in contrast to many other cancers that are difficult to treat, prostate cancer can be completely cured if it is detected in its early stage. Many prostate cancer markers including prostate-specific antigen (PSA), prostate specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), and prostate stem cell antigen (PSCA) have been identified so far (Truong *et al.*, 1993; Hobisch *et al.*, 1998; Bussemakers *et al.*, 1999; Gupta *et al.*, 2009; Madu and Lu, 2010; Batta *et al.*, 2012), which all together can help to increase the chance of earlier detection of prostate cancer (Table 1).

In 1938, which is 85 years after first identification of prostate cancer (Fig. 1), it was discovered that the activity of prostatic acid phosphatase (PAP) was increased in the circulation of the patients with prostate cancer (Gutman and Gutman, 1938). This elevated PAP activity was especially higher in those patients with bone metastasis (Small *et al.*, 2006; Sheridan *et al.*, 2007). Later on, it was established that blood PAP activity

correlates with prostate cancer progression in prostate cancer patients and that PAP could serve as a biochemical indicator for cancer treatment (Veeramani *et al.*, 2005). Subsequently, serum PAP was widely studied as a surrogate marker for prostate cancer until the establishment of prostate-specific antigen (PSA) as the new standard (Veeramani *et al.*, 2005). The introduction of total PSA testing in blood has revolutionized the detection and management of patients with prostate cancer. Indeed, PSA has been regarded as a strong prognostic marker for long-term risk of prostate cancer. The patients who will eventually develop prostate cancer have increased total PSA levels years or decades before the cancer is diagnosed. However, there is a growing need for novel biomarkers that could aid in clinical decision making about biopsy and initial treatment. This is due to the inherent biological variability of total PSA levels which inevitably affects the interpretation of clinical data. For example, total PSA velocity improves the predictiveness of total PSA only marginally, limiting its value for prostate cancer screening and prognostication (Shariat *et al.*, 2011). In this regard, it is encouraging that Swedish group recently developed a novel miRNA index quote (miQ = [miR-96-5p×miR-183-5p]/[miR-145-5p×miR221-5p]) as an early marker for prostate cancer with aggressive progression char-

Open Access <http://dx.doi.org/10.4062/biomolther.2012.095>

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Received Dec 4, 2012 Revised Jan 10, 2013 Accepted Jan 14, 2013

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Table 1. Biomarkers of prostate cancer. Different classes of prostate cancer biomarkers are shown. The list is not exhaustive. The markers are mostly proteins in blood. DNA, RNA, and metabolite are also shown

Biomarker	Composition	Change	Purpose	Reference
Prostatic acid phosphatase (PAP)	Protein	Increase	Diagnosis/ Prognosis	Taskén <i>et al.</i> , 2005; Veeramani <i>et al.</i> , 2005; Makarov <i>et al.</i> , 2009
Prostate-specific antigen (PSA)	Protein	Increase	Diagnosis	Li and Beling, 1973; Ercole <i>et al.</i> , 1987; Stamey <i>et al.</i> , 1987
Biomarker candidate				
α -Methylacyl coenzyme A racemase (AMACR)	Protein	Increase	Diagnosis	Rogers <i>et al.</i> , 2004
B7-H3	Protein	Increase	Diagnosis/ Prognosis	Roth <i>et al.</i> , 2007
Caveolin-1 (Cav-1)	Protein	Decrease	Prognosis	Thompson <i>et al.</i> , 2010
Chromogranin A (CGA, GRN-A)	Protein	Increase	Prognosis	Defetos, 1998
DAB2 interacting protein (DAB2IP)	Protein	Decrease	Diagnosis	Chen <i>et al.</i> , 2002
Endoglin (CD 105)	Protein	Increase	Prognosis	Wikström <i>et al.</i> , 2002
Early prostate cancer antigen (EPCA)	Protein	Increase	Diagnosis	Getzenberg <i>et al.</i> , 1991
Goligiphosphoprotein 2 (GOLPH2)	Protein	Increase	Diagnosis	Kristiansen <i>et al.</i> , 2008
Glutathione S-transferase P1 gene (GSTP1)	DNA	Hypermethylation (decrease)	Diagnosis	Lee <i>et al.</i> , 1994
Human kallikrein 2 (hK2)	Protein	Increase	Diagnosis	Becker <i>et al.</i> , 2000
Interleukin-6 (IL-6)	Protein	Increase	Prognosis	Hobisch <i>et al.</i> , 1998
Ki-67	Protein	Increase	Diagnosis	Gerdes <i>et al.</i> , 1984;
P504S/p63	Protein	Increase	Diagnosis	Harvey <i>et al.</i> , 2010
Prolactin-inducible protein (PIP/GCTFP15)	Protein	Increase	Diagnosis	Tian <i>et al.</i> , 2004
Prostate cancer antigen-1 (PCA-1)	Protein	Increase	Diagnosis	Liu <i>et al.</i> , 2007
Prostate cancer antigen 3 (PCA3 or DD3)	RNA	Increase	Diagnosis	Bussemakers <i>et al.</i> , 1999
PDLIM4 gene (PDLIM4)	DNA	Hypermethylation (decrease)	Diagnosis	Vanaja <i>et al.</i> , 2006
Prostate stem cell antigen (PSCA)	Protein	Increase	Prognosis	Reiter <i>et al.</i> , 1998
Prostate-specific membrane antigen (PSMA)	Protein	Increase	Diagnosis	Brawer <i>et al.</i> , 1992
Sarcosine	Metabolite (Chemical)	Increase (in urine)	Diagnosis	Sreekumar <i>et al.</i> , 2009
STAMP1	Protein	Increase	Diagnosis	Korkmaz <i>et al.</i> , 2002
STAMP2	Protein	Increase	Diagnosis	Korkmaz <i>et al.</i> , 2005
STEAP	Protein	Increase	Diagnosis	Hubert <i>et al.</i> , 1999
Transforming growth factor- β 1 (TGF- β 1)	Protein	Increase	Prognosis	Truong <i>et al.</i> , 1993
Urokinase plasminogen activation (uPA)	Protein	Increase	Diagnosis/ Prognosis	Gupta <i>et al.</i> , 2009

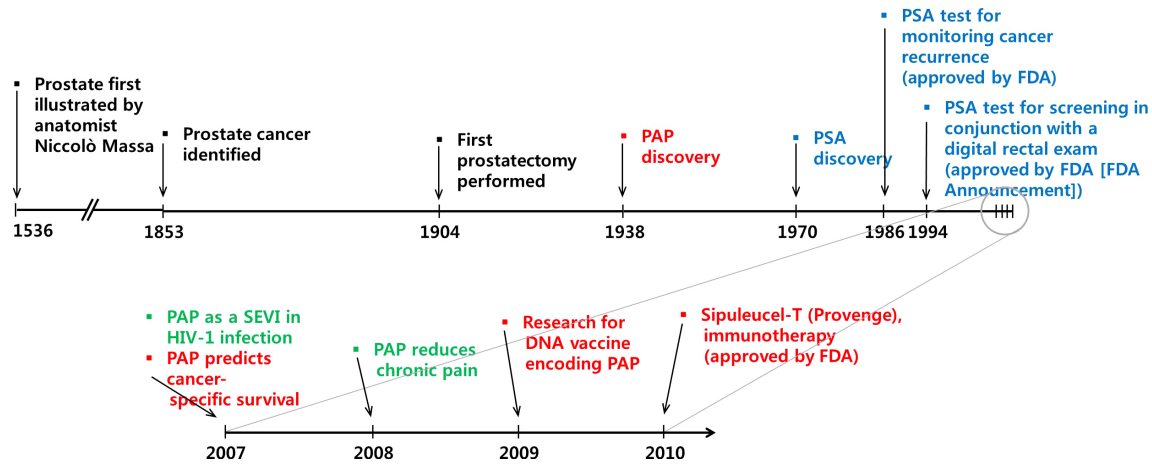


Fig. 1. History of PAP development. Illustrated timelines for prostate cancer and its biomarkers. The major breakthroughs and advances in prostate cancer research are shown. The rise, fall, and revival of PAP along with its emerging diverse roles are also depicted.

acteristics (Larne *et al.*, 2012). They measured the expression of microRNAs (miRNA) using qRT-PCR in FFPE prostatic tissue samples from a Swedish cohort of 49 patients with prostate cancer and 25 without cancer and found eight of 14 preselected miRNAs to discriminate between the two groups. Subsequently four discriminatory miRNAs were combined to a quota. The miQ was found to successfully predict diagnosis ($p < 0.0001$) with high accuracy (AUC=0.931), significantly outperforming PSA. On the other hand, novel blood-based biomarkers including human glandular kallikrein 2 (hK2, Becker *et al.*, 2000), urokinase plasminogen activator (uPA) and its receptor (uPAR, Gupta *et al.*, 2009), transforming growth factor-beta 1 (TGF- β 1, Truong *et al.*, 1993), interleukin-6 (IL-6) and its receptor (IL-6R, Hobisch *et al.*, 1998) were identified and they also are expected to supplement or replace PSA for better diagnosis, staging, prognostication, and monitoring (Kraus *et al.*, 2010). Recently, PCA3 (Bussemakers *et al.*, 1999), and T2:ERG (Young *et al.*, 2012) was suggested to be a novel biomarker for prostate cancer. PCA3 is a noncoding RNA that is found at high levels in prostate cancer compared to non-cancerous prostate cells. T2:ERG is a fusion of the TMPRSS2 gene that is regulated by androgens and ERG oncogene. Found in 50% of prostate cancers, this fusion gene is hypothesized to have a role in the development of prostate cancer (Young *et al.*, 2012).

In this review, however, we will focus on PAP molecule because an increasing number of studies have identified PAP as a significant prognostic factor for patients with intermediate- to high-risk prostate cancer (Taira *et al.*, 2007) and it is recently used as a therapeutic target for immunotherapy (Drake, 2012; Sims, 2012). In addition, novel non-canonical functions of PAP such as pain suppression and involvement in viral infection will be discussed.

HUMAN PAP

Human PAP, also known as Acpp or prostatic specific acid phosphatase (PSAP), is a secreted glycoprotein (100 kDa) enzyme (E.C. 3.1.3.2) that is synthesized in the prostate gland's epithelial cells (Vihko *et al.*, 1978). Although used as a

prostate cancer marker and studied widely in the past few decades, its physiological role is not clearly understood yet. As the name suggests, PAP shows phosphatase activity in acidic condition (pH 4-6) (Zelivianski *et al.*, 1998). PAP enzymatic activity occurs when PAP forms a homodimer that consists of two catalytically inactive subunits (50 kDa) bound by non-covalent bonds (Kuciel *et al.*, 1990; Lee *et al.*, 1991). Each subunit comprises two domains. The larger domain is an α/β type composed of a central seven-stranded mixed β -sheet with α -helices on both sides, while the smaller α -domain contains six α -helices and is formed mostly by long-chain excursions from the first domain (Ortlund *et al.*, 2003; Hassan *et al.*, 2010). The inter-subunit hydrogen bonds observed are the side chain of Gln 33 to main chain His 67 interactions, side chain of Gln 40 to main chain of Val 97, and side chain of His 112 to side chain of Asp 76 (Jakob *et al.*, 2000).

After cleavage of the 32 amino acids that make up PAP's signal peptide, PAP becomes approximately 41 kDa which is its mature form (Roiko *et al.*, 1990; Zelivianski *et al.*, 1998). The PAP monomer has 6 conserved cysteine residues that form 3 disulfide bonds (Cys129-Cys340, Cys183-Cys281 and Cys315-Cys319) together with three putative N-linked glycosylation sites (Van Etten *et al.*, 1991). A high mannose-type carbohydrate binds to Asn61 and Asn301, while Asn188 residue partially sialylates (Jakob *et al.*, 2000). Interestingly, these glycosylation sites and active sites are conserved in all mammalian PAPs (Ostanin *et al.*, 1994). The structure and active site of PAP has been extensively characterized from various species (Hassan *et al.*, 2010). Multiple sequence analyses of human PAP with that of other mammalian PAPs revealed close resemblance among one another. Interestingly, the human PAP showed approximately 99% sequence homology with the panther, 94% with the monkey, 81% with the cow, 83% with the mouse and 80% with the rat.

The major action of PAP is to dephosphorylate macromolecules with the help of catalytic residues (His¹² and Asp²⁵⁸) that are located in the cleft between two domains (Hassan *et al.*, 2010). Site-directed mutagenesis of amino acid residues of PAP revealed that His12 and Asp 258 are critical residues for enzymatic activity of PAP because H12D and/or D258A mutant could not decrease phosphorylation level of ErbB-

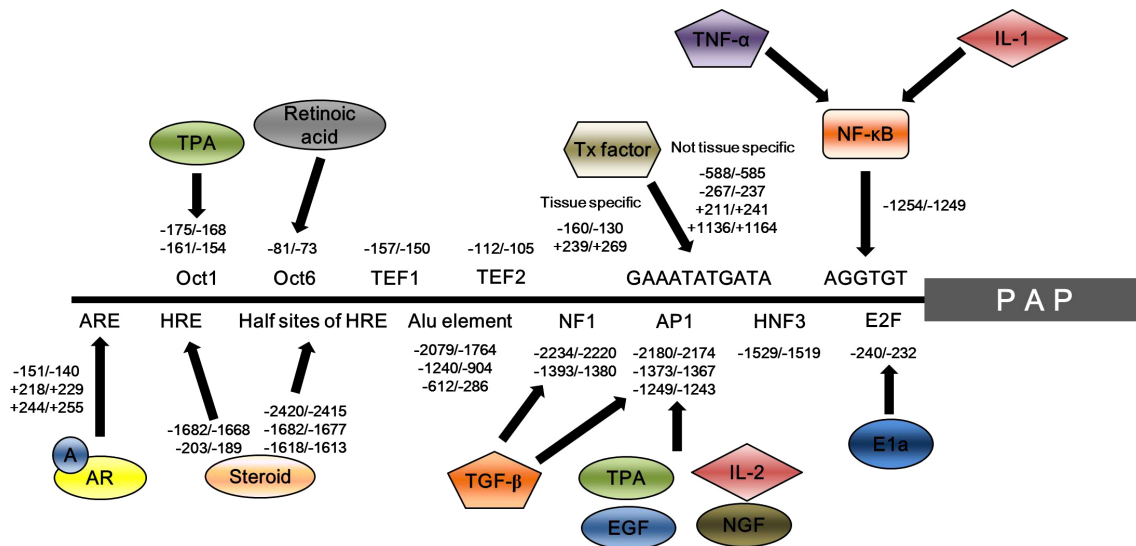


Fig. 2. Binding sites and their corresponding factors which regulate human PAP expression. *Trans*-acting factors involved in the regulation of PAP are schematically represented together with their binding sites. NF- κ B binds to AGGTGT motif at the -1254/-1249 region, which acts as a *cis*-acting element. This leads to tissue-specific upregulation of PAP expression through TNF- α or IL-1. Transcription factors enhance PAP transcription through the GAAATATGATA motif. -588/-585, -267/-237, +211/+241 and +1136/+1164 regions are associated with not tissue-specific upregulation of PAP, whereas -160/-130 and +239/+269 regions are associated with prostate tissue-specific upregulation. Androgen (A) - androgen receptor (AR) complex binds to androgen response element (ARE) for positive and/or negative regulation of PAP transcription. ARE located in -151/140 region is involved in enhancing PAP transcription, whereas, +218/+229 and +244/+255 regions are associated with transcriptional inhibition of PAP. A: androgen; AR: androgen receptor; NGF: nerve growth factor; EGF: epidermal growth factor; TPA: tissue plasminogen activator; IL: interleukin; TGF- β : Transforming growth factor- β ; NF- κ B: nuclear factor kappa B; TNF- α : tumor necrosis factor- α .

2 (Zhang *et al.*, 2001). Histidine (H257) and arginine (R11, R15, R54, R79) residues are also important for PAP activity (Ostanin *et al.*, 1994). The dephosphorylation mechanism of PAP is similar to that of fructose-2,6-bisphosphatase (Okar *et al.*, 2000). The His12 acts as a nucleophile and conjugates with the substrate to form a phosphohistidine intermediate. Then, for recycling of the enzyme and dephosphorylation, Asp258 hydrolyzes phosphohistidine (Ostanin *et al.*, 1994; Sharma and Juffer, 2009). Although the dephosphorylation mechanisms and catalytic active site of PAP are well-known, there are very few substrates that have been identified so far. The few that have been identified include AMP, phosphotyrosine, phosphocholine, phosphocreatine and ErbB-2 (Dave and Rindani, 1988). Because PAP has the potential to act as the protein tyrosine phosphatase, there could be many other substrates that have yet to be identified. Identification of such substrates would help to delineate the signal transduction pathways of PAP, which can contribute to better diagnosis, treatment and prevention of prostate cancer.

TISSUE EXPRESSION OF PAP

In human, PAP is one of the major proteins secreted by prostate columnar epithelium secretory cells following puberty (Graddis *et al.*, 2011). PAP protein has been determined to be about 0.5 mg/g wet weight of prostate tissue (Goldfarb *et al.*, 1986) and approximately 1 mg/ml in seminal fluid (Ronberg *et al.*, 1981). PAP expression is associated with the sex hormone testosterone which determines secondary sexual characteristics (Goldfarb *et al.*, 1986). PAP may be found in

increased amounts in men who have prostate cancer. Indeed, robust expression of PAP was detected in high Gleason score prostate cancer (Gunia *et al.*, 2009). But PAP expression is also enriched in normal prostate cells as well as in prostate cancer tissue as determined by real-time qPCR (Graddis *et al.*, 2011). When compared with other tissue, PAP mRNA level is 50-5,000 fold higher in normal prostate tissue, and 110-6,000 fold higher in prostate cancer tissue. PAP can also be detected in various tissues other than prostate such as brain, kidney, liver, lung, placenta, salivary gland, spleen, thyroid and thymus cells (Solin *et al.*, 1990). PAP is absent in breast carcinoma tissue in contrast to normal breast tissue where PAP can be detected (Wang *et al.*, 2005). More recently, however, PAP was discovered in large quantities in breast cyst fluid (BCF), especially in metaplastic epithelium (intracystic Na/Klessthan, 3 type I), suggesting the role of PAP in protecting several carcinomas by activating TGF- β as a similar molecule to PSA (Erbas *et al.*, 2007). Further study is needed to elucidate the role of PAP on TGF- β activation. In colon carcinoma, PAP was detected in only 40% of samples at a lower level than normal prostate or prostate carcinoma (Wang *et al.*, 2005). The acid phosphatase that is expressed in placenta and liver is mostly located in lysosome, and are therefore termed lysosomal acid phosphatase (LAP) (Shan *et al.*, 2003).

REGULATION OF PAP GENE EXPRESSION

The PAP gene is located in chromosome 3q21-23 in humans (Winqvist *et al.*, 1989). Alternative splicing generates two types of PAP transcripts; transmembrane PAP which

consists of 11 exons (exon 1-9, 10a and 11), and cellular and secretory PAPs having 10 exons (exon 1-9 and 10b) (Zelivianski *et al.*, 1998; Veeramani *et al.*, 2005; Kong *et al.*, 2011). The length of 3'-UTR of transmembrane PAP is shorter than those of cellular and secretory PAPs: 405 bp versus 874 bp (Kong *et al.*, 2011). The molecular mechanisms underlying PAP gene regulation are not fully understood. However, many *trans*-acting factors including androgen/androgen receptor, NF- κ B, TNF- α and IL-1 are involved in the regulation of PAP gene expression (Fig. 2). In human prostatic carcinoma, two major transcription initiation sites exist, one at 56bp and the other 91bp upstream of ATG codon (Zelivianski *et al.*, 1998). The androgen and androgen receptor (AR) complex seems to be essential for prostate development and function. In PAP regulatory regions, an androgen response element (ARE) exists in 3 sites (-151/-140, +218/+229, +244/+255) which are androgen-AR complex binding motifs. When androgen levels are normal, the -151/-140 site is involved in enhancing PAP transcription level. However, when androgen level is low, the +218/+229 and +244/+255 sites act as negative regulators of PAP transcription (Porvari *et al.*, 1995; Kong *et al.*, 2011). These findings imply that the androgen is an essential factor for human PAP expression. Upstream deletion analysis confirmed that 577bp in -1356/-799 region is *cis*-acting enhancer region. Furthermore, prostate cancer specific PAP expression is increased due to NF- κ B binding to AGGTGT motif in -1254/-1249 region that is located in the PAP promoter region (Zelivianski *et al.*, 2004). Chloramphenicol acetyl transferase (CAT) reporter gene assay of the human PAP promoter revealed that the -1258/-779 elements are essential for cell type-specific PAP expression (Zelivianski *et al.*, 2004). In addition, GAAATATGATA-like element necessary for transcription factor binding were found to exist in 6 sites in PAP regulatory regions (Shan *et al.*, 2005). Based on electrophoretic mobility shift assay data, it was confirmed that -160/-130 and +239/+269 sites are related to tissue-specific PAP expression (Shan *et al.*, 2003; Shan *et al.*, 2005). Additionally, other sites should exist that regulate tissue-specific regulation of PAP in promoter regions.

DIFFERENT FORMS OF PAP

Although the level of PAP is increased in the circulation of patients with prostate cancer, its intracellular level and activity are greatly diminished in prostate cancer cells. This apparent discrepancy can be explained by the fact that there are two forms of PAP in prostate epithelial cells; the cellular form (cPAP) and secretory form (sPAP). The two forms of PAP differ in their biochemical properties such as hydrophobicity, isoelectric points and glycosylation patterns (Van Etten, 1982; Veeramani *et al.*, 2005). sPAP is expressed only in the prostate (Solin *et al.*, 1990) and is mostly released into seminal fluid (Ronnberg *et al.*, 1981). The expression of cPAP becomes very high in normal prostate epithelial cells. But its level decreases in prostate cancer cells compared to neighboring normal cells (Reif *et al.*, 1973; Lin *et al.*, 2001). This decreased expression of cPAP results in hyperphosphorylation of HER-2 at tyrosine residues and activation of downstream extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signaling, which can lead to androgen-independent cell growth and prostate cancer development. Consistent

with this, prolonged passage of LNCaP cells led to a decrease in the cPAP level, which corresponds to the loss of their androgen sensitivity and an increase in the growth rate and tumorigenicity. Conversely, ectopic expression of cPAP in AR-positive, PAP-null cells could restore their androgen sensitivity and a decrease in their growth rate and tumorigenicity (Lin *et al.*, 1998; Meng *et al.*, 2000; Lin *et al.*, 2001). Moreover, the level of cPAP inversely correlated with prostate cancer progression despite an elevated level of blood sPAP (Abrahamsson *et al.*, 1988). In another aspect, PAP proteins isolated from prostate cancer patients had lower pI values and longer half-lives than normal tissues (Veeramani *et al.*, 2005). Lin *et al.* could show that the decreased clearance rate of this cancerous PAP in animal model can be explained by altered post-translational modification such as increased sialylation (lower pI). Based on these findings, it was suggested that the elevated blood PAP in prostate cancer patients is due to combined effects of increased tumor mass and increased half-life of sPAP (Veeramani *et al.*, 2005). On the other hand, recent studies revealed that the splice variant (TM-PAP) was expressed in nonprostatic tissues, including brain, kidney, liver, lung, skeletal muscle, placenta, salivary gland, spleen, thyroid, and thymus (Azumi *et al.*, 1991; Hsing and Chokkalingam, 2006). TM-PAP was also expressed in fibroblast, Schwann, and LNCaP cells, but not in PC-3 cells. This type I transmembrane (TM) protein had the extracellular NH₂-terminal phosphatase activity and the COOH-terminal lysosomal targeting signal (Yxx Φ). TM-PAP was localized in the plasma membrane-endosomal-lysosomal pathway and found to colocalize with the lipid raft marker flo-

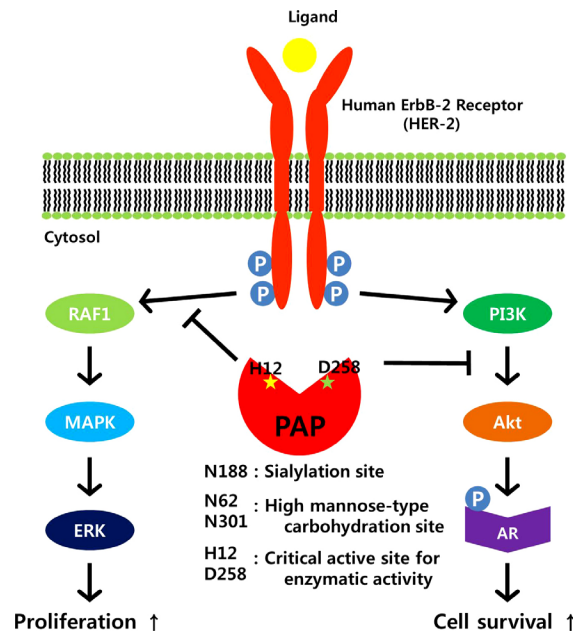


Fig. 3. Molecular inhibitory mechanism of cPAP to block proliferation and survival of prostate cells. When human ErbB-2 receptor (HER-2) is activated by hyperphosphorylation, it transduces signaling for two pathways: RAF1/MAPK/ERK pathway and PI3K/Akt/AR pathway. Blocking of these two pathways by prostatic acid phosphatase (PAP) can lead to inhibition of cell proliferation and survival. Functionally important residues for PAP activity together with critical active sites (H12 & D258) are indicated. AR: androgen receptor.

tillin-1 (Quintero *et al.*, 2007). These findings emphasize the fact that the expression of PAP may not be exclusive to prostatic tissue, and that this issue together with non-canonical functions of PAP has to be taken into account for the success of PAP-based immunotherapy without unwanted side effects (Antonarakis and Drake, 2010; Garcia, 2011; Gerritsen, 2012).

CELL SIGNALING REGULATION BY PAP

PAP can regulate prostate cell growth in two signaling pathways (Fig. 3). Human ErbB-2 (HER-2) can be homodimerized when it is phosphorylated at a tyrosine residue in early developmental stages of prostate cancer (Lin *et al.*, 1994). Dimerized HER-2 then activates downstream ERK1/2 and MAPK, which in turn increases cell proliferation (Meng *et al.*, 2000). In another pathway, activated HER-2 stimulates PI3K signaling. Upon activation, PI3K accumulates and activates Akt. Activated Akt then leads to phosphorylation and activation of AR and phosphorylated AR stimulates cell proliferation (Vihko *et al.*, 2005). PAP can act as a negative regulator of both pathways of HER-2 by dephosphorylation. By blocking Akt, PAP can inhibit androgen-independent prostate cell growth. This is consistent with the observation that PAP expression has a negative correlation with prostate cancer development (Saito *et al.*, 2007). Indeed, late stage prostate cancer had a low level of PAP, suggesting a high risk for malignant tumor formation (Merrick *et al.*, 2005). In this regard, PAP can be regarded as a tumor suppressor mediating inhibition of cell growth (Veeramani *et al.*, 2005).

Measuring levels of the active form of the protein EGFR in the tumor and its vicinity can provide a more reliable prognosis for individuals with prostate cancer. EGFR belongs to the same family as the prognosis marker HER-2 (Rubenstein *et al.*, 2012), which is used today for breast cancer to determine the aggressiveness of a tumor that is to be treated with inhibitors of HER2 (Herceptin). In a similar way, it may be possible in the future to screen for the active form of EGFR to select patients with a poor prognosis and are suitable for treatment with inhibitors of EGFR. In order to use EGFR as a prognosis marker clinically in the future, further studies will need to target its expressions in other and larger material in prostate tumors.

PAP AS A USEFUL MARKER FOR PROSTATE CANCER

Despite the great progress in our understanding of the disease process and standardization of diagnostic criteria for prostate cancer, the majority of prostate tumors are detected at early stages with uncertain prognosis (Larne *et al.*, 2012). Previous studies have shown that PAP can serve as a prostate cancer marker by proportionally increasing secretory PAP expression as prostate cancer progresses (Azumi *et al.*, 1991; Wang *et al.*, 2005; Gunia *et al.*, 2009). High levels of PAP expression were detected in high Gleason score prostate cancers as determined by immunohistochemistry (Gunia *et al.*, 2009). However, the introduction and widespread adoption of PSA has largely displaced PAP in the diagnosis and treatment of prostate cancer. This was because PSA was more sensitive than PAP in the detection of prostate cancer in the serum. However, the use of PSA has also led to over-diagnosis and overtreatment of prostate cancer resulting in contro-

versy about its use for screening (Vihko *et al.*, 2005; Shariat *et al.*, 2011). Indeed, there are still some significant controversies over PSA screening because no study has successfully shown any significant correlation between such screening and a decline in mortality rate. (Madu and Lu, 2010). PSA also has limited predictive accuracy for predicting outcomes after treatment and for making clinical decisions about adjuvant and salvage therapies (Huang *et al.*, 1993; Madu and Lu, 2010). Hence, there has been an urgent need for novel biomarkers to supplement PSA for detection and management of prostate cancer. Under these circumstances, there is now a renewed interest in PAP again because it has significantly higher correlation with prostate cancer progression (Zimmermann, 2009). The cancer-specific survival (CSS) study, which tested 193 patients' serum, showed that, when PAP concentration is <1.5 U/L, 1.5-2.4 U/L and >2.5 U/L, the progression of prostate cancer is 93%, 87% and 75% ($p=0.013$), respectively. However, when PSA concentration is <10 ng/ml, 10-20 ng/ml and > 20 ng/ml, the progression of prostate cancer is 92%, 76% and 83% ($p=0.393$), respectively (Fang *et al.*, 2008). These results strongly suggest that PAP may be a more suitable marker for prostate cancer and CSS than PSA. PAP appears to be particularly valuable in predicting distant failure in higher-risk patients for whom high levels of local control are achieved with aggressive initial local treatment. As prostate cancer care becomes increasingly focused on identifying the minority of patients who would benefit from aggressive systemic therapy,

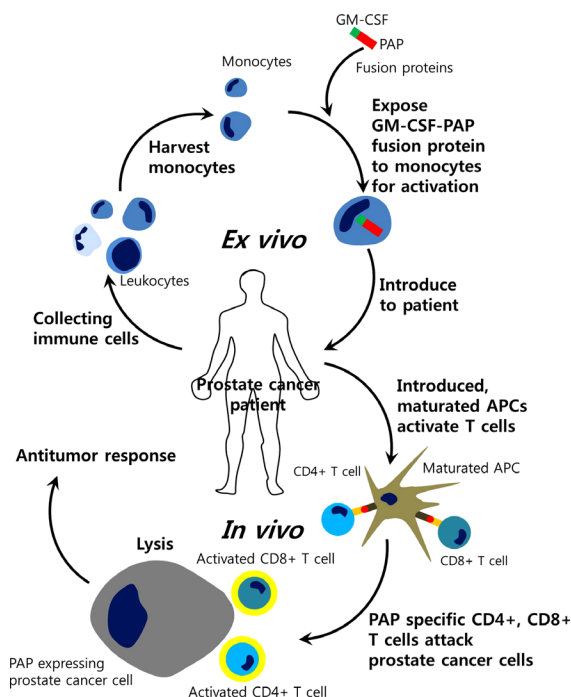


Fig. 4. Schematic diagram of Provenge trial. The stages of Sipuleucel-T treatment for patients with prostate cancer are shown. Sipuleucel-T treatment is similar to a dendritic cell (DC) vaccine. It is a United States Food and Drug Administration (FDA)-approved autologous cell-based immunotherapy that targets prostatic acid phosphatase (PAP) as a treatment for advanced prostate cancer. Modified from Garcia (2011) and Gerritsen (2012). GM-CSF: granulocyte-macrophage colony-stimulating factor; APC: antigen-presenting cells.

a reevaluation of the potential contribution of the PAP test seems timely (Taira *et al.*, 2007). Investigation of any potential interplay between PAP and PSA and between PAP and other markers are also warranted. Recently, diagnostic utility of P504S/p63 cocktail in verifying prostatic carcinoma involvement in seminal vesicles were evaluated (Harvey *et al.*, 2010). The use of the single-color P504S/p63 immunohistochemical stain cocktail was recommended for identifying prostatic carcinoma involving the seminal vesicle and for distinguishing benign prostatic glands from prostatic carcinoma when there is a question of seminal vesicle invasion. It was argued that P504S/p63 cocktail is superior to PSA or PAP when sections contain both seminal vesicle and benign glands because PSA and PAP cannot distinguish benign from malignant glands (Harvey *et al.*, 2010).

PAP AS A USEFUL ANTIGEN FOR PROSTATE CANCER THERAPY

Based on the good prognostic value of PAP and the potential usefulness of PAP as an antigen, an immunotherapy employing autologous PAP-loaded dendritic cells was initiated (Drake, 2010). This FDA-approved therapy termed PROVENGE (Sipuleucel-T) works on the basic idea that over 95% of prostate cancer cells express PAP (Drake, 2012). Treatment with sipuleucel-T comprises a number of stages (Fig. 4). First, autologous peripheral blood mononuclear cells (PBMCs) including antigen-presenting cells (APCs) are pulsed *ex vivo* and activated *in vitro* with a recombinant fusion protein (PA2024) that couples the vaccine target (PAP) to granulocyte-macrophage colony-stimulating factor (GM-CSF) (Garcia, 2011; Gerritsen, 2012). This PAP and GM-CSF fusion protein is presented to antigen presenting cells (APCs) that are collected from the patient. These activated APCs are then introduced to the patient for induction of T cells *in vivo*. Activated T cells now attack prostate cancer cells in the patient, thus treating the cancer (Cheever and Higano., 2011; Sims, 2012). In the phase III IMPACT (Immunotherapy Prostate AdenoCar-

cinoma Treatment) trial of 512 patients with asymptomatic or minimally symptomatic metastatic CRPC, which served as the basis for the licensing approval of sipuleucel-T, vaccine treatment resulted in a 4.1 month improvement in median overall survival compared with placebo (25.8 months versus 21.7 months, respectively) with a 22% relative reduction in the risk of death [hazard ratio (HR): 0.78, *p*=0.03] (Gerritsen, 2012). The delayed onset of response reflected in the late separation of survival curves has been reported in many clinical trials of immunotherapeutic agents and is known to impact on clinical dynamics, highlighting the need for suitable end points to assess efficacy (Gerritsen, 2012). On the other hand, the PAP encoded DNA vaccine is currently undergoing clinical trials that aim to prevent and treat prostate cancer. Ten of twenty-two patients showed antigen-specific T cell proliferation and upregulation of CD8⁺INF γ (McNeel *et al.*, 2009, Lubaroff, 2012). DNA vaccines encoding PAP are expected to be an effective way to prevent and treat prostate cancer. To date, a lot of prostate cancer-associated antigens such as PSA, PAP, or PSMA have been cloned and are being tested as a component of investigational therapeutic cancer vaccines (Vieweg and Dannull, 2005). But because prostate cancer is known to be a heterogeneous disease with a number of different genetic make-ups, personalized therapeutic strategies guided by the use of novel molecular imaging will be necessary to successfully test the utility of such targeted agents in patients whose tumors will depend upon that antigen target for tumor growth and/or survival.

INVERSE CORRELATION BETWEEN PAP AND OLIGO-SPERMIA

PAP has an essential role not only in prostate cancer but also in many other physiological functions (Fig. 5). PAP is also expressed in normal prostate tissue, which is an indication that PAP has a prostate-specific physiological role. PAP is abundant in seminal fluid and is therefore thought to be an important factor in fertilization, helping to increase the mobility

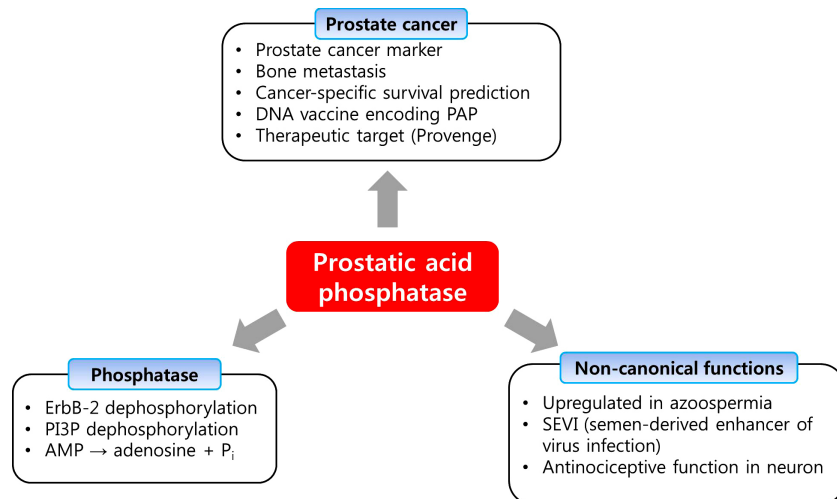


Fig. 5. Diverse roles of PAP including non-canonical functions. Traditionally, PAP was a molecule mainly involved in prostate cancer diagnosis and treatment. Recently, however, inherent phosphatase activity of PAP broadens its role in other areas such as oligospermia, SEVI, and pain suppression.

of sperm (Afzal *et al.*, 2003). On the other hand, a previous study of 365 semen samples has shown that PAP concentration is inversely associated with sperm concentration (Dave and Rindani, 1988; Singh *et al.*, 1996). Moreover, other group showed that the highest phosphatase activity was detected in azoospermic men and, when phosphatase activity was decreased, the concentration of sperm tended to recover to normal concentration (Dave and Rindani, 1988; Collins and Bennett, 2011). Although molecular mechanisms are still unclear as to how PAP induces oligospermia, PAP can be used as an effective marker for oligospermia (Coussens and Werb, 2002).

ANTINOCICEPTIVE EFFECT OF PAP

While PAP was classically considered to be a non-specific phosphomonoesterase (E.C. 3.1.3.2) (Ostrowski and Kuciel, 1994), sPAP and transmembrane PAP could function as ectonucleotidases that hydrolyzes extracellular adenosine 5'-monophosphate (AMP) to adenosine and Pi. This extracellular adenosine leads to a decrease in chronic pain by activating A1R in nociceptive neurons (Zylka *et al.*, 2008). sPAP is glycosylated at three asparagine residues (N62, N188, N301) and has potent antinociceptive effects when administered to mice (Hurt *et al.*, 2012a). Secretion and post-translational carbohydrate modifications were found to be required for PAP protein stability and catalytic activity. Also, it was found that deletion of PAP reduces extracellular AMP hydrolysis in nociceptive neurons and in the dorsal spinal cord (Street *et al.*, 2011). Intrathecal injection of sPAP had three day long antinociceptive effects in mouse models of inflammatory pain and neuropathic pain (Sowa *et al.*, 2009). In addition, sPAP had enduring (>7 days) A1R-dependent antinociceptive effects if injected intrathecally before nerve injury or inflammation (Sowa *et al.*, 2010). These findings altogether suggest that a recombinant version of human sPAP could be used as a treatment for chronic pain or for preemptive analgesia (Hurt *et al.*, 2012b).

SEMEN-DERIVED ENHANCER OF VIRUS INFECTION (SEVI)

PAP may play an important role in the transmission of HIV. By screening a complex peptide/protein library derived from human semen, German group could show that naturally occurring fragments of the abundant semen marker prostatic acidic phosphatase (PAP) form amyloid fibrils (Münch *et al.*, 2007). These fibrils, termed Semen-derived Enhancer of Virus Infection (SEVI), capture HIV virions and promote their attachment to target cells, thereby enhancing the infectious virus titer by several orders of magnitude. Physiological concentrations of SEVI amplified HIV infection of T cells, macrophages, *ex vivo* human tonsillar tissues, and transgenic rats *in vivo*, as well as *trans*-HIV infection of T cells by dendritic or epithelial cells. Since amyloidogenic PAP fragments are abundant in seminal fluid and boost semen-mediated enhancement of HIV infection, PAP may be a future target to combat the spread of HIV infection. In another instance, SEVI greatly increased xenotropic murine leukemia virus-related virus (XMRV) infections of primary prostatic epithelial and stromal cells (Hong *et al.*, 2009). Recently, it was shown that Cu(II) and Zn(II) inhibit fibrillization of SEVI, suggesting that the metals may modulate

SEVI fibrillization under physiological conditions (Sheftic *et al.*, 2012).

SUMMARY AND CONCLUSIONS

Prostate cancer research in the past decade has made huge stride in the understanding of the disease process and standardization of diagnostic criteria. Although great progress has been made, there still remain many areas of uncertainty and debate. The revolution towards a synthesis of diagnosis and therapy together with sound prognostic models is only just beginning. One of the major hurdles in prostate cancer therapy is that more than 70% of patients fall into a group where very little can be said about their prognosis with today's markers. This in turn means that certain patients are over-treated with therapies that can lead to serious side effects and that other patients who really need intensive treatment do not get it or get it too late. Therefore, a panel of sound biomarkers will be needed to achieve sufficient degree of certainty in guiding clinical decisions. PAP has a significantly higher correlation with the morphological characteristics of prostate cancer and can provide a more efficient prognosis than any other markers currently available. Since PAP is a proportional measure of prostate cancer progression, it can also be used in immunotherapy of prostate cancer. However, utility of PSA and other potential markers must also be considered to ensure best diagnosis and prognosis of prostate cancer. More molecular studies on PAP increase in prostate cancer and different forms of PAP including transmembrane PAP are needed to unveil the detailed mechanism of PAP in prostate cancer. Although PAP has been used as a marker of prostate cancer for decades, normal physiological functions of PAP must still be identified. Recent characterization of PAP's involvement in pain suppression, oligospermia, and viral infection is shedding newer lights on the role played by PAP. To better understand the diverse roles of PAP *in vivo*, a systematic and integrated approach will be needed.

ACKNOWLEDGMENTS

The present research was conducted by the research fund of Dankook University in 2010.

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