Chapter 12
Human Prostatic Acid Phosphatase in Prostate Carcinogenesis

Sakthivel Muniyan, Yu-Wei Chou, Shou-Qiang Ou-Yang, and Ming-Fong Lin

Abstract Human prostatic acid phosphatase (PAcP) is classically known as a prostate epithelium-specific differentiation antigen and was used as a surrogate marker for detecting prostate cancer (PCa) and monitoring its progression until the availability of prostate-specific antigen. Mature human PAcP protein is a 100 kDa glycoprotein containing two subunits of approximately 50 kDa each. Classically, two forms of human PAcP proteins have been identified: the cellular form (cPAcP) and the secretory form (sPAcP). Recent studies reveal the existence of a transmembrane form (TM-PAcP). While the function of sPAcP and TM-PAcP in human remains under further investigation, cPAcP functions as a neutral protein tyrosine phosphatase in PCa cells and dephosphorylates human epidermal growth factor receptor-2 (HER-2/ErbB-2/Neu) resulting in decreased cell growth as well as tumor suppression. Clinically, cPAcP levels decrease in PCa tissues and correlate with PCa progression, despite elevated levels of sPAcP in circulation. Data from xenograft animal models validate the tumor suppressor activity of cPAcP in prostate carcinomas. Further, activation of ErbB-2 upon knockdown of cPAcP expression results in a castration-resistant phenotype. Expression of PAcP is regulated by different factors in human PCa cells. PAcP is also a useful immunogen in PCa immunotherapy. Further investigation of the regulatory mechanism of cPAcP expression will likely provide valuable insights into novel PCa therapy.

S. Muniyan, Ph.D. • Y.-W. Chou, Ph.D. • S.-Q. Ou-Yang, Ph.D.
Department of Biochemistry and Molecular Biology, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA

M.-F. Lin, Ph.D. (✉)
Department of Biochemistry and Molecular Biology, College of Medicine, University of Nebraska Medical Center, 985870 Nebraska Medical Center, Omaha, NE 68198, USA
e-mail: mlin@unmc.edu

D.J. Tindall (ed.), *Prostate Cancer: Biochemistry, Molecular Biology and Genetics*, Protein Reviews 16, DOI 10.1007/978-1-4614-6828-8_12, © Mayo Clinic 2013

Abbreviations

Ab	Antibody
AcP	Acid phosphatase
ADT	Androgen deprivation therapy
APCs	Antigen-presenting cells
CRPCa	Castration-resistant prostate cancer
DHT	5α-dihydrotestosterone
EGF	Epidermal growth factor
EGFR	EGF receptor
FBS	Fetal bovine serum
HDAC	Histone deacetylase
HER-2/ErbB-2/neu	Human epidermal growth factor receptor-2
PAcP	Prostatic acid phosphatase
PCa	Prostate cancer
PI3K	Phosphoinositide 3-kinase
pIs	Isoelectric point
PKC	Protein kinase C
PSA	Prostate-specific antigen
PTP	Protein tyrosine phosphatase
p-Tyr	Phosphotyrosine
TM-PAcP	Transmembrane PAcP
Tyr-P	Tyrosine phosphorylation

Introduction

Prostatic acid phosphatase (PAcP; E.C.3.1.3.2) is known classically as a prostate-specific differentiation antigen in differentiated prostate epithelia [1, 2]. Human PAcP protein is synthesized in differentiated columnar epithelia of the prostate gland [2–6]; some of which is secreted into prostatic fluid as the secretory form (sPAcP) and the rest is retained intracellularly as the cellular form (cPAcP) [7]. Recent studies have revealed a transmembrane form of PAcP (TM-PAcP) [8]. Since PAcP biochemically hydrolyzes a broad variety of small organic phosphomonoesters under acidic conditions, this enzyme is known as an AcP [3, 9–11].

PAcP levels are very low in normal circulation. In 1936, Gutman and colleagues made the seminal observation that human PAcP activity in serum is significantly increased in PCa patients, especially those with bone metastases [12]. Subsequently, Huggins and colleagues reported that the circulating PAcP activity correlates with prostate tumor progression [13]. Since then, serum PAcP has been studied extensively as a surrogate marker for the diagnosis of PCa until the availability of PSA [14].

PAcP expression is positively associated with prostate epithelial cell differentiation. Prior to puberty, cPAcP is expressed at a low level. In normal, well-differentiated human prostate epithelial cells, the high level of cPAcP protein is in agreement with slow cellular growth [1, 2, 15]. In prostate adenocarcinoma cells cPAcP expression is lower than in adjacent noncancerous cells, despite elevated sPAcP activity in circulation [6, 16–18]. Studies have suggested that cPAcP acts as a tumor suppressor in PCa cells [19–21]. Several lines of evidence collectively support the concept that cPAcP functions as a histidine-dependent PTP in prostate epithelia and regulates its tumorigenicity by dephosphorylating p-Tyr of human ErbB-2 (also known as HER-2 or neu protein) at physiological pH [19, 21–24]. cPAcP also plays a critical role in regulating the cross-talk between androgens and Tyr-P signaling. In parallel, numerous studies have shown a therapeutic potential of PAcP for the treatment of PCa [19, 21, 25–28]. Hence, in this chapter, we review the structure and regulation of PAcP isoforms in prostate epithelia and the function and therapeutic role of cPAcP in human PCa.

Human Prostatic Acid Phosphatase

Physiology of PAcP

AcPs are a group of enzymes that biochemically hydrolyze phosphomonoesters optimally at acidic pH. In human cells, AcPs can be divided into at least five isoforms, including erythrocytic, lysosomal, prostatic, macrophagic, and testicular AcPs [2, 29, 30]. The mature form of PAcP is a glycoprotein consisting of two subunits of approximately 50 kDa each and is synthesized in differentiated columnar prostate epithelia [3, 5, 17, 31]. There are two forms of PAcP protein in well-differentiated human prostate epithelia: the cellular form (cPAcP) and the secretory form (sPAcP). Recent results reveal the possible existence of a transmembrane form (TM-PAcP) [8].

The physiological level of PAcP is negligible in fetal tissue and young males. After puberty, cPAcP level can reach approximately 0.5 mg/g of wet tissue in normal, well-differentiated prostate epithelia [2, 15]. sPAcP is predominantly secreted into seminal fluid at approximately 1 mg/ml and has been used as a marker in forensic medicine [7, 14]. In PCa patients, the circulating level of sPAcP is elevated and correlates with the stage of PCa. Hence, PAcP has received much attention and has served as a surrogate marker for PCa [12–14].

Expression and Distribution of PAcP in Human Tissues

While PAcP is considered as a prostate-specific differentiation antigen, studies of PAcP expression in non-prostate tissues have yielded inconsistent results. Solin et al. [32] showed by RNA blot analysis that there is no detectable hPAcP mRNA in

human liver, lung, pancreatic cancer tissue, placenta, breast cancer cells, mononuclear blood cells, or acute promyelocytic leukemia cells. Similarly, Zelivianski et al. [33] could not detect hPAcP mRNA expression in spleen, thymus, testis, ovary, small intestine, colon, or peripheral blood leukocyte by northern blotting.

On the contrary, immunologic studies demonstrate the positive reactivity of hPAcP Ab in some non-prostate cells such as leukocytes, kidney, spleen, placenta, pancreas, liver, stomach, granulocytes, neutrophils [34–38], male anal gland and in urethral gland of both sex [39], crypt epithelium of the duodenum [40], pancreatic islet cell carcinomas [41], and breast tumor cells [34]. Nevertheless, it has been proposed that the reactivity of hPAcP Ab in non-prostatic cells does not recognize the authentic hPAcP, but an immunologically cross-reactive AcP [22, 32, 42, 43], e.g., lysosomal acid phosphatase, a transmembrane phosphatase expressed in almost all tissues and cell types [38]. Supportively, purified AcPs from human spleen and lung having a similar molecular weight as hPAcP share at least one common antigenic epitope with hPAcP [22].

Recently, Graddis et al. [44] using quantitative RT-PCR reported that hPAcP is expressed at moderate to high levels in both normal and malignant prostate tissues. In non-prostate normal tissues examined, bladder cells express the highest ratio relative to prostate, though the expression level is still 50-fold lower than prostate [44]. The ratio of normal prostate PAcP mRNA to normal kidney PAcP mRNA is 178, which is comparable to the ratio of 192 reported previously [45]. The hierarchical tissue distribution of hPAcP mRNA by PCR in normal tissues is prostate >>>bladder>kidney>pancreas>cervix = testis>lung = ovary [44], which is similar to previous analyses prostate>>>placenta>kidney>testis>pancreas>small intestine = leukocytes>lung>ovary [45]. Among the tumor samples analyzed, cervical tumors express PAcP mRNA at the level similar to that seen in normal bladder; i.e., the level is less than 2 % of that in normal prostate [44]. Due to the clinical importance of hPAcP in PCa therapy and other medical applications, further experiments should clarify the identities of these proteins by determining their sequence.

Expression of PAcP in Prostate Epithelia

Immunohistochemistry staining has demonstrated that hPAcP is primarily localized in the differentiated columnar epithelial cells of prostate [37, 43, 46–48]. In situ hybridization analyses confirmed that hPAcP mRNA is detected in the glandular, ductal epithelial cells of prostate, and that the stromal cells are devoid of this mRNA [6]. An electron microscopic study showed that hPAcP is in the microvilli lining and vesicular bodies of apical cells in normal prostate [46]. The existence of human cPAcP in the cytosolic fraction has been clearly demonstrated by various biochemical approaches including sub-fractionation [9, 21]. Due to the importance of cPAcP in regulating Tyr-P signaling in PCa cells, it is imperative to clarify the subcellular localization of cPAcP where it interacts with ErbB-2 for growth regulation.

Structure of Human Prostatic Acid Phosphatase

Biochemical Characterization of Human PAcP Gene and mRNA

The human PAcP gene is located at chromosome 3q21–q23 [49] and has a size of more than 40 kb and distributed over 10 exons [50, 51]. Exon 1 encodes for the signal peptide and the first eight amino acids of the protein. Exons 2–10 encode the rest of the coding regions and 3′-untranslated region (Fig. 12.1). Several PAcP cDNA clones have been obtained in which additions, deletions, and/or substitutions of nucleotides [49, 52–54] lead to the heterogeneity of amino acid residues. Interestingly, two different signal peptide sequences have been identified [52–54]. The biological significance of these heterogeneities in PAcP sequence requires further investigations.

In human LNCaP prostate carcinoma cells, the major transcription start site is located at 50 nucleotides upstream of the gene's ATG codon [55]. In normal differentiated human prostate epithelia, two species of PAcP mRNA are detected by Northern blot analysis with molecular sizes of 2.4 kb and 3.3 kb, essentially due to the variation in the number of Alu repeats in the 3′-noncoding sequence [32]. In prostate carcinomas, only expression of the 3.3 kb species is detected, which is lower than in noncancerous cells [32, 33, 52, 56]. The biological significance and the molecular mechanism of the loss of 2.4 kb PAcP mRNA expression in PCa cells is not clear.

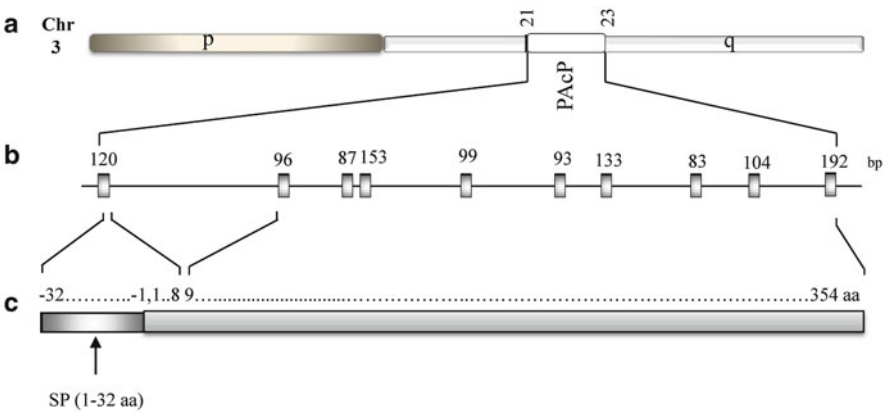

Fig. 12.1 Schematic diagram of human prostatic acid phosphatase (PAcP) gene and protein. (**a**) Localization of the hPAcP gene in the q-arm of the chromosome 3 (Chr 3). (**b**) hPAcP gene encoded by 10 exons. The number of nucleotides in each exon was noted. (**c**) The full length of hPAcP protein consisting of 386 amino acids with 32 amino acid signal peptides (SP). The signal peptide and the first eight amino acids were encoded by exon 1 and the rest of amino acids were coded by exons 2–10

Structure of the PAcP Protein

The PAcP protein is initially translated as a precursor form with 386 amino acids containing a 32-amino acid signal peptide, and the mature PAcP protein of 354 amino acids has a calculated molecular mass of 41,126 Da (Fig. 12.1). The signal peptide directs the nascent PAcP polypeptide into rough endoplasmic reticulum, which provides an environment for posttranslational modifications of PAcP protein [52, 57]. These modifications provide stability for the PAcP protein, where deletion of the signal peptide sequence from cDNA results in an extreme low level or nondetectable PAcP protein [58, 59], (Lingappa, Vishwanath and Lin, Ming-Fong, Unpublished observations). It has been proposed that the signal peptide directs secretion of PAcP protein [59]; moreover, no cPAcP protein is found intracellularly [58, 59]. These data support the concept that the function of signal peptide is primarily responsible for directing the nascent peptide via rough endoplasmic reticulum for its various post-translational modifications, including glycosylation, which stabilizes PAcP protein [58].

Sequence analysis has revealed that hPAcP protein contains three asparagine-linked glycosylation sites (62Asn–Glu–Ser64, 188Asn–Phe–Thr190, and 301Asn–Glu–Thr303) and 6 cysteine residues forming two disulfide bonds (Cys129–340 and Cys314–319) and two free residues (Cys183 and Cys281). The glycosylation and disulfide linkages support the structural conformation and the stability of PAcP protein. Molecular sieving under native and denaturing conditions indicates that hPAcP is a dimer consisting of two subunits of similar molecular size [3, 9, 11, 60]. Analyses of the crystal structure of hPAcP reveal that each subunit has two domains: the larger domain is α/β type composed of a central seven-stranded mixed β-sheet with helices on both sides; while, the second, smaller one contains six α-helices and is formed mostly by long-chain excursions (residues 125–227) from the first domain and α-loop between residues 16–38 with no secondary structural elements [61].

PAcP Isoforms: Cellular, Secretory, and Transmembrane Forms

Recent studies have revealed that the signal peptide of hPAcP protein can direct differential biosynthetic pathways, which results in different biological functions of PAcP protein, depending on the growth environments [56], (Lingappa, Vishwanath and Lin, Ming-Fong, Unpublished observations). cPAcP and sPAcP proteins exhibit unique antigenic epitope(s), yet they share partial cross-reactivities [9]. Biochemically, they exhibit different, while overlapping p*I*s [9, 62].

The level of PAcP in normal circulation is negligible, while it is elevated in PCa patients and correlates with clinical progression [12, 13, 63, 64]. Thus, circulating PAcP has served as a surrogate marker for PCa detection prior to the

availability of PSA [14, 65, 66]. The tumor-associated PAcP exhibits different biochemical properties from normal species and is hyper-glycosylated, including sialylation [11]. This hyper-sialylation prolongs the half-life of sPAcP protein in circulation, which contributes to the elevated PAcP level in the circulation of PCa patients despite its decreased mRNA level [11, 67]. sPAcP and cPAcP exhibit different sensitivities to endoglycosidase [Garcia-Arenas, Renee and Lin, Ming-Fong, Unpublished observations]. The biological significance of glycosylation involved in PAcP function and subcellular localization deserves further studies.

Large amounts of PAcP protein are found in normal prostate tissues by immunohistochemistry staining and biochemical analysis of tissue homogenates [9, 62, 68, 69]. The presence of cytosolic cPAcP protein is further validated by sub-fractionation approaches, including ultracentrifugation [21]. Additionally, immunocytochemistry staining of intact, nonpermeabilized LNCaP PCa cells shows no significant staining of hPAcP. On the contrary, intensive staining is seen in the cytosolic area of permeabilized cells with higher intensity of staining in higher density cells [21, 70, 71]. Therefore, cPAcP is localized intracellularly and has served as a useful marker for identifying the prostate origin of metastatic cancer [1, 2, 14]. In prostate carcinomas, intracellular PAcP protein level decreases, correlating with PCa progression [6, 16]. The decreased protein level is at least in part by the decrease of mRNA [32]. Because of the importance of cPAcP in tumorigenicity and androgen sensitivity of PCa cells, the molecular structure of cPAcP relating to sPAcP should be further investigated.

Quintero et al. [8] reported the existence of a PAcP-spliced variant, which is a type I transmembrane protein in many mouse tissues. However, its expression profile in human tissues other than prostate is not yet known. Immunostaining with an anti-PAcP Ab showed PAcP expression in human skeletal muscle cells. They further demonstrated that PAcP colocalizes with lysosomal associated membrane protein 2. None-the-less, expression of PAcP is extremely low in skeletal muscle cells, and importantly, exhibits no lysosomal localization [1, 2, 38]. These observations have raised a concern that the staining may be due to a partial cross-reactivity of polyclonal Ab [22]. It has been further proposed that the active site of TM-PAcP is localized and functioning extracellularly [20]. Biochemical analyses have demonstrated that no phosphatase activity is significantly detected when small organophosphate substrates are incubated with intact LNCaP cells. These results collectively suggest that in prostate epithelia only a very low amount, if any, of the active domain of hPAcP faces extracellularly. While the decreased expression of classical PAcP mRNA and protein correlates with PCa progression, the expression of TM-PAcP mRNA is not significantly changed, indicating that TM-PAcP is not involved in prostate carcinogenesis [8]. A functional characterization of TM-PAcP in prostate epithelia utilizing its specific monoclonal Ab is required for further investigation.

Biological Function of Prostatic Acid Phosphatase Isozymes

Cellular PAcP (cPAcP) as a Growth Regulator and Tumor Suppressor in Prostate Cancer Cells

Expression of PAcP is correlated with the differentiation of normal prostate cells. In PCa, cPAcP levels of both mRNA and protein are decreased, compared to nonmalignant cells [6, 16, 63, 69, 72–74]. It has been proposed that normal prostate epithelia, having a low level of cPAcP, are at high risk of carcinogenesis [72]. In human PCa cell lines, cPAcP levels are correlating inversely with cell growth rates [16, 19, 21, 56]. Results of increased PAcP expression by cDNA transfection and decreased cPAcP expression by antisense cDNA or shRNA knockdown in PCa cells validate the growth regulatory role of cPAcP in PCa cells [17, 21, 71].

Several studies of clinical archival specimens showed that decreased cPAcP expression correlates with increased tumorigenicity and cancer progression [2, 6, 16, 17, 75]. Conversely, expression of cPAcP correlates with decreased tumorigenicity of PCa cells in xenograft animals [16]. Igawa et al. [19] further explored the direct tumor suppression activity of cPAcP in a xenograft animal model. Importantly, in a PAcP-knockout mouse model, the prostate develops adenocarcinomas [20]. The data collectively demonstrate that cPAcP expression suppresses the growth and tumorigenicity of PCa cells. This provides an explanation for the clinical phenomenon that the expression levels of cPAcP inversely correlate with the stage of PCa as well as its advanced progression under androgen deprivation therapy (ADT) [6, 72].

Further studies have revealed that cPAcP is involved in regulating androgen-stimulated proliferation of human PCa cells [76–78]. Expression of cPAcP by cDNA transfection in androgen receptor (AR)-positive, androgen-independent LNCaP C-81 cells results in restoring the androgen sensitivity, i.e., the cell growth is sensitive to androgen treatment [16, 78]. Conversely, an androgen-independent phenotype is obtained by knockdown of cPAcP expression by shRNA in androgen-senstitive LNCaP C-33 cells [21]. Thus, androgen-induced proliferation of prostate epithelia is at least in part due to an androgen effect on decreasing cPAcP activity [16, 71, 78]. The data taken together support the concept that cPAcP plays a critical role in regulating the basal as well as the androgen-stimulated proliferation of human PCa cells.

Transmembrane PAcP (TM-PAcP) as an Analgesic in Mice

While TM-PAcP is detected in several tissues from mice, thus far it has been detected only in prostates of humans [8]. Interestingly, PAcP knock-out ($PAcP^{-/-}$)

mice display enhanced noxious thermal sensitivity and sensitization through inactivation of adenosine A1 receptor (A1R) and phospholipase C-mediated elevation of phosphatidylinositol 4,5-bisphosphate (PIP2) [20, 79, 80]. It has been proposed that TM-PAcP is the fluoride-resistant acid phosphatase and functions as phosphoadenosine phosphatase upon expression in human embryonic kidney HEK293 cells [20]. Considering the long-lasting antinocieptive effect of secretory PAcP in the naïve mouse model, it has been proposed that recombinant PAcP can be used to treat chronic pain [20, 81, 82]. However, it is not known if TM-PAcP is indeed expressed in the corresponding human tissues and cells. The expression profile of TM-PAcP in human tissues and cells should be analyzed.

Role of Secretory PAcP (sPAcP) in Sperm Motility, HIV Transmission, and Forensic Marker

Due to the large amount of sPAcP protein in seminal fluid [83], it has been suggested that PAcP plays a physiological role in fertility [84] and may affect the motility of sperm [20, 85]. Dave and Rindani [86] observed that phosphatase activity is maximal in azoospermic men, and that this activity is decreased as the sperm number (concentration) increases. However, there is no significant difference in PAcP activity in seminal plasma between normal and vasectomized patients [87].

Importantly, a proteolytically cleaved PAcP peptide, PAPf39, which forms amyloid fibrils called Semen-derived Enhancer of Viral Infection (SEVI), can enhance the HIV's ability to infect human cells by five orders of magnitude [88]. This observation is in agreement with a previous report that HIV replication component is detected at a tenfold higher concentration in seminal fluid than in blood, even in the presence of an antiretroviral drug [89]. Alternatively, PAcP may increase the pH of the vagina [90]. Thus, this postintercourse neutralization of pH may allow a female-to-male transmission of HIV [91]. The role of sPAcP in sexually transmitted diseases requires further investigation.

It should be noted that secretory PAcP can also serve as a forensic marker. Due to the large quantity of sPAcP in seminal fluid, and also due to its specificity of expression in males, secretory PAcP was investigated and served as a surrogate marker in forensic medicine for sexual assault [92]. This was supported by the observations that elevated levels of AcP activity persist in the vaginal pool after sexual intercourse and in semen stains [93–100]. Nevertheless, there are some concerns, for example, the potential cross reactivity with other acid phosphatases and the instability of its enzymatic activity. The subsequent advancements including the enhancement in enzymatic assays and the development of detection methods such as ELISA, counter-immunoelectrophoresis, and radioimmunoassays made PAcP as a useful surrogate marker in forensic medicine [101–104]. Nevertheless, the discovery of PSA (also called γ-seminoprotein; γ-SM) in seminal fluid, due to its

long-term stability (identifiable for as long as 1 year) and easy identification, replaced PAcP as the forensic marker [14, 105–108].

Biochemical Characterization of Human Prostatic Acid Phosphatase Protein

PAcP: A Histidine-Dependent Neutral Protein Tyrosine Phosphatase

PAcP, which belongs to the histidine phosphatase superfamily, uses an active-site histidine in catalyzing the transfer of a phosphoryl group from phosphomonoesters to water at acidic pH. The copurification of cPAcP protein with the majority of PTP activity from noncancerous prostate tissue and purified PAcP protein from seminal fluid and tissue exhibiting the endogenous PTP activity together indicate that PAcP is an authentic PTP [109, 110]. Biochemical characterizations show that PAcP dephosphorylates p-Tyr of EGFR with a neutral pH optimum, supporting the concept that cPAcP can indeed function as a neutral PTP in cells [111]. Further, in PAcP K/O mice, Tyr-P activity is increased in prostate cells, suggesting that intracellular cPAcP functions as PTP [20, 112]. Several lines of evidence together support the notion that cPAcP indeed functions as a neutral PTP in prostate epithelia [17, 110, 113].

Structural analyses of PAcP protein reveal that it contains neither the PTP signature motif, $C(X)_5R(S/T)$, nor the extended active site signature sequence for the dual-specificity phosphatases, $VXVHCXXGXXRS(X)_5AY(L/I)M$ [52, 57, 114, 115]. Chemical titration experiments revealed that PAcP has two reactive sulfhydryl groups [116]. It was hypothesized that Cys183 is essential for the PTP activity of PAcP. Nevertheless, studies by site-directed mutagenesis determined that neither Cys183 nor Cys281 plays a role in the phosphatase enzymatic activity [58].

Covalent modifications and phosphoenzyme trapping studies revealed that PAcP contains histidine and carboxylic acid residues in the active site [117–119]. The role of His12 in both AcP and PTP activities is clearly evidenced by site-directed mutagenesis [58]. The His12 imidazole ring provides a pair of electrons for nucleophilic attack to the phosphate group. Cooperatively, Asp258 donates a proton from its carboxyl group to the substrate resulting in the formation of the phosphoenzyme intermediate and the liberation of dephosphorylated substrate. Additionally, Asp258 might also stabilize the phospho-His12 intermediate. Subsequently, the nucleophilic attack of the phosphoenzyme intermediate occurs through a water molecule to release the phosphate group and to return a proton to the Asp258 carboxyl group [116, 120, 121]. The data collectively from chemical modification, site-directed mutagenesis, and X-ray crystallographic approaches suggest the importance of His12 and Asp258 in both AcP and PTP activity of PAcP protein [58, 61, 116,

120–122]. These results further support the concept that PAcP represents a novel histidine-dependent PTP, which uses the same active site as well as the catalytic mechanism of AcP to execute its PTP activity.

ErbB-2/HER-2/neu: An Intracellular Substrate of cPAcPin Prostate Epithelia

Several lines of evidence validate cPAcP as an authentic PTP [17, 21, 109–111, 113, 121, 123]. In PCa cells, cPAcP activity inversely correlates with the p-Tyr level of a 185 kDa protein [23, 111]. The incorporation of purified PAcP protein into PAcP-null DU145 PCa cells results in decreased Tyr-P of 185 kDa protein [23]. The 185 kDa protein was identified to be the ErbB-2 [24]. The notion of cPAcP dephosphorylating ErbB-2 Tyr-P is further supported by ectopic expression of the wild-type cPAcP but not its phosphatase-inactive mutant in PCa cells [16, 58]. Conversely, small interfering RNA or antisense-mediated PAcP knockdown in LNCaP cells results in increased ErbB-2 Tyr-P and subsequently cell proliferation [17, 21]. Additionally, an intratumoral injection of the wild type PAcP, but not phosphatase-inactive mutant, cDNA expression vector in xenograft tumors results in decreased ErbB-2 Tyr-P as well as tumorigenicity [19].

Transient expression of PAcP in PAcP-null PCa cells is associated with decreased Tyr1221/2 and Tyr1248 phosphorylation at ErbB-2 and reduced Tyr-P of p52Shc and cell growth [21]. Knockdown of endogenous PAcP expression by shRNA is associated with elevated Tyr-P of ErbB-2 at Tyr1221/2 as well as Tyr1248 and activation of downstream signaling, including Akt, STAT-3, and STAT-5 [21]. Importantly, reciprocal co-immunoprecipitation analyses showed an interaction between PAcP and ErbB-2 in the same complex under a nonpermissive growth condition [21]. This interaction by co-immunoprecipitation was decreased upon growth stimulation [124]. Thus, the effect of cPAcP on downregulation of PCa cell growth is at least in part through dephosphorylating the p-Tyr of ErbB-2 protein in those cells [16, 21, 24, 58, 124]. ErbB-2 serves as an in vivo substrate of cPAcP in PCa cells [17, 19, 21, 120, 124] (Fig. 12.2).

The cPAcP dephosphorylation model indicates that dimeric cPAcP dephosphorylates two autophosphorylated residues on an activated receptor simultaneously because the presence of a second phosphorylated tyrosyl residue at the C terminus of ErbB-2 can enhance the binding affinity considerably [120]. Phosphopeptide-binding analyses showed that cPAcP has the most favorable binding energy toward the synthesized peptide DNLpYYWD, corresponding to Tyr1221/2 phosphorylation of ErbB-2, with the possibility of acting on Tyr1248 as the additional site [120]. This is supported by kinetic studies on ErbB-2 activation that phosphorylation of Tyr1221/2 is elevated prior to Tyr1248 activation in PAcP-knockdown PCa cells [21]. Alternatively, due to the close proximity of Tyr1221/2 and Tyr1248, elevated phosphorylation on Tyr1248 in PAcP-knockdown cells may be secondary to the

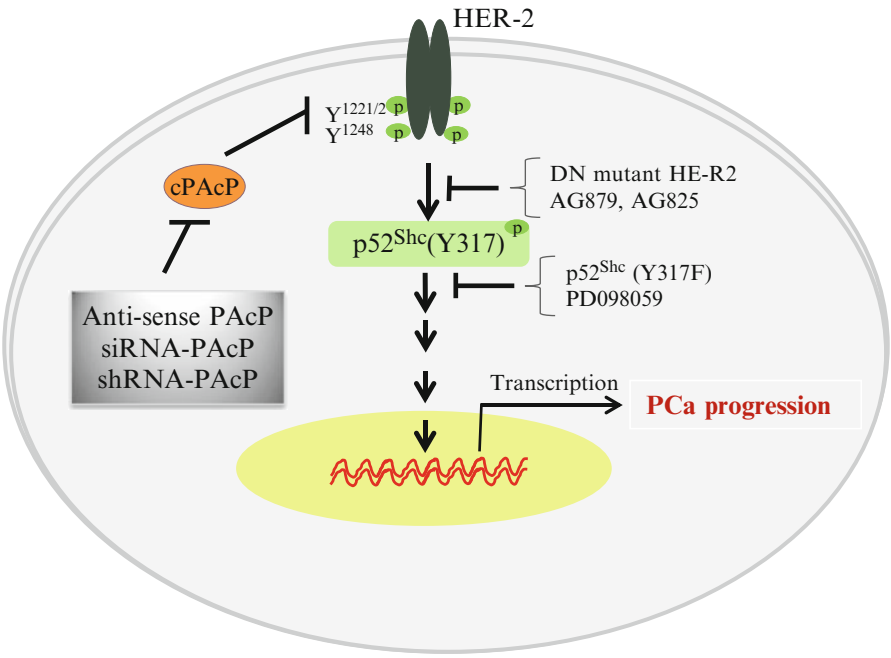

Fig. 12.2 Schematic representation of cPAcP interaction with ErbB-2 in prostate cancer cells. Progression of androgen-sensitive prostate cancer cells towards androgen independence is accompanied by early decrease/loss of cPAcP expression in prostate cancer cells results in hyperphosphorylation of HER-2 on tyrosine residues including Y1221/2 and Y1248 leading to androgen-independent cellular proliferation. Activated HER-2 can transduce its signals via p52Shc (blocked by dominant-negative (DN) HER-2 cDNA transfection or HER-2 inhibitors, AG825, AG879) to activate the downstream pathway (blocked by p52Shc Y317F mutant cDNA transfection or MEK inhibitors)

removal of cPAcP from Tyr1221/2. Further experiments should clarify the molecular mechanism.

ErbB-2 Signaling

Results of several studies clearly support the notion that ErbB-2 plays a critical role in PCa progression despite the fact that ErbB-2 gene is not amplified nor ErbB-2 protein is elevated in most carcinomas. It should be noted that ErbB-2-specific activity is increased as shown by increased overall Tyr-P and downstream signaling in AI PCa cells, higher than that in corresponding AS cells, and this increased Tyr-P is inversely correlated with cPAcP activity [21, 23, 24, 77]. Evidently, knockdown of cPAcP expression by antisense cDNA and siRNA in AS PCa cells leads to increased Tyr-P of ErbB-2, activation of downstream signaling and increased cell growth both in regular medium and in steroid-reduced condition [17, 21]. Thus, increased ErbB-2 protein-specific activity contributes to

advanced CRPCa progression primarily by phosphorylation regulation, including ERK/MAPK, Akt as well as STAT-3 and STAT-5 activation, which leads to advanced PCa cell survival, proliferation, and PSA production under androgen-deprived conditions. In summary, the interaction between cPAcP and ErbB-2 is involved in controlling the basal as well as the androgen-stimulated proliferation of human PCa cells [21, 78]. Aberrant regulation of this interaction can lead to CRPCa progression under ADT.

Regulation of PAcP Expression

The expression of PAcP protein is regulated by multi factors at different levels. Due to the importance of cPAcP protein as a growth regulator in PCa cells, it is vital to delineate its regulatory mechanism for potential clinical applications.

Androgen Regulation of PAcP Expression and Secretion in Prostatic Carcinoma Cells

Since PAcP expression correlates with the differentiation of prostate epithelia after puberty, it has been proposed that the expression and secretion of PAcP is regulated by androgens [2]. The stimulated secretion of PAcP has served as a hallmark of androgen action in prostate epithelial cells for over 6 decades, and the circulating PAcP in PCa patients has been used as a surrogate marker in ADT for about five decades [1, 2, 13].

Lin and Garcia-Arenas [125] made the seminal observation that depending on the cultured cell density, DHT can upregulate or downregulate PAcP mRNA levels in LNCaP cells. These results clarify the inconsistent reports of the opposite regulations of PAcP mRNA by DHT [54, 126]. Nuclear run-on experiments showed that DHT regulation of PAcP expression can occur at the transcriptional level [33]. Further investigation is needed to delineate the molecular mechanism by which cell density modulates androgen regulation of PAcP mRNA level and to examine whether androgens regulate the stability of PAcP mRNA in addition to the transcriptional rate [33].

Sequence analyses revealed that the human PAcP gene promoter DNA within 3 kb upstream of the coding region lacks the canonical TATA box and the GC box, where there are five putative AREs [55, 127]. In prostate carcinoma cells, although the PSA promoter is regulated by androgens [128–130], PAcP expression is not androgen dependent [54, 126, 131]. Utilizing AR-negative, androgen-independent PC-3 and DU145 PCa cell lines, in the absence of androgen receptor or the addition of androgen, the PAcP promoter is highly active as determined by reporter assay. These results demonstrate that in PCa cells, the PAcP gene is regulated in an androgen-independent, responsive manner [70, 126, 132]. Supportively, secretion

of PAcP protein is observed when LNCaP cells are cultured in media supplemented with charcoal-stripped FBS [133] or dialyzed FBS [77, 134] in which steroids and growth factors are significantly reduced. Further, PAcP protein expression is at a high level in cells with serum-free media in the absence of added DHT and even higher than with regular medium containing FBS [54, 77]. These data together support the notion that in LNCaP cells, androgen-stimulated PAcP secretion is via two regulatory pathways: increasing levels of secretory PAcP mRNA and at the same time, promoting the secretory pathway [54].

It has been shown that JFC1 [also designated as synaptotagmin-like protein (slp1)], a Rab27a and PtdIns(3,4,5)P3-binding protein, can modulate androgen-stimulated secretion of sPAcP in LNCaP cells [135–137]. In parallel, phosphoinositide 3-kinase (PI3K) also plays a critical role in regulating the exocytosis of sPAcP [135]. Additionally, PKC activator and androgens both increase PAcP secretion, which are blocked by PKC inhibitors [138]. These results together suggest that sPAcP secretion is mediated by a regulatory process including Rab27a, PI3K, and PKC, differing from PSA secretion primarily in response to androgens [135, 138, 139].

Effects of Growth Factors on PAcP Expression

Factors other than androgens can regulate PAcP gene expression in LNCaP cells. Interestingly, the effect of EGF is more pronounced than DHT in determining PAcP mRNA expression and can potentiate the downregulation by androgens, but there is no added effect by androgens on EGF suppression [54, 70, 140]. EGF treatment also results in decreased cPAcP activity, which may be due to its phosphorylation inactivation by EGFR or oxidation inactivation [124]. While both EGF and TGF-α can bind to the EGF receptor [141, 142], TGF-α has a less inhibitory effect than EGF on reducing hPAcP mRNA [71]. On the other hand, TGF-β_1, which is inhibitory to normal prostatic epithelial cells [143, 144], upregulates the expression of PAcP mRNA [140]. Due to the importance of PAcP in clinical applications, further experiments should clarify the regulation of PAcP in prostate epithelia.

In the presence of androgens, the expression of growth factors, e.g., EGF, TGF-α, TGF-β_1, and TGF-β_3, are also modulated. Androgens and growth factors and their receptors, represent cross-talk at several levels. Additional experiments are required for elucidating the role of this cross-talk on the regulation of PAcP gene expression.

Epigenetic Regulation of PAcP Expression in Prostate Carcinoma Cells

DNA methylation and histone modification, two common epigenetic mechanisms, play vital roles in regulating PCa cell growth and metastasis [145]. Histone modification, primarily by acetylation and deacetylation, leads to altered gene expression by changing chromosome structure and the level of gene transcription. Histone

deacetylase (HDAC) activity is enhanced and upregulated in PCa and other carcinomas [146–148]. Hence HDAC is recognized as a promising target for cancer therapy, although the exact role of specific HDACs in the pathophysiology of PCa is still not well understood.

HDAC inhibitors have been shown to induce PCa cell growth arrest, differentiation, and apoptosis. cPAcP functions as a tumor suppressor in prostate carcinomas, and its decreased expression correlates with PCa progression. However, the molecular mechanism of its reduced expression in PCa remains an enigma [16, 19, 21]. Importantly, HDAC inhibitors, including sodium butyrate, trichostatin A (TSA), and valproic acid (VPA), suppress the growth of PCa cells and concurrently, cPAcP mRNA and protein expression are increased and ErbB-2 Tyr-P is decreased [149]. Conversely, knockdown of cPAcP expression by shRNA reduces the efficacy of HDAC inhibitor-induced growth suppression. Therefore, PAcP is involved in HDAC inhibitor-induced growth suppression and functions as a tumor suppressor gene in regulating PCa progression and metastasis (Fig. 12.3). Importantly, HDAC inhibitor-treated PCa cells increase their androgen responsiveness [149]. Understanding the regulation of cPAcP expression by HDACs may lead to improved CRPCa therapy by HDAC inhibitors.

Prostatic Acid Phosphatase as a Therapeutic Agent as Well as a Target for Prostate Cancer Treatment

While the majority of patients with metastatic prostate cancer have an initial response to ADT, most patients will eventually relapse with castration-resistant tumors. With the limited efficacy of conventional therapeutic approaches and also with significant morbidities of surgical and radiation treatments in advanced PCa, other avenues for treating advanced prostate carcinoma are actively under investigation.

PAcP Per Se as a Therapeutic Agent

Several lines of biochemical evidence have demonstrated that cPAcP functions as a tumor suppressor. A single intratumoral injection of an expression vector encoding the wild-type PAcP protein into xenograft tumors results in the suppression of tumor growth and progression [19]. In PAcP-knockout mice, the prostate develops carcinomas in situ, indicating that cPAcP functions as a tumor suppressor [20]. In parallel, cPAcP plays a critical role in HDAC inhibitor-induced PCa cell growth suppression [149]. Importantly, the HDAC inhibitor-treated PCa cells exhibit an increase in androgen responsiveness, suggesting that intermittent treatment with HDAC inhibitors may prolong the duration of ADT [149]. Thus, the restoration of cPAcP expression in PCa cells may provide a novel avenue for treating CRPCa.

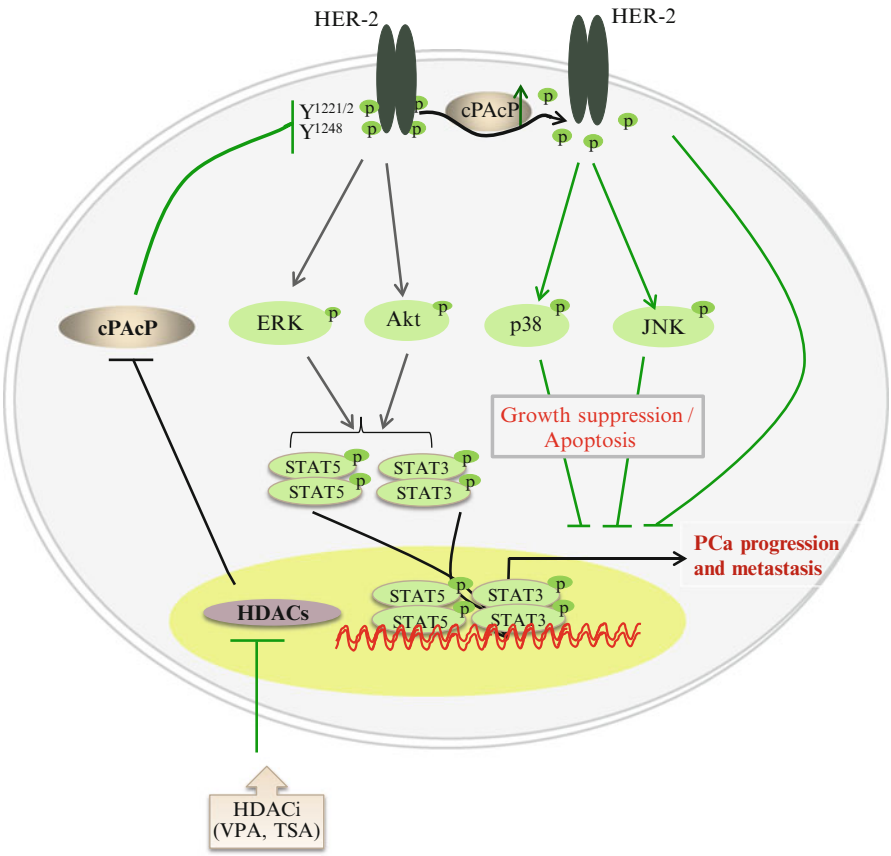

Fig. 12.3 Epigenetic regulation of prostate cancer cells. We propose that in PCa cells, upregulated histone deacetylases (HDACs) downregulate PAcP expression. This PAcP suppression leads to aberrant activation of ErbB2/HER-2/neu by Tyr-P followed by ERK/MAPK and Akt activation, leading to cell survival, proliferation, adhesion, and migration. Conversely, HDACs inhibitors (HDACi) restore cPAcP expression. This restored cPAcP dephosphorylates ErbB-2, which leads to inhibit prostate cancer progression and metastasis by p38 and JNK activation

PAcP as an Antigen for Immunotherapy of Prostate Cancer

Immunotherapeutic vaccines induce an antitumor response [150] by targeting tumor-associated antigens (TAAs) or by disrupting molecular pathways that promotes tumor growth [151, 152]. Therefore, the primary goal of immunotherapy is to activate the effector T cells that can migrate to the developing tumors and facilitate the damage of individual cancer cells.

Prostate cells express several specific biomarkers, including PSA, PAcP, and prostate-specific membrane antigen, which serve as TAAs and can serve as

immunogens. Previous studies demonstrated naturally occurring PAcP-specific binding IgG in human serum [65], the induction of a destructive prostatitis by PAcP-specific CTLs in rodents [153], and T-helper cell responses in men with PCa [154]. Studies have also identified Abs and circulating T cells against TAAs in PCa patients [155, 156]. These findings suggest that T-cells can break the tolerance and induce an immune response against tumor cells [155, 156]. These phenomena collectively indicate that an immune environment capable of supporting antigen-specific CTL may exist in vivo [154]. Small et al. [157] observed that dendritic cells loaded with an engineered antigen–cytokine fusion protein consisting of PAcP and granulocyte macrophage colony-stimulating factor (GM-CSF) are capable of inducing a potent cellular immune response in vivo to rodent tissues and tumors that express PAcP [157]. Subsequently, a dendritic cell product consisting of autologous dendritic cells loaded with the human PAcP–GM-CSF fusion protein was developed. It is hypothesized that when the vaccine is infused into the patient, the activated antigen-presenting cells (APCs) displaying the fusion protein will induce an immune response against the TAA. Phase I/II clinical trials with a dendritic cell-based PAcP vaccine in CRPCa patients led to a greater than 50 % decrease in PSA [157, 158]. The study showed that all patients developed specific immune responses to the recombinant fusion protein, and 38 % developed immune responses to PAcP. The time to disease progression correlated with the development of an immune response to PAcP and with the dose of dendritic cells received. There were minimal side effects of the therapy.

Highlight: Sipuleucel-T – An Autologous Dendritic Cell Product

Sipuleucel-T (Provenge, the commercial name) has become the first vaccine in the class of T cell-associated cancer immunotherapeutic agents approved by the United States Food and Drug Administration in April 2010 for the treatment of metastatic CRPCa. Sipuleucel-T is composed of autologous peripheral blood mononuclear cells (PBMCs), including APCs with a recombinant fusion protein PA2024 (full-length PAcP) linked to an adjuvant (GM-CSF). Currently, Sipuleucel-T is reserved for patients with documented metastatic PCa who have progressed on ADT with a documented testosterone level of less than 50 ng/dL. Sipuleucel-T treated patients demonstrated an additional 4.1-month median survival compared to the placebo group, which was statistically significant (HR = 0.78; 95 % CI, 0.61–0.98; $p = 0.03$). This increase in survival correlated with a 22 % decrease in mortality with the use of Sipuleucel-T [159, 160]. There is a need of surrogate markers for determining a patient's response to therapy. Clearly, the identification of predictive biomarkers will help practitioners select patients who are most likely to benefit from therapy [160].

Conclusion and Perspectives

Until the availability of PSA, circulating PAcP activity has served as a surrogate marker for diagnosing PCa and also has been used to monitor the efficacy of androgen deprivation therapy in treating PCa [14, 66]. In contrast, expression of PAcP and its intracellular level (cPAcP) is diminished in prostate carcinomas. Recent advances emphasize that cPAcP is an authentic protein tyrosine phosphatase and functions as a negative growth regulator in PCa cells. Importantly, cPAcP represents a novel subfamily of PTP super family [121]. The expression of PAcP is regulated at different levels as well as by different factors including androgens and growth factors in prostate carcinoma cells. The androgenic regulation of PAcP expression and secretion has been known to be a hallmark of androgen action for over six decades [2]. Nevertheless, the results of molecular studies demonstrate that the promoter activity of the PAcP gene is regulated in an androgen-independent manner [54, 126, 132].

Several lines of evidence support the importance of cPAcP enzyme in regulating PCa cell proliferation, particularly during the castration-resistant progression, at least in part by dephosphorylating p-Tyr of ErbB-2 intracellularly. It has been speculated that PAcP can also function as a phospholipid phosphatase because in cPAcP-knockdown cells, phospholipid activity is enhanced and also PAcP has an open active domain [80, 121]. Additionally, PAcP expression is in part regulated by epigenetic mechanism including histone acetylation and possibly methylation. These emerging data support PAcP as a potential therapeutic target for advanced PCa. The recent clinical immunotherapy trial with PAcP protein as a vaccine is promising. Further studies are needed to improve the clinical efficacy, for example, by effective intracellular delivery of antigenic peptides into dendritic cells.

Taken together, the data clearly show that cPAcP functions as an authentic tumor suppressor in PCa. Due to the importance of the PAcP gene in prostate carcinogenesis, investigation of the basic biochemistry and molecular biology of cPAcP including its interaction with other oncogenic proteins should provide valuable insights into its potential therapeutic applications.

Acknowledgments This book chapter preparation was supported in part by Grants CA88184 (NIH), PC050769 (DoD), PC074289 (DOD) and the Nebraska Research Initiative. We also thank previous lab members for their contributions and efforts on prostatic acid phosphatase project.

References

1. Gyorkey F (1973) Some aspects of cancer of the prostate gland. Methods Cancer Res 10:279–368
2. Yam LT (1974) Clinical significance of the human acid phosphatases: a review. Am J Med 56:604–616
3. Derechin M, Ostrowski W, Galka M, Barnard EA (1971) Acid phosphomonoesterase of human prostate: molecular weight, dissociation and chemical composition. Biochim Biophys Acta 250:143–154

4. Ostrowski W, Bhargava AK, Dziembor E, Gizler M, Gryszkiewicz J, Barnard EA (1976) Acid phosphomonoesterase of human prostate. Carbohydrate content and optical properties. Biochim Biophys Acta 453:262–269
5. Risley MJ, Van Etten RL (1987) Structures of the carbohydrate moieties of human prostatic acid phosphatase elucidated by 1H nuclear magnetic resonance spectroscopy. Arch Biochem Biophys 258:404–412
6. Hakalahti L, Vihko P, Henttu P, Autio-Harmainen H, Soini Y, Vihko R (1993) Evaluation of PAP and PSA gene expression in prostatic hyperplasia and prostatic carcinoma using northern-blot analyses, in situ hybridization and immunohistochemical stainings with monoclonal and bispecific antibodies. Int J Cancer 55:590–597
7. Ronnberg L, Vihko P, Sajanti E, Vihko R (1981) Clomiphene citrate administration to normogonadotropic subfertile men: blood hormone changes and activation of acid phosphatase in seminal fluid. Int J Androl 4:372–378
8. Quintero IB, Araujo CL, Pulkka AE, Wirkkala RS, Herrala AM, Eskelinen EL, Jokitalo E, Hellstrom PA, Tuominen HJ, Hirvikoski PP, Vihko PT (2007) Prostatic acid phosphatase is not a prostate specific target. Cancer Res 67:6549–6554
9. Vihko P (1979) Human prostatic acid phosphatases: purification of a minor enzyme and comparisons of the enzymes. Invest Urol 16:349–352
10. Van Etten RL (1982) Human prostatic acid phosphatase: a histidine phosphatase. Ann NY Acad Sci 390:27–51
11. Lin MF, Lee CL, Li SS, Chu TM (1983) Purification and characterization of a new human prostatic acid phosphatase isoenzyme. Biochemistry 22:1055–1062
12. Gutman EB, Sproul EE, Gutman AB (1936) Significance of increased phosphatase activity at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. Am J Cancer 28:485–495
13. Huggins C, Hodges CV (1941) Studies on prostatic cancer: the effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293–297
14. Chu TM, Lin MF (1998) PSA and acid phosphatase in the diagnosis of prostate cancer. J Clin Lig Assay 21:24–34
15. Goldfarb DA, Stein BS, Shamszadeh M, Petersen RO (1986) Age-related changes in tissue levels of prostatic acid phosphatase and prostate specific antigen. J Urol 136:1266–1269
16. Lin MF, Lee MS, Zhou XW, Andressen JC, Meng TC, Johansson SL, West WW, Taylor RJ, Anderson JR, Lin FF (2001) Decreased expression of cellular prostatic acid phosphatase increases tumorigenicity of human prostate cancer cells. J Urol 166:1943–1950
17. Veeramani S, Yuan TC, Chen SJ, Lin FF, Petersen JE, Shaheduzzaman S, Srivastava S, MacDonald RG, Lin MF (2005) Cellular prostatic acid phosphatase: a protein tyrosine phosphatase involved in androgen-independent proliferation of prostate cancer. Endocr Relat Cancer 12:805–822
18. Hassan MI, Aijaz A, Ahmad F (2010) Structural and functional analysis of human prostatic acid phosphatase. Expert Rev Anticancer Ther 10:1055–1068
19. Igawa T, Lin FF, Rao P, Lin MF (2003) Suppression of LNCaP prostate cancer xenograft tumors by a prostate-specific protein tyrosine phosphatase, prostatic acid phosphatase. Prostate 55:247–258
20. Zylka MJ, Sowa NA, Taylor-Blake B, Twomey MA, Herrala A, Voikar V, Vihko P (2008) Prostatic acid phosphatase is an ectonucleotidase and suppresses pain by generating adenosine. Neuron 60:111–122
21. Chuang TD, Chen SJ, Lin FF, Veeramani S, Kumar S, Batra SK, Tu Y, Lin MF (2010) Human prostatic acid phosphatase, an authentic tyrosine phosphatase, dephosphorylates ErbB-2 and regulates prostate cancer cell growth. J Biol Chem 285:23598–23606
22. Lin MF, Li SS, Chu TM, Lee CL (1990) Comparison of prostate acid phosphatase with acid phosphatase isoenzymes from the lung and spleen. J Clin Lab Anal 4:420–425
23. Lin MF, Meng TC (1996) Tyrosine phosphorylation of a 185 kDa Phosphoprotein (pp 185) inversely correlates with the cellular activity of human prostatic acid phosphatase. Biochem Biophys Res Commun 226:206–213

24. Meng TC, Lin MF (1998) Tyrosine phosphorylation of c-ErbB-2 is regulated by the cellular form of prostatic acid phosphatase in human prostate cancer cells. J Biol Chem 273:22096–22104
25. Johnson LE, Frye TP, Chinnasamy N, Chinnasamy D, McNeel DG (2007) Plasmid DNA vaccine encoding prostatic acid phosphatase is effective in eliciting autologous antigen-specific CD8+ T cells. Cancer Immunol Immunother 56:885–895
26. Becker JT, Olson BM, Johnson LE, Davies JG, Dunphy EJ, McNeel DG (2010) DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer. J Immunother 33:639–647
27. Spies E, Reichardt W, Alvarez G, Groettrup M, Ohlschläger P (2012) An artificial PAP gene breaks self-tolerance and promotes tumor regression in the TRAMP model for prostate carcinoma. Mol Ther 20:555–564
28. Sims RB (2011) Sipuleucel-T: autologous cellular immunotherapy for men with asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer. J Cancer 2:357–359
29. Moss DW, Raymond FD, Wile DB (1995) Clinical and biological aspects of acid phosphatase. Crit Rev Clin Lab Sci 32:431–467
30. Yousef GM, Diamandis M, Jung K, Diamandis EP (2001) Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis. Genomics 74:385–395
31. Yam LT, Li CY, Lam KW (1980) The nonprostatic acid phosphatases. In: Spring Mills E, Hafez ESE (eds) Male accessory sex glands. Elsevier/North-Holland Biomedical, Amsterdam
32. Solin T, Kontturi M, Pohlmann R, Vihko P (1990) Gene expression and prostate specificity of human prostatic acid phosphatase (PAP): evaluation by RNA blot analyses. Biochim Biophys Acta 1048:72–77
33. Zelivianski S, Comeau D, Lin MF (1998) Cloning and analysis of the promoter activity of the human prostatic acid phosphatase gene. Biochem Biophys Res Commun 245:108–112
34. Li CY, Lam WK, Yam LT (1980) Immunohistochemical diagnosis of prostatic cancer with metastasis. Cancer 46:706–712
35. Shaw LM, Yang N, Brooks JJ, Neat M, Marsh E, Seamonds B (1981) Immunochemical evaluation of the organ specificity of prostatic acid phosphatase. Clin Chem 27:1505–1512
36. Yam LT, Janckila AJ, Li CY, Lam WK (1981) Presence of "prostatic" acid phosphatase in human neutrophils. Invest Urol 19:34–38
37. Seitz J, Aumüller G (1985) Cytochemistry and biochemistry of acid phosphatases V: electrophoretic studies on the heterogeneity of acid phosphatases from human prostate, seminal fluid, and leukocytes. Prostate 7:73–90
38. Waheed A, Van Etten RL, Gieselmann V, von Figura K (1985) Immunological characterization of human acid phosphatase gene products. Biochem Genet 23:309–319
39. Kamoshida S, Tsutsumi Y (1990) Extraprostatic localization of prostatic acid phosphatase and prostate-specific antigen: distribution in cloacogenic glandular epithelium and sex-dependent expression in human anal gland. Hum Pathol 21:1108–1111
40. Drenckhahn D, Waheed A, Van Etten R (1987) Demonstration of prostatic-type acid phosphatase in non-lysosomal granules in the crypt epithelium of the human duodenum. Histochemistry 88:47–52
41. Choe BK, Pontes EJ, Rose NR, Henderson MD (1978) Expression of human prostatic acid phosphatase in a pancreatic islet cell carcinoma. Invest Urol 15:312–318
42. Hoyhtya M, Vihko P, Vuolas L, Tryggvason K, Vihko R (1987) High-affinity monoclonal antibodies specific for human prostatic acid phosphatase. Clin Chem 33:103–107
43. Lam KW, Li CY, Yam LT, Sun T, Lee G, Ziesmer S (1989) Improved immunohistochemical detection of prostatic acid phosphatase by a monoclonal antibody. Prostate 15:13–21
44. Graddis TJ, McMahan CJ, Tamman J, Page KJ, Trager JB (2011) Prostatic acid phosphatase expression in human tissues. Int J Clin Exp Pathol 4:295–306
45. Cunha AC, Weigle B, Kiessling A, Bachmann M, Rieber EP (2006) Tissue-specificity of prostate specific antigens: comparative analysis of transcript levels in prostate and non-prostatic tissues. Cancer Lett 236:229–238

46. Mori K, Wakasugi C (1985) Immunocytochemical demonstration of prostatic acid phosphatase: different secretion kinetics between normal, hyperplastic and neoplastic prostates. J Urol 133:877–883
47. Lilja H, Abrahamsson PA (1988) Three predominant proteins secreted by the human prostate gland. Prostate 12:29–38
48. Sinha AA, Gleason DF, Wilson MJ, Wick MR, Reddy PK, Blackard CE (1988) Relationship of prostatic acid phosphatase localization in human prostate by a monoclonal antibody with the Gleason grading system. Prostate 13:1–15
49. Sharief FS, Lee H, Leuderman MM, Lundwall A, Deaven LL, Lee CL, Li SS (1989) Human prostatic acid phosphatase: cDNA cloning, gene mapping and protein sequence homology with lysosomal acid phosphatase. Biochem Biophys Res Commun 160:79–86
50. Sharief FS, Li SS (1992) Structure of human prostatic acid phosphatase gene. Biochem Biophys Res Commun 184:1468–1476
51. Sharief FS, Mohler JL, Sharief Y, Li SS (1994) Expression of human prostatic acid phosphatase and prostate specific antigen genes in neoplastic and benign tissues. Biochem Mol Biol Int 33:567–574
52. Vihko P, Virkkunen P, Henttu P, Roiko K, Solin T, Huhtala ML (1988) Molecular cloning and sequence analysis of cDNA encoding human prostatic acid phosphatase. FEBS Lett 236:275–281
53. Van Etten RL, Davidson R, Stevis PE, MacArthur H, Moore DL (1991) Covalent structure, disulfide bonding, and identification of reactive surface and active site residues of human prostatic acid phosphatase. J Biol Chem 266:2313–2319
54. Lin MF, Garcia-Arenas R, Chao YC, Lai MM, Patel PC, Xia XZ (1993) Regulation of prostatic acid phosphatase expression and secretion by androgen in LNCaP human prostate carcinoma cells. Arch Biochem Biophys 300:384–390
55. Banas B, Blaschke D, Fittler F, Horz W (1994) Analysis of the promoter of the human prostatic acid phosphatase gene. Biochim Biophys Acta 1217:188–194
56. Lin MF, DaVolio J, Garcia-Arenas R (1992) Expression of human prostatic acid phosphatase activity and the growth of prostate carcinoma cells. Cancer Res 52:4600–4607
57. Roiko K, Janne OA, Vihko P (1990) Primary structure of rat secretory acid phosphatase and comparison to other acid phosphatases. Gene 89:223–229
58. Zhang XQ, Lee MS, Zelivianski S, Lin MF (2001) Characterization of a prostate-specific tyrosine phosphatase by mutagenesis and expression in human prostate cancer cells. J Biol Chem 276:2544–2550
59. Hurt JK, Fitzpatrick BJ, Norris-Drouin J, Zylka MJ (2012) Secretion and N-linked glycosylation are required for prostatic acid phosphatase catalytic and antinociceptive activity. PLoS One 7:e32741
60. Kuciel R, Bakalova A, Mazurkiewicz A, Bilska A, Ostrowski W (1990) Is the subunit of prostatic phosphatase active? reversible denaturation of prostatic acid phosphatase. Biochem Int 22:329–334
61. Jakob CG, Lewinski K, Kuciel R, Ostrowski W, Lebioda L (2000) Crystal structure of human prostatic acid phosphatase. Prostate 42:211–218
62. Lad PM, Learn DB, Cooper JF, Reisinger DM (1984) Distribution of prostatic acid phosphatase isoenzymes in normal and cancerous states. Clin Chim Acta 141:51–65
63. Foti AG, Cooper JF, Herschman H, Malvaez RR (1977) Detection of prostatic cancer by solid-phase radioimmunoassay of serum prostatic acid phosphatase. N Eng J Med 297:1357–1361
64. Heller JE (1987) Prostatic acid phosphatase: its current clinical status. J Urol 137:1091–1103
65. Papsidero LD, Wojcieszyn JW, Horoszewicz JS, Leong SS, Murphy GP, Chu TM (1980) Isolation of prostatic acid phosphatase-binding immunoglobulin G from human sera and its potential for use as a tumor-localizing reagent. Cancer Res 40:3032–3035
66. Wang MC, Papsidero LD, Kuriyama M, Valenzuela LA, Murphy GP, Chu TM (1981) Prostate antigen: a new potential marker for prostatic cancer. Prostate 2:89–96
67. Lin MF, Lee CL, Wojcieszyn JW, Wang MC, Valenzuela LA, Murphy GP, Chu TM (1980) Fundamental biochemical and immunological aspects of prostatic acid phosphatase. Prostate 1:415–425

68. Foti AG, Cooper JF, Herschman H (1979) Prostatic acid phosphatase and prostatic cancer. Recent Results Can Res 67:45–49
69. Loor R, Wang MC, Valenzuela L, Chu TM (1981) Expression of prostatic acid phosphatase in human prostate cancer. Cancer Lett 14:63–69
70. Lin MF, Garcia-Arenas R, Kawachi M, Lin FF (1993) Regulation of the expression of prostatic acid phosphatase in LNCaP human prostate carcinoma cells. Cell Mol Biol Res 39:739–750
71. Lin MF, Garcia-Arenas R, Xia XZ, Biela B, Lin FF (1994) The cellular level of prostatic acid phosphatase and the growth of human prostate carcinoma cells. Differentiation 57:143–149
72. Reif AE, Schlesinger RM, Fish CA, Robinson CM (1973) Acid phosphatase isozymes in cancer of the prostate. Cancer 31:689–699
73. Vihko P, Kostama A, Janne O, Sajanti E, Vihko R (1980) Rapid radioimmunoassay for prostate-specific acid phosphatase in human serum. Clin Chem 26:1544–1547
74. Vihko P, Lukkarinen O, Kontturi M, Vihko R (1981) Effectiveness of radioimmunoassay of human prostate-specific acid phosphatase in the diagnosis and follow-up of therapy in prostatic carcinoma. Cancer Res 41:1180–1183
75. Isaacs JT (1983) Prostatic structure and function in relation to the etiology of prostatic cancer. Prostate 4:351–366
76. Dionne FT, Chevalier S, Bleau G, Roberts KD, Chapdelaine A (1983) Induction of acid phosphatase synthesis in canine prostatic epithelial cells in vitro. Mol Cell Endocrinol 33:113–126
77. Lin MF, Meng TC, Rao PS, Chang C, Schonthal AH, Lin FF (1998) Expression of human prostatic acid phosphatase correlates with androgen-stimulated cell proliferation in prostate cancer cell lines. J Biol Chem 273:5939–5947
78. Meng TC, Lee MS, Lin MF (2000) Interaction between protein tyrosine phosphatase and protein tyrosine kinase is involved in androgen-promoted growth of human prostate cancer cells. Oncogene 19:2664–2677
79. Wu WP, Hao JX, Halldner L, Lovdahl C, DeLander GE, Wiesenfeld-Hallin Z, Fredholm BB, Xu XJ (2005) Increased nociceptive response in mice lacking the adenosine A1 receptor. Pain 113:395–404
80. Sowa NA, Street SE, Vihko P, Zylka MJ (2010) Prostatic acid phosphatase reduces thermal sensitivity and chronic pain sensitization by depleting phosphatidylinositol 4,5-bisphosphate. J Neurosci 30:10282–10293
81. Sowa NA, Vadakkan KI, Zylka MJ (2009) Recombinant mouse PAP has pH-dependent ectonucleotidase activity and acts through A(1)-adenosine receptors to mediate antinociception. PLoS One 4:e4248
82. Zylka MJ (2011) Pain-relieving prospects for adenosine receptors and ectonucleotidases. Trends Mol Med 17:188–196
83. Brillard-Bourdet M, Rehault S, Juliano L, Ferrer M, Moreau T, Gauthier F (2002) Amidolytic activity of prostatic acid phosphatase on human semenogelins and semenogelin-derived synthetic substrates. Eur J Biochem 269:390–395
84. Ostrowski WS, Kuciel R (1994) Human prostatic acid phosphatase: selected properties and practical applications. Clin Chim Acta 226:121–129
85. Singh G, Adaikan PG, Ng YK (1996) Is seminal prostatic acid phosphatase a reliable marker for male infertility? Singapore Med J 37:598–599
86. Dave BN, Rindani TH (1988) Acid phosphatase activity in human semen. Int J Fertil 33:45–47
87. Saha A, Basu J, Bhattacharyya AK (1981) Seminal acid phosphatase from normal, oligospermic, vasectomized, and azoospermic men. Int J Fertil 26:124–127
88. Münch J, Rücker E, Ständker L, Adermann K, Goffinet C, Schindler M, Wildum S, Chinnadurai R, Rajan D, Specht A, Giménez-Gallego G, Sánchez PC, Fowler DM, Koulov A, Kelly JW, Mothes W, Grivel JC, Margolis L, Keppler OT, Forssmann WG, Kirchhoff F (2007) Semen-derived amyloid fibrils drastically enhance HIV infection. Cell 131:1059–1071

89. Pomerantz RJ (2003) HIV: cross-talk and viral reservoirs. Nature 424:136–137
90. Collins KA, Bennett AT (2001) Persistence of spermatozoa and prostatic acid phosphatase in specimens from deceased individuals during varied postmortem intervals. Am J Forensic Med Pathol 22:228–232
91. Bouvet JP, Grésenguet G, Bélec L (1997) Vaginal pH neutralization by semen as a cofactor of HIV transmission. Clin Microbiol Infect 3:19–23
92. Sensabaugh GF (1979) The quantitative acid phosphatase test. A statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. J Forensic Sci 24:346–365
93. Fischer RS (1949) Acid phosphatase tests as evidence of rape. N Engl J Med 240:738–739
94. Pinto FC (1959) Rape for the defense - acid phosphatase. J Forensic Med 6:147–159
95. Enos WF, Mann GT, Dolen WD (1963) A laboratory procedure for the identification of semen. Am J Clin Pathol 39:316–320
96. Walther G (1971) Acid phosphatase. Its significance in the determination of human seminal traces. J Forensic Med 18:15–17
97. Gomez RR, Wunsch CD, Davis JH, Hicks DJ (1975) Qualitative and quantitative determinations of acid phosphatase activity in vaginal washings. Am J Clin Pathol 64:423–432
98. McCloskey KL, Muscillo GC, Noordewier B (1975) Prostatic acid phosphatase activity in the postcoital vagina. J Forensic Sci 20:630–636
99. Schumann GB, Badawy S, Peglow A, Henry JB (1976) Prostatic acid phosphatase. Current assessment in vaginal fluid of alleged rape victims. Am J Clin Pathol 66:944–952
100. Enos WF, Beyer JC (1980) Prostatic acid phosphatase, aspermia, and alcoholism in rape cases. J Forensic Sci 25:353–356
101. Killian CS, Vargas FP, Lee CL, Wang MC, Murphy GP, Chu TM (1980) Quantitative counter immunoelectrophoresis assay for prostatic acid phosphatase. Invest Urol 18:219–224
102. Kloosterman AD, Pouw-Arnou M, Persijn JP (1984) Comparison of enzyme assay and radioimmunoassay for the measurement of human acid phosphatase in cases of sexual assault. Forensic Sci Int 25:45–55
103. Okamura K, Yamamoto Y, Ishizu H (1987) Medicolegal identification of seminal fluid by the radioimmunoassay of prostatic acid phosphatase. Nihon Hoigaku Zasshi 41:385–398
104. Allen SM (1995) An enzyme linked immunosorbent assay (ELISA) for detection of seminal fluid using a monoclonal antibody to prostatic acid phosphatase. J Immunoassay 16:297–308
105. Hara M, Koyanagi Y, Inoue T, Fukuyama T (1971) Some physico-chemical characteristics of "-seminoprotein", an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII. Nihon Hoigaku Zasshi 25:322–324
106. Wang MC, Valenzuela LA, Murphy GP, Chu TM (1979) Purification of a human prostate specific antigen. Invest Urol 17:159–163
107. Graves HC, Sensabaugh GF, Blake ET (1985) Postcoital detection of a male-specific semen protein. Application to the investigation of rape. N Engl J Med 312:338–343
108. Armbruster DA (1993) Prostate-specific antigen: biochemistry, analytical methods, and clinical application. Clin Chem 39:181–195
109. Li HC, Chernoff J, Chen LB, Kirschonbaum A (1984) A phosphotyrosyl-protein phosphatase activity associated with acid phosphatase from human prostate gland. Eur J Biochem 138:45–51
110. Lin MF, Clinton GM (1986) Human prostatic acid phosphatase has phosphotyrosyl protein phosphatase activity. Biochem J 235:351–357
111. Lin MF, Clinton GM (1988) The epidermal growth factor receptor from prostate cells is dephosphorylated by a prostate-specific phosphotyrosyl phosphatase. Mol Cell Biol 8:5477–5485
112. Vihko PT, Quintero I, Ronka AE, Herrala A, Jantti P, Porvari K, Lindqvist Y, Kaija H, Pulkka A, Vuoristo J Sormunen R, Soini Y, Halmekyto M, Janne J, Luokkala T Kurkela R (2005) Prostatic acid phosphatase (PAcP) is PI(3)P-phosphatase and its inactivation leads to change of cell polarity and invasive prostate cancer (Abstract 5239). Proceedings of the AACR, 96th Annual Meeting, Anaheim, CA

113. Lin MF, Clinton GM (1987) Human prostatic acid phosphatase and its phosphotyrosyl-protein phosphatase activity. Adv Protein Phosphatases 4:199–228
114. Jackson MD, Denu JM (2001) Molecular reactions of protein phosphatases - insights from structure and chemistry. Chem Rev 101:2313–2340
115. Rudolph J (2002) Catalytic mechanism of Cdc25. Biochemistry 41:14613–14623
116. Ostanin K, Saeed A, Van Etten RL (1994) Heterologous expression of human prostatic acid phosphatase and site-directed mutagenesis of the enzyme active site. J Biol Chem 269:8971–8978
117. McTigue JJ, Van Etten RL (1978) An essential active-site histidine residue in human prostatic acid phosphatase. Ethoxyformylation by diethyl pyrocarbonate and phosphorylation by a substrate. Biochim Biophys Acta 523:407–421
118. Ostrowski W (1978) Isolation of tau-phosphohistidine from a phosphoryl-enzyme intermediate of human prostatic acid phosphatase. Biochim Biophys Acta 526:147–153
119. Saini MS, Van Etten RL (1979) An essential carboxylic acid group in human prostate acid phosphatase. Biochim Biophys Acta 568:370–376
120. Sharma S, Pirila P, Kaija H, Porvari K, Vihko P, Juffer AH (2005) Theoretical investigations of prostatic acid phosphatase. Proteins 58:295–308
121. Veeramani S, Lee MS, Lin MF (2009) Revisiting histidine-dependent acid phosphatases: a distinct group of tyrosine phosphatases. Trends Biochem Sci 34:273–278
122. Ortlund E, LaCount MW, Lebioda L (2003) Crystal structures of human prostatic acid phosphatase in complex with a phosphate ion and a-benzylaminobenzylphosphonic acid update the mechanistic picture and offer new insights into inhibitor design. Biochemistry 42:383–389
123. Bourassa C, Nguyen LT, Durocher Y, Roberts KD, Chevalier S (1991) Prostatic epithelial cells in culture: phosphorylation of protein tyrosyl residues and tyrosine protein kinase activity. J Cell Biochem 46:291–301
124. Veeramani S, Chou YW, Lin FC, Muniyan S, Lin FF, Kumar S, Xie Y, Lele SM, Tu Y, Lin MF (2012) Reactive oxygen species induced by p66Shc longevity protein mediate nongenomic androgen action via tyrosine phosphorylation signaling to enhance tumorigenicity of prostate cancer cells. Free Radic Biol Med 53:95–108
125. Lin MF, Garcia-Arenas R (1994) Effect of cell density on androgen regulation of the mRNA level of human prostatic acid phosphatase. Mol Cell Endocrinol 99:R21–R24
126. Shan JD, Porvari K, Ruokonen M, Karhu A, Launonen V, Hedberg P, Oikarinen J, Vihko P (1997) Steroid-involved transcriptional regulation of human genes encoding prostatic acid phosphatase, prostate-specific antigen, and prostate-specific glandular kallikrein. Endocrinology 138:3764–3770
127. Virkkunen P, Hedberg P, Palvimo JJ, Birr E, Porvari K, Ruokonen M, Taavitsainen P, Jänne OA, Vihko P (1994) Structural comparison of human and rat prostate-specific acid phosphatase genes and their promoters: identification of putative androgen response elements. Biochem Biophys Res Commun 202:49–57
128. Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J (1991) The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930
129. Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J (1996) Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 271:6379–6388
130. Gotoh A, Ko SC, Shirakawa T, Cheon J, Kao C, Miyamoto T, Gardner TA, Ho LJ, Cleutjens CB, Trapman J, Graham FL, Chung LW (1998) Development of prostate-specific antigen promoter-based gene therapy for androgen-independent human prostate cancer. J Urol 160:220–229
131. Zelivianski S, Larson C, Seberger J, Taylor R, Lin MF (2000) Expression of human prostatic acid phosphatase gene is regulated by upstream negative and positive elements. Biochim Biophys Acta 1491:123–132

132. Zelivianski S, Igawa T, Lim S, Taylor R, Lin MF (2002) Identification and characterization of regulatory elements of the human prostatic acid phosphatase promoter. Oncogene 21:3696–3705
133. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP (1983) LNCaP model of human prostatic carcinoma. Cancer Res 43:1809–1818
134. Lin MF, Lee MS, Garcia-Arenas R, Lin FF (2000) Differential responsiveness of prostatic acid phosphatase and prostate-specific antigen mRNA to androgen in prostate cancer cells. Cell Biol Int 24:681–689
135. Johnson JL, Ellis BA, Noack D, Seabra MC, Catz SD (2005) The Rab27a-binding protein, JFC1, regulates androgen-dependent secretion of prostate-specific antigen and prostatic-specific acid phosphatase1. Biochem J 391:699–710
136. Strom M, Hume AN, Tarafder AK, Barkagianni E, Seabra MC (2002) A family of Rab27-binding proteins. Melanophilin links Rab27a and myosin Va function in melanosome transport. J Biol Chem 277:25423–25430
137. Tolmachova T, Anders R, Stinchcombe J, Bossi G, Griffiths GM, Huxley C, Seabra MC (2004) A general role for Rab27a in secretory cells. Mol Biol Cell 15:332–344
138. Lin MF, Zhang XQ, Dean J, Lin FF (2001) Protein kinase C pathway is involved in regulating the secretion of prostatic acid phosphatase in human prostate cancer cells. Cell Biol Int 25:1139–1148
139. Andrews PE, Young CY, Montgomery BT, Tindall DJ (1992) Tumor-promoting phorbol ester down-regulates the androgen induction of prostate-specific antigen in a human prostatic adenocarcinoma cell line. Cancer Res 52:1525–1529
140. Henttu P, Vihko P (1993) Growth factor regulation of gene expression in the human prostatic carcinoma cell line LNCaP. Cancer Res 53:1051–1058
141. Massagué J (1983) Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. J Biol Chem 258:13614–13620
142. Yarden Y, Ullrich A (1988) Growth factor receptor tyrosine kinases. Annu Rev Biochem 57:443–478
143. Sporn MB, Roberts AB, Wakefield LM, Assoian RK (1986) Transforming growth factor-beta: biological function and chemical structure. Science 233:532–534
144. Martikainen P, Kyprianou N, Isaacs JT (1990) Effect of transforming growth factor-beta 1 on proliferation and death of rat prostatic cells. Endocrinology 127:2963–2968
145. Albany C, Alva AS, Aparicio AM, Singal R, Yellapragada S, Sonpavde G, Hahn NM (2011) Epigenetics in prostate cancer. Prostate Cancer 2011:580318
146. Waltregny D, North B, Van Mellaert F, de Leval J, Verdin E, Castronovo V (2004) Screening of histone deacetylases (HDAC) expression in human prostate cancer reveals distinct class I HDAC profiles between epithelial and stromal cells. Eur J Histochem 48:273–290
147. Nakagawa M, Oda Y, Eguchi T, Aishima S, Yao T, Hosoi F, Basaki Y, Ono M, Kuwano M, Tanaka M, Tsuneyoshi M (2007) Expression profile of class I histone deacetylases in human cancer tissues. Oncol Rep 18:769–774
148. Weichert W, Röske A, Gekeler V, Beckers T, Stephan C, Jung K, Fritzsche FR, Niesporek S, Denkert C, Dietel M, Kristiansen G (2008) Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. Br J Cancer 98:604–610
149. Chou YW, Chaturvedi NK, Ouyang S, Lin FF, Kaushik D, Wang J, Kim I, Lin MF (2011) Histone deacetylase inhibitor valproic acid suppresses the growth and increases the androgen responsiveness of prostate cancer cells. Cancer Lett 311:177–186
150. Doehn C (2008) Immunotherapy of prostate cancer. Eur Urol 53:681–683, discussion 684–685
151. Vieweg J (2007) Immunotherapy for advanced prostate cancer. Rev Urol 9(Suppl 1):S29–S38
152. Drake CG (2010) Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol 10:580–593

153. Fong L, Ruegg CL, Brockstedt D, Engleman EG, Laus R (1997) Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization: implications for immunotherapy of prostate cancer. J Immunol 159:3113–3117
154. McNeel DG, Nguyen LD, Ellis WJ, Higano CS, Lange PH, Disis ML (2001) Naturally occurring prostate cancer antigen-specific T cell responses of a Th1 phenotype can be detected in patients with prostate cancer. Prostate 47:222–229
155. Houghton AN (1994) Cancer antigens: immune recognition of self and altered self. J Exp Med 180:1–4
156. Alam S, McNeel DG (2010) DNA vaccines for the treatment of prostate cancer. Expert Rev Vaccines 9:731–745
157. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, Valone FH (2000) Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol 18:3894–3903
158. Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, Richardson RL, Valone FH, Vuk-Pavlović S (2004) Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a phase 2 trial. Prostate 60:197–204
159. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF, IMPACT Study Investigators (2010) Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 363:411–422
160. Fong MK, Hare R, Jarkowski A (2012) A new era for castrate resistant prostate cancer: a treatment review and update. J Oncol Pharm Pract 18:343–354