

Structural and molecular biology of PSP94: Its significance in prostate pathophysiology

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1. ABSTRACT

Prostate secretory protein of 94 amino acids (PSP94), primarily found in the prostatic secretion, was originally isolated and purified from human seminal plasma. PSP94 has several putative biological functions and is considered a marker of prostate cancer (PCa). Here, we review the structural-functional relationships of PSP94, address its fungicidal activity and role as an inhibitor of sperm motility and protection from female immune surveillance, and review its role in tumor suppression. We also review the diagnostic assays that are developed for PSP94 for use in the diagnosis of PCa and use of such tests in the

differential diagnosis of PCa from benign prostatic hyperplasia (BPH).

2. INTRODUCTION

Prostate secretory protein of 94 amino acids (PSP94) is a predominant protein secreted by the epithelial cells of the prostate which is also known to be a candidate prostate cancer (PCa) biomarker. Worldwide, PCa is the leading cause of cancer-related deaths in men. While, the incidence rate of PCa is lower in India as compared to its rate in Western

countries, this rate has been steadily rising (1,2). The difference in the incidence rates of PCa throughout the world is mainly dependent on the coverage of prostate-specific antigen (PSA, human kallikrein-3) screening. Although serum PSA (sPSA) testing has been used routinely for screening and has significantly reduced PCa mortality (3), increased sPSA levels are also reported in non-malignant conditions such as benign prostatic hyperplasia (BPH) and prostatitis (4). Thus, sPSA has low specificity for PCa, especially in the diagnostic gray zone (sPSA levels between 4–10 ng/ml) which may lead to unnecessary biopsies. Hence, there is a constant need for exploring alternate markers. Potential of PSP94 is being investigated in PCa progression by several investigators worldwide. The various studies on PSP94 with respect to isolation, structural characterization, identification of various binding partners and putative functions so far are described below.

3. HISTORICAL PERSPECTIVE

PSP94 earlier referred to as human seminal plasma inhibin (HSPI) or prostatic inhibin peptide (PIP) was originally believed to be of testicular origin, with a function to regulate circulatory levels of follicle stimulating hormone (FSH) (5–7). Seidah *et al.* delineated the complete amino acid sequence of HSPI which was purified from human seminal plasma and predicted that the FSH inhibiting activity may reside within the C-terminal region (8). Concurrently, the sequence of inhibin from ovarian follicular fluid was delineated and found to be a glycosylated heterodimer (9) having no sequence similarity to HSPI. Subsequently, the ability of HSPI to suppress FSH secretion by the pituitary gland was later contradicted, wherein investigators observed that highly purified fractions of HSPI were devoid of inhibin-like activity (10,11). Later, studies showed that the amino acid sequence of HSPI was identical to that of a sperm coating antigen of prostatic origin reported by Johansson *et al.* (1984) and was proposed to play a role in reproduction (12). On complete amino acid sequencing by automated Edman degradation, this protein isolated from seminal plasma was shown to consist of 93 amino acid residues with a molecular mass of 10652 Da and was referred to as beta-microseminoprotein (beta-MSP) (13). At the same time, a 16 kDa protein was identified from human seminal plasma, which on N-terminal sequencing was found to be identical to HSPI. This protein consisted of 94 amino acids and hence was referred to as PSP94 (14). PSP94 is a non-glycosylated, cysteine rich protein having a molecular mass of ~10 kDa with an apparent molecular size of ~16 kDa on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) due to anomalous migration. PSP94 is believed to be involved in various biological processes including prostate pathophysiology and male reproduction. Further observation showed that PSP94 levels are significantly lower in tissue of

PCa patients as compared to BPH patients and healthy men (15). Early studies have indicated PSP94 to exist as a bound form in serum (16,17) and at the cell surface of pituitary, prostate and sperm (18,19,20). The identification and characterization of proteins interacting with PSP94 has been the subject of numerous research studies to understand the biology behind the many reported functions of PSP94.

4. PROSTATIC AND NON-PROSTATIC ORIGIN OF PSP94

PSP94 is mainly secreted by the prostatic epithelial cells in seminal fluid (~1 mg/ml) (14,15) and these concentrations are similar to that of PSA and prostatic acid phosphatase (PAP) in the seminal plasma (21). PSP94 has also been shown to be present in the epithelial tissue of porcine epididymis, seminal vesicles and Cowper's gland (22). Several studies have revealed the presence of PSP94 on rat (23), porcine (24,25) and macaque (26) sperm as well. PSP94 transcripts were demonstrated in human uterine, breast and other female reproductive tissues as well in endometrial cancer cell lines (27) indicating that the protein may have functions other than those related to male reproduction as a local autocrine/paracrine factor. Low concentrations of human PSP94 have also been reported in non-reproductive tissues like the gastric, tracheobronchial (28) and in uterine cervix secretions (29), suggesting an overall protective role. Thus, various functions have been attributed to PSP94 based on the difference in localization.

5. PSP94 HOMOLOGS

Several studies were undertaken to identify the presence of PSP94 homologs in other species. Cloning of cDNA and sequencing of the gene for PSP94, referred to as *MSMB*, was reported by Mbikay *et al.* (30). They observed that PSP94 encodes for a 114 amino acid polypeptide which includes a 20 amino acid signal sequence and the same has been confirmed by Nolet *et al.* (31). Additional studies defined the promoter structure and mapped the chromosomal location of the gene to the 10q11.2. region (32,33). Besides the old world monkeys like rhesus (31) and baboon (34), PSP94 homologs were identified in new world monkey species such as cotton-top tamarin and common marmoset (35). Likewise, based on amino acid and cDNA sequence comparison, homologs were identified in pigs (36,37), rat (38) and mice (39) as well. The sequence analysis did not show homology with any other protein sequences from the database indicating that these homologs constitute a unique protein family, collectively referred to as beta-microseminoprotein.

Subsequently, two avian species, namely ostrich (40) and chicken (41,42) were identified to contain a protein which was structurally related to

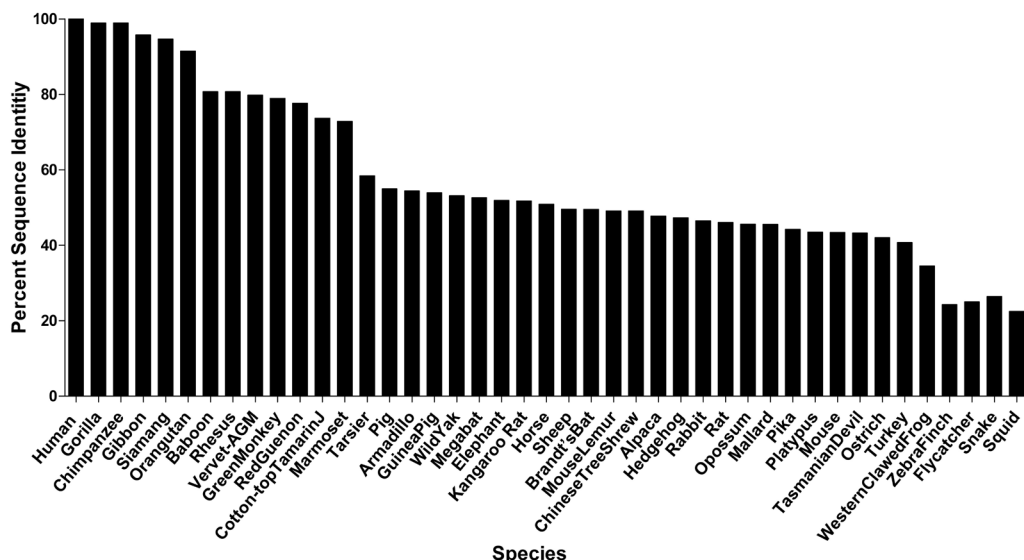


Figure 1. Percent sequence identity created by Clustal2.1. between reported PSP94/beta-MSP amino acid sequences. The sequence identity were determined following multiple sequence alignment of full length sequences of PSP94/beta-MSP (signal peptides included) using the Clustal Omega (CLUSTAL O (1.2.1.)) multiple sequence alignment program at <http://www.ebi.ac.uk/Tools/msa/clustalo/>

mammalian beta-MSP. Database searches using the amino acid sequence of ostrich beta-MSP allowed identification of related proteins in numerous species such as cow, African clawed frog, zebrafish and Japanese flounder (40). Presence of beta-MSP was also explored in invertebrates, wherein cDNA encoding beta-microseminoprotein-like (beta-MSPL) protein from the gut cDNA library of amphioxus was amplified (43). In the recent past, snake serum has also been shown to contain homolog of PSP94 called small serum protein (SSP2) (44). Interestingly, all the ten cysteine residues present in PSP94 are conserved in the homologs from all the species (40). However, the primary structure of the protein revealed a remarkably low level of conservation in amino acids. Further, antibodies against PSP94 from different species (human, primate, rodents) have poor cross-reactivity indicative of PSP94 being a rapidly evolving protein across species (45). We searched for beta-microseminoprotein related proteins in the various databases like UniProt and SWISS PROT and orthologues of *MSMB* gene reported in Ensembl. Figure 1 provides the multiple sequence alignment percentages between the various PSP94-related proteins exhibiting considerable amino acid sequence variation (signal peptides included).

6. PSP94 GENE VARIANTS AND THEIR SIGNIFICANCE IN PROSTATE TUMORIGENESIS

The gene for PSP94, *MSMB*, has two transcript variants—these splice variants represent all the isoforms reported in Ensembl genebuild 52 (46).

The longer transcript is *MSMB1* (for full length PSP94 or PSP94 protein) and the shorter transcript is *MSMB2* (for PSP57 protein which has a deleted Exon III) (47). *MSMB1* and *MSMB2* transcripts have both been shown in prostate (predominantly, *MSMB1*). However, *MSMB2* was not detected in breast and lung, but was largely found in kidney and bladder. Hence, the researchers proposed that in kidney and bladder, the majority of *MSMB1* was alternatively spliced resulting in *MSMB2*. This aberrantly spliced *MSMB2* mRNA was localized in the nuclear fraction of the cell. On the other hand, no alternative splicing was observed in lung and breast and only the full length *MSMB1* mRNA exists in these tissues. This study led to the conclusion that alternative splicing of *MSMB* occurs primarily in urogenital tissues. The same study suggested presence of both forms of *MSMB* mRNA in normal, benign and malignant prostate tissues. They later showed both *MSMB1* (487 bp) and *MSMB2* (381 bp) transcripts in endometrium and myometrium. However, breast, ovary and placenta showed presence of *MSMB1*, but not *MSMB2* (27).

Comparison of the predicted amino acid composition of PSP94 and PSP57 showed that the proteins shared the first 16 amino acids. Unlike PSP94, PSP57 was predicted to be a highly basic protein with hydrophobic regions and would harbor only one cysteine residue as compared to ten in PSP94. Because of this basic nature of PSP57 and presence of hydrophobic regions, PSP57 could have a role in anti-microbial host defense functions (47). There are several reports showing increased PSP94 mRNA levels in benign tumors, which are decreased or

lost in PCa (48). Transcript abundance studies in our laboratory have also shown that the mean fold-change in *MSMB1*, as well as *MSMB2* mRNA levels were lower in prostate samples from PCa as compared to BPH (49). Interestingly, we observed similar results with the sPSP94 protein levels, which were significantly lower in patients with PCa than in BPH (50). Similar to our results, Ohnuma *et al.* had also shown *MSMB1* and *MSMB2* transcripts to be present in the normal prostate and showing diminished presence in most of the PCas (51). Additionally, expression of either of the transcripts was not seen in normal colon, uterus and breast. On the contrary, Harries *et al.* found that *MSMB2* is predominant in benign prostate tissue, whereas, *MSMB1* represents the majority of the total *MSMB* expression in PCa tissues (52). Their results suggested that the isoform *MSMB2* may render a tumor suppressor property and the *MSMB1* isoforms could be pro-carcinogenic. Nevertheless, in another recent study, the absolute expression of both *MSMB1* and *MSMB2* in malignant prostate tissue has been shown to be decreased as compared to normal prostate tissue even though both the transcripts were comparable to each other in all the studied tissues (46). Concurrently, the transcript levels of both splice variants of PSP94, *MSMB1* and *MSMB2*, have also been demonstrated to strongly correlate with each other not only in PCa but also in BPH (49). This may suggest that the *MSMB2* transcript could have a physiological role in the prostate. Also, since a diverse presence of the aberrantly spliced *MSMB2* transcript is seen in various organs, *MSMB2* may be involved in regulation of maintenance of the varied levels of the PSP94 protein seen in different organs. Importantly, no PSP57 protein has been detected in the prostate tissue so far (27) due to which no function can be associated with PSP57.

The level of the PSP94 protein is also driven by the presence of the allele at -57 position of the *MSMB* promoter. Presence of T-allele at this single nucleotide polymorphism (SNP) (rs10993994), results in up to 13 percent decrease in *MSMB* promoter activity as compared to the C-allele (53). This SNP is in a binding site for cAMP response element-binding protein (CREB), and the decrease in activity may be attributed to the effect of the marginal decrease in DNA affinity on the CREB activation cascade like protein kinase A (PKA) phosphorylation (54). Several SNPs have been identified as predisposition loci for PCa (55). However, among these SNPs, genome-wide association studies (GWAS) have identified risk allele rs10993994 (g.-57C>T), to be associated with PCa risk (56,57). This association has also been ascertained in varied ethnicities and the risk allele has been established as a causal variant for PCa (46,53,58–60). Similarly, the sPSP94 levels significantly associate with the rs10993994 genotype (53) and both hold potential as biomarkers for PCa

risk. Of the 30 SNPs reported in the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref) corresponding to the *MSMB* proximal promoter region, rs10993994 is well established as a susceptibility locus for PCa. However, the T allele of rs10993994 may not be predisposing to PCa risk in Chinese Han population (61). Also, this SNP was not found to be a predisposing factor for BPH development (62,63). In a recent study by our group, we observed that the frequency of rs10993994 was similar in healthy participants as well as BPH and PCa patients (64). Another Asian study, which included 122 PCa patients from North India, suggested that the T risk allele was associated with risk for metastatic PCa (65). Two independent studies have shown a higher frequency of the T risk allele in aggressive PCa (56,66). However, contrary results have been observed in other studies (67,68). Hence, more replicative studies of larger sample size can ascertain whether Asian Indian ethnicity harbors rs10993994 as a PCa risk factor. Nonetheless, three large functional studies show that the T allele at rs10993994 is associated with lower sPSP94 levels as compared to the C allele, in PCa cases (69) as well as healthy controls (70,71). Our study also showed the T risk allele to be associated with reduced PSP94 mRNA and protein expression, in both malignant and non-malignant cases and the rs10993994 was found to be a functional SNP in Asian Indians (64). Nevertheless, rs10993994 had been shown to be associated with both the altered sPSP94 expression and PCa risk (69,72). Interestingly, the rs10993994 risk allele is common with a frequency of about 30–40 percent in Europeans and 70–80 percent in men of African ancestry (59). However, PCa is not that common, thus decreased sPSP94 levels may not be the sole causal event in PCa and could be only one of the contributing factors (71). More studies to elucidate the biological significance of influence of rs10993994 on sPSP94 levels and prostate pathophysiology need to be undertaken, since this SNP influences the PSP94 expression in healthy men in multiple populations. Significant findings of the *MSMB* promoter polymorphisms from various studies are summarized in Table 1.

7. REGULATION OF PSP94

Different study groups have gained insight in understanding the hormonal regulation of the PSP94 gene, *MSMB*. Intense immunohistochemical staining observed for PSP94, as compared to PSA and PAP, in 3 androgen independent human and rat prostate carcinoma cell lines, suggested that the synthesis and secretion of PSP94 is not solely dependent on androgens (90). Unlike PSA, PSP94 is not affected by loss of androgen receptor activation and continues to be expressed despite hormone manipulation, indicating possible alternative pathways in regulation of the gene. Thus, PSP94 was proposed to be a

Table 1. Major findings of the *MSMB* promoter SNPs rs10993994

Sr. No.	Population studied (n)	Salient findings
1.	European (145)	T allele is positively associated with decreased PSP94 expression in normal as well as malignant prostate tissue (46)
2.	Swedish (4621) European (2009) Caucasian (3530) European (2329)	SNP found to be positively associated with PCa risk (53)
3.	European (10377)	Functionally active SNP and a probable causal variant for PCa, a higher frequency of the PCa risk allele T, was seen in patients with more aggressive PCa (56)
4.	European (3748) Australian (6634)	GWAS identified MSMB locus at rs10993994 for rendering PCa susceptibility (57)
5.	European (12213)	T allele of rs10993994 found to be most strongly associated marker with PCa risk. C allele binds to the CREB transcription factor and shows more PSP94 expression than T allele (60)
6.	Chinese (320)	SNP positively associated with biochemical relapse after radical prostatectomy (61)
7.	Scottish (646)	Increased PCa risk associated with TT genotype at rs10993994, however no significant association found between rs10993994 and BPH (62)
8.	Korean (173)	Significant positive association seen between rs12770171 and BPH development, while rs10993994 did not show any association with BPH (63)
9.	Asian Indian (112)	No significant association of rs12770171 / rs10993994 with BPH or PCa development was observed. However, the rs10993994 was found to be a functional SNP in Asian Indians with respect to sPSP94 levels (64)
10.	Caucasians (1675) Asian Indians (444) African Americans (296)	T allele found to be associated with increased risk for metastatic PCa, but not with recurrence (65)
11.	European (5486)	T allele of rs10993994 found to be positively associated with less aggressive PCa (66)
12.	European (2045)	SNP not associated with aggressiveness of PCa or with Gleason scores, pathologic stage, or age at diagnosis of PCa (67)
13.	African Americans (1435) European Americans (887) Latinos (1175) Japanese Americans (1409) Native Hawaiians (221)	SNP positively associated with PCa, could be a global marker of risk of PCa (68)
14.	African Americans (649) Japanese (998) Latino (521) Native Hawaiian (173) Caucasians (575)	Low blood levels of PSP94 increased the risk of PCa irrespective of race, ethnicity or rs10993994 genotype (69)
15.	European Americans (125) African Americans (125) Latinos (125) Japanese Americans (125)	T allele accounts for 30–50 percent of the variation in PSP94 levels in blood plasma of healthy men. PSP94 levels were found to be highest in African Americans and lowest in Japanese Americans (70)
16.	Swedish (304)	TT genotype was associated with lower PSP94 but higher PSA levels. This SNP is significantly correlated with levels of blood and semen PSP94, free and total PSA, and semen levels of hK2 at normal physiological levels (71)
17.	Han Chinese (509)	T allele found to be positively associated with decreased sPSP94 levels and increased PCa risk (72)
18.	European and others (70)	Study did not find additional SNPs in perfect linkage disequilibrium (LD) with rs10993994 which was suggested as the probable variant associated with PCa (73)
19.	Swedish (2875)	SNP not associated with death from PCa in a cohort of men diagnosed with PCa (74)
20.	European (168)	TT genotype linked with moderate to low PSP94 expression in prostate tissue (75)
21.	Swedish (4323)	SNP found to be PCa susceptibility locus, significantly associated with total PSA (tPSA), free PSA (fPSA) and human kallikrein 2 (hK2) levels in plasma (76)
22.	European (384)	In silico studies predict that rs10993994 changes the binding site for the ubiquitous CCAAT and Gli-Kreupel Transcription Factors and is positively associated with PCa risk and is in LD with rs12770171 ($r^2=0.3.2$), however no SNPs in perfect LD with rs10993994 (77)
23.	African American (136)	rs10993994 not significantly associated with PCa (78)
24.	European Americans (296) Japanese (298) African Americans (168)	T allele significantly associated with less PSP94 expression in all 3 ethnicities (79)
25.	Western European (165)	T allele associated positively with DNA repair capacity (80)
26.	Chinese (3492)	SNP genotype found to be associated with increased levels of PSA (81)
27.	Swedish (3412)	SNP found to be positively associated with PCa diagnosis, and independently associated with PCa risk. SNP was not shown to enhance predictive power of PSA testing (82)

28.	Caucasian (729)	T allele is associated with decreased PSP94 protein expression, and the association is stronger in tumor compared to normal prostate tissue. (83)
29.	European American (2471)	Positive association seen between number of sexual partners and PCa risk in men carrying the T allele (84)
30.	Han Chinese (720)	Increased risk of idiopathic infertility with azoospermia was associated with T allele of rs10993994, where these males have significantly higher PSP94 expression levels than fertile controls (85)
31.	European (21511)	T allele of rs10993994 inversely associated with risk of PCa-specific mortality and found to be positively associated with both fatal and nonfatal PCa (86)
32.	European (424)	SNP found to be significantly associated with MSMB expression in tumor or normal prostate tissue (87)
33.	Swedish (13)	Low PSP94 expression found to be associated with the TT genotype at rs10993994 (88)
34.	Finnish (1272)	Significantly lower levels of sPSP94 were associated with SNP in both PCa patients and healthy controls, however, no association found with PCa risk (89)

useful biomarker following hormone ablation therapy (91). Concurrently, *in vivo* hormonal regulation of PSP94 mRNA in long-term castrated rats revealed that PSP94 is under androgen regulation in the rat lateral prostate. In addition, this protein could also be regulated by glucocorticoid and progestin; though not zinc (92). As direct evidence of the androgen responsiveness of PSP94, increasing concentrations of a synthetic androgen was shown to result in a dose-dependent increase in promoter activity of the *MSMB* gene (53). Short- and long-term androgen deprivation therapy (ADT) showed decrease in PSP94 expression in these patients, indicating androgen-dependent expression. However, the expression was not fully repressed (88). Thus, androgen-independent expression seen of PSP94 is likely mediated by alternate pathways like regulation by other steroids, in absence of androgens.

Further, PSP94 expression is low in progressive and metastatic disease which is in contrast to PSA expression indicating that both proteins are differentially regulated in progressive PCa (88). This could be due to gene silencing mediated by the putative oncogene, enhancer of zeste homolog 2 (*EZH2*) (93). *EZH2* is a Polycomb protein which represses transcription via trimethylation of histone H3 on Lys27 (H3K27). Knockdown of *EZH2* resulted in a loss of H3K27 trimethylation and an increased expression of the *MSMB* gene, while overexpression of *EZH2* was associated with a decreased expression of the *MSMB* gene (93). PSP94 expression is also found to be controlled by the SNP rs10993994 on the *MSMB* promoter as discussed in Section 6. This SNP regulates the PSP94 levels in normal as well abnormal prostate (64) by altering the transcription factor binding site (53). Thus, PSP94 expression may be regulated by several mechanisms and may be under tight control and needs to be investigated further.

8. PURIFICATION AND CHARACTERIZATION OF PSP94 FROM SEMINAL PLASMA

PSP94 has been isolated, purified and characterized from human seminal plasma using various methods (8,14,94). Dubé *et al.* (1987) reported the purification of a protein from human seminal plasma which was found to be identical to PSP94 (14). Ohkubo *et al.* purified PSP94 which resolved at a molecular weight of 19 kDa and 17 kDa on gel filtration and reduced SDS-PAGE respectively (95). Another procedure for the purification of PSP94 from human seminal plasma was reported by Baijal-Gupta *et al.* (96). Cation and anion exchange chromatography were used, resulting in a yield of 18–25 mg PSP94 per 50 ml of seminal plasma. Our group has also successfully purified PSP94 from human seminal plasma by ammonium sulphate precipitation, hydrophobic interaction chromatography followed by preparative reversed phase-high performance liquid chromatography (RP-HPLC) which resulted in 35 mg of pure PSP94 per 100 ml of seminal plasma (97). An in-depth characterization of purified PSP94 was carried out by amino acid analysis, N-terminal sequencing and molecular mass estimation which confirmed the homogeneity and identity of the protein. This preparation was used for elucidating the 3D structure of PSP94 by X-ray crystallography.

9. RECOMBINANT PSP94 AND ITS EXPRESSION

The earliest preparation of recombinant PSP94 was reported by Linard *et al.* (98) in *E. coli*. A clone expressing PSP94 epitopes in periplasmic extracts was identified. Further, this periplasmic PSP94 was shown to be similar to natural PSP94 indicating correct processing and folding of the cysteine-rich protein. Attempts were also made to

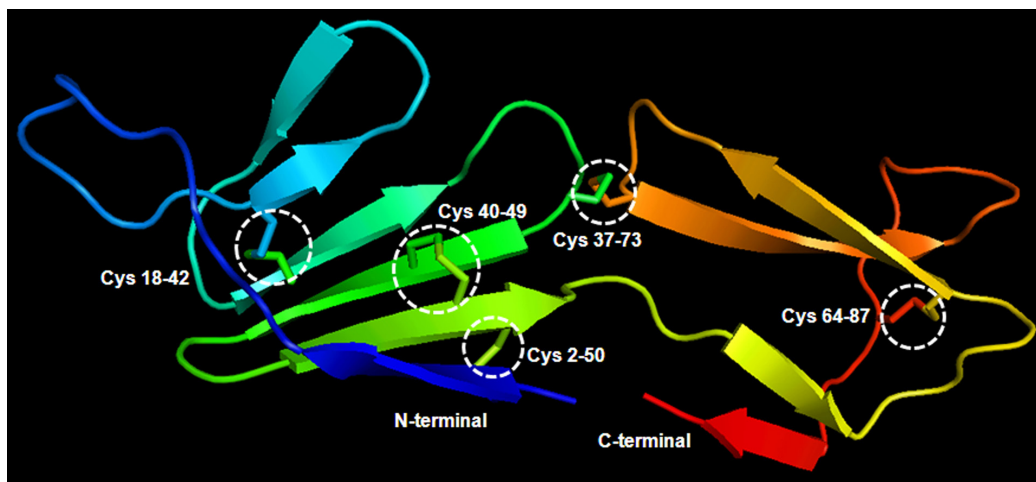


Figure 2. Cartoon representation of PSP94 with polypeptide chain in rainbow color from blue (N-terminus) to red (C-terminus). Five disulfides are shown in stick representation. Adapted with permission from Elsevier (106).

produce recombinant human PSP94 to evaluate its clinical and functional role in PCa (99). The PSP94 cDNA was cloned and expressed in yeast using *Pichia pastoris* expression system and showed functional similarity with that of native PSP94. The production and purification of recombinant PSP94 in insect cells using baculovirus system has also been reported (100). The protein was recovered using ethanol extraction and the yield in culture medium was ~35 mg/L.

10. STRUCTURAL STUDIES OF PSP94

Characterization of the primary structure of PSP94 purified from human seminal plasma has been undertaken since last four decades. The amino acid sequence for ostrich MSP was established through a combination of automated Edman degradation and matrix assisted laser desorption ionization–time of flight mass spectrometric (MALDI-TOF MS) analysis (40). Homology-based computational methods could not be applied for determining the structure of PSP94, due to difficulties in extracting PSP94 in the pure form and lack of sequence homology with known structures. Hence, an *ab initio* structure was generated as one of the first attempts to understand the biophysical properties of PSP94 (101). A detailed secondary structure study was later reported using native and recombinant porcine beta-MSPs by Wang *et al.* (102). Both native and recombinant proteins were shown to exhibit a very similar structure based on circular dichroism and nuclear magnetic resonance data. However, determination of the tertiary structure of PSP94 was evaded for a long time due to difficulties in crystallizing PSP94. In 2005, Wang *et al.* determined the three dimensional solution structure of porcine beta-MSP using nuclear magnetic resonance (NMR) spectroscopy (103). Another study by Ghasriani *et al.* reported the solution structures of porcine and

human beta-MSPs, which on comparison showed conservation of the ten cysteine residues maintaining the three-dimensional conformation between the species (104). However, the orientation of the protein was entirely different compared to the one reported by Wang *et al.*

Using X-ray crystallography, our group has successfully determined the crystal structure of PSP94 (PDB id. 3IX0) (105, 106). The protein was determined to have a two-domain structure rich in beta-sheets with an identical pattern of five disulfide linkages as reported for porcine beta-MSP (104). However, this structure is completely different compared to that of the structure predicted by the *ab initio* method (101). PSP94 exists as a monomer which consists of two domains held together by the disulfide linkage between Cys37-Cys73; where the N- (residues 1–52) and C-terminal (residues 53–94) are held in close proximity via a strong hydrogen bond (106). The ten cysteine residues were found to be paired between ²Cys-⁵⁰Cys, ¹⁸Cys-⁴²Cys, ³⁷Cys-⁷³Cys, ⁴⁰Cys-⁴⁹Cys and ⁶⁴Cys-⁸⁷Cys (Figure 2). The disulphide alignments of beta-MSPs from different species obtained by means of different methods are compared in Table 2.

The C-terminal domain has two double-stranded antiparallel beta-sheets, which has no structural similarity with any known proteins, but the N-terminal (15–52 residues) has a fold similar to Fibronectin type I (FnI) module (106, 107). The N-terminal domain has four antiparallel beta-strands (beta1: 1–6, beta4: 30–34, beta5: 38–42 and beta6: 46–51) arranged in the form of Greek-key motif and two small antiparallel beta-strands (beta2: 18–21 and beta3: 23–27) forming a flap on top of the Greek key motif. The edge-to-edge interaction of two monomers by the sequential alignment of terminal antiparallel

Table 2. Comparison of disulphide alignments of cysteine at position 2, 18, 37, 40, 42, 49, 50, 64, 73, 87 of different beta-MSPs by different methods

Ostrich MALDI-TOF (40)	Human Ab initio (101)	Porcine NMR (102)	Human NMR (104)	Human X-ray (106)
2–18	2–50	2–50	2–50	2–50
37–73	18–64	18–42	18–42	18–42
40–49/50	37–87	37–73	37–73	37–73
42–49/50	40–42	40–49	40–49	40–49
64–87	49–73	64–87	64–87	64–87

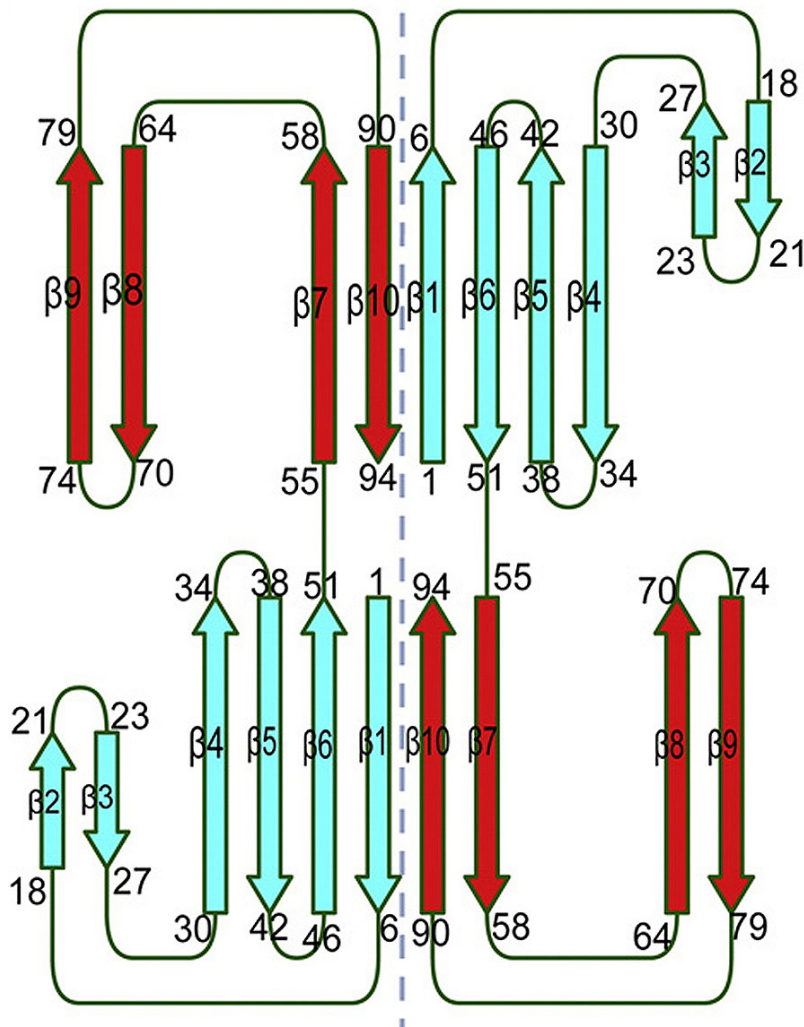


Figure 3. PSP94 dimer. Topology diagram of dimeric interaction showing extension of beta-sheet across the dimer interface (broken vertical line). The beta-strands in the N-terminal domain are shown in cyan, and those in the C-terminal domain are shown in red. Reproduced with permission from Elsevier (106).

beta-strands has been shown to aid in the formation of PSP94 homodimer. The N-terminal Greek key motif in each monomer gets extended across the dimer interface by the C-terminal strands beta10 and beta7 of the adjacent monomer (Figure 3). This monomer/

dimer transition was attributed to difference in pH conditions; wherein the monomeric form was observed in acidic pH. The structure of monomeric PSP94 as well as its dimeric form; and possible mode of interaction with different binding proteins may provide clues to

the biological functioning of this important family of proteins.

11. PSP94 INTERACTOME

11.1. Prostate secretory protein binding protein (PSPBP) (CRISP-9/PI16)

In late 1980s, Abrahamsson *et al.* indicated variable concentrations of PSP94 to be present in the serum of PCa patients (108). Further studies revealed the presence of a higher molecular weight form of the native protein (between 60 and 150 kDa) which was not efficiently detected by antibodies raised against free PSP94 (109). Thus, investigators began to work towards identifying putative binding molecules of PSP94 that would enable development of serum immunoassays for PCa management by utilizing the bound and free forms of PSP94. Higher ratio of bound/free forms of serum PSP94 (sPSP94) compared to sPSA level in pretreatment samples was demonstrated to be a significant predictor of relapse post radiotherapy (110). Serum PSP94-bound complexes were later considered for evaluating the clinical utility of PSP94 as a marker for PCa progression. PSP94 interacting protein purified from human serum was named as PSP94-binding protein (PSPBP), which showed significant amino acid sequence similarity to CRISP family of proteins harboring the SCP (sperm coating protein) domain (111). A specific enzyme-linked immunosorbent assay (ELISA) was developed, where bound/free PSP94 and PSPBP were ascertained to be independent prognostic markers following radical prostatectomy (112).

11.2. Immunoglobulin G (IgG)

Efforts to study the immunoglobulin binding proteins from human seminal plasma led to the identification of a protein that binds to human immunoglobulin Fc region and was designated as immunoglobulin binding factor (IgBF) (113,114). IgBF was considered a soluble form of immunoglobulin (Ig) Fc binding protein that may be related to the Fc gamma receptor type III (FcγRIII/ CD16) and could modulate the local immune system (113,114,115,116). Under reducing condition, 16 kDa form of IgBF was shown to interact with monoclonal natural killer-associated anti-Leu-11b antibody; whereas the 27 kDa form was found to be inactive as determined by Western blot analysis (117,118). This protein was presumed to probably be a non-glycosylated protein since it failed to interact with concanavalin A under reducing condition (114). IgBF showed a broad binding activity with immunoglobulins obtained from several species and had the greatest affinity for human IgG1; but did not interact with human IgM or IgA (118).

This 16 kDa protein in seminal plasma with IgG binding property was later characterized by mass

spectrometry and found to be identical to PSP94 (119). Thus, PSP94 was identified as a member of IgBF family found in human seminal plasma that specifically suppressed pokeweed mitogen stimulated lymphocyte blastogenesis indicative of a role in local immunity (120). Further, IgBF was hypothesized to suppress activation of B cells in the male and female genital tract. Later studies revealed IgBF to be present in cervical mucus besides human seminal plasma, where this protein interacts with IgG as a monomer under reducing conditions (121). This observation led to the hypothesis that the conversion of IgBF to the active form may play a role in preventing antibody production against allogeneic sperms in the female reproductive tract.

Our group has reported the binding of native PSP94 purified from seminal plasma to IgG *in vitro* (97). Further, the reduction of disulfide bonds of PSP94 affected the secondary and tertiary structure, but not the IgG binding ability; suggesting involvement of sequential epitopes of PSP94 in IgG binding (97). In continuance, real time SPR analysis were undertaken to understand the kinetics of PSP94 interaction with IgG subdomains and it was observed that the Fab domains, but not the Fc region interacted with the terminal beta-strands of PSP94 and that the disulfide linkages within PSP94 are not essential for this interaction (133).

11.3. Cysteine rich secretory protein (CRISP)

Extensive studies have shown CRISP-3 to be interacting with PSP94 in human seminal plasma (122–124). Unlike PSP94, CRISP-3 expression is upregulated during prostate tumorigenesis and both have been considered for diagnosis and prognosis of PCa (88). CRISP-3 belongs to CRISP family of proteins that are found in vertebrates, mainly expressed in the male reproductive tract in mammals, while homologs of CRISP-3 have been identified in many snake venoms (125,126). Porcine and human PSP94 which are evolutionarily diverse have also been shown to bind to snake venom CRISP (126). The interaction between the two families appears to be quite general and may have functional implications. Also, affinity of snake CRISP is less than that of human CRISP-3 for PSP94 suggesting that PSP94-CRISP interaction affinities differ between species. CRISP-3 was first identified in human neutrophilic granulocytes as SGP28 (Specific Granule protein of 28 kDa). This protein is shown to be present in saliva, blood and seminal plasma (127,128). CRISP family of proteins consists of two domains connected by a short hinge region. The N-terminal large globular domain shares sequence similarity to the PR-1 (pathogenesis related proteins of group 1) domain in plants; suggesting a role in innate immunity (129,130). The amino terminal of PSPBP (residues 6–170) shows sequence similarity with corresponding

part of CRISP-3 (residues 19–183) (112), suggestive of other proteins possessing SCP domain to be involved in binding with PSP94. On the other hand, several snake venom CRISPs show ion-channel regulatory activity which is associated with the C-terminal cysteine-rich domain (CRD) known as ion-channel regulatory (ICR) domain (130,131). *In silico* models have also displayed the formation of PSP94 heterodimers with CRISP3 (106,132) and IgG (133). Recent studies from our lab have delineated the specific residues involved in PSP94-CRISP3 interaction, where CRISP3 interacts with PSP94 through the same edge involved in PSP94 dimeric interface (123). Using site directed mutagenesis, the first alpha-helix of the N-terminal domain and the hinge region of the C-terminal CRISP domain of CRISP3 have been shown to interact with the terminal beta-sheets of PSP94.

The interaction of CRISP-3 and PSP94 was earlier hypothesized to inhibit the ICR domain activity (130). The NMR model and X-ray crystal structure proposed for PSP94-CRISP-3 interaction suggest involvement of one of the beta strands in the SCP domain of CRISP-3 with the terminal beta-strands of PSP94 (106, 132). Recent studies by our group have shown the hinge region along with first alpha-helix of CRISP-3 as the binding interface for terminal beta-strands of PSP94 (123). Thus, the involvement of hinge region of CRISPs in interaction with PSP94 may affect the domain movement of CRISPs regulating the ion-channel regulatory activity. A high sequence similarity between the first alpha-helix of CRISPs as well as the structural similarity in the hinge region due to four conserved cysteine residues indicates that other CRISP family proteins might be interacting with PSP94 through the same binding interface. In continuation, CRISP-2 was also shown to interact with PSP94 *in vitro*, wherein both proteins were overexpressed in PC3 cells (123). CRISP-2, previously referred to as testis-specific protein 1 (Tpx-1), is a non-glycosylated testis-enriched member that is produced during spermatogenesis (134). Interestingly, human CRISP-3 and CRISP-2 share 71.4. percent sequence identity. In this direction, using affinity pull down, we identified CRISP-2 as one of the probable binding proteins from human sperm to bind to PSP94 (135). Further, we pursued the development of indigenous anti-peptide antibodies against the least conserved ICR region of the CRISP domain in an attempt to distinguish human CRISP-2 from CRISP-3. The presence of CRISP-2 on human sperm was validated using this anti-peptide antibody against peptide 219–231 of CRISP-2. CRISP-2 has not only been shown to have a role in sperm egg interaction (136), but mammalian CRISPs are reported to be involved in sperm maturation, gamete fusion and host defense as well (137). Therefore, there is a possibility that PSP94 may also have a role in sperm function through its interaction with CRISP-2.

11.4. Prostatic acid phosphatase (PAP)

Based on the findings so far, it could be inferred that PSP94 may exist either as a homodimer or heterodimer complexed with its interacting proteins in human semen. Also, the concentration of PSP94 (600–900 mg/L) (21) is many fold higher than CRISP-3 (3–30 mg/L) in seminal plasma (122), which led our group to identify other binding proteins of PSP94 that could help in delineating the mechanism of action of PSP94. During the isolation and purification of PSP94 from human seminal plasma (97), it was observed that the fractions collected subsequent to PSP94-containing fraction continued to show detectable levels of PSP94 when subjected to SDS-PAGE followed by western blotting. This suggested that PSP94 may be present in the bound form with other proteins in these fractions. Subsequently, one of the binding proteins was identified to be PAP and the interaction was predicted to be through the terminal beta sheets of PSP94. Further, this binding interface was revealed to be similar to that of other PSP94 binding partners as CRISP-3 and IgG (124).

Human PAP is one of the predominant proteins secreted by the epithelial cells of the prostate (21,138). PAP has been suggested to have a physiological role in fertility facilitating liquefaction of semen (139). The serum level of PAP is frequently elevated in patients with PCa and the protein is correlated with tumor progression (140). Previous reports indicate that native PAP exists as a dimer of two catalytically inactive subunits, non-covalently bound together to form an active enzyme (141,142). Further, PAP is known to inactivate lysophosphatidic acid (LPA) (143), a protein involved in cell proliferation and anti-apoptotic activities (144). Therefore, it could be hypothesized that the PSP94-PAP interaction may prevent dimerization of PAP (active enzyme) and may have a role in PCa progression.

12. PUTATIVE FUNCTIONS OF PSP94

12.1. Sperm motility inhibitor

Some of the early studies reported the *in vitro* agglutination of human spermatozoa using anti-PSP94 antibodies (145). Further, PSP94 exhibited a dose-related suppression of ascorbate-induced lipid peroxidation in human spermatozoa and was hypothesized to be one of the factors involved in the regulation of lipid peroxidation and sperm motility (146). In early 1990's, Jeng *et al.* purified a protein from porcine seminal plasma shown to inhibit sperm motility (24). This protein on further characterization was found to competitively block Na⁺/K⁺-ATPase channel and displayed sequence similarity to PSP94 (25). Later studies showed PSP94 to be localized on the human spermatozoa surface, involved in the

inhibition of spontaneous acrosome reaction (147). PSP94 has also been shown to be released in the spent medium post capacitation from macaque and porcine sperm (22,26), but was still present in the sperm acidic extract in case of porcine sperm (22), suggesting intra-acrosomal localization, though this property appears to vary from species to species. On the basis of the suggested structure of porcine PSP94, its interaction with the sperm plasma membrane is likely due to the electrostatic interactions between PSP94 and integral proteins (103, 147). Thus, PSP94 attached to the sperm surface in head/tail region might have a protective role or affect sperm motility.

12.2. Protection of sperm from female immune surveillance

Several anti-sperm antibodies have been identified against human alloantigens, causing agglutination of spermatozoa and/or inhibiting their functions (148). Many studies have demonstrated PSP94 as an IgG-binding protein having some apparent role in immunity (114,117,133). It was further reported that IgG forms a high affinity complex with the terminal beta-strands of PSP94 through its Fab domain (133). In the female reproductive tract, the acidic pH of the vagina may facilitate formation of PSP94 monomers (106). This interaction of free PSP94 with IgG could circumvent the binding of IgG to the sperm. Thus, the presence of large quantities of PSP94 in seminal plasma could confer immune protection to the sperm in the female genital tract, thereby facilitating fertilization.

12.3. Fungicidal activity

In 2010, Edström Hägerwall *et al.* demonstrated the fungicidal property of PSP94 in post coital seminal plasma and proposed that PSP94 may improve fertility (149). The activity was attributed to an 11 amino acid (residues 66 to 76) peptide located on the C-terminus of PSP94. The fungicidal activity was demonstrated against *Candida albicans* and other fungi only in the acidic environment of the human vagina. The presence of calcium ions was shown to inhibit this activity through interaction with glutamic acid (E) at position 71. This amino acid when substituted with glutamine (Q), maintained the fungicidal activity of PSP94, but the activity was no longer inhibited by the presence of calcium ions. Further experiments revealed that porcine and human PSP94 showed similar activity despite having only 51 percent sequence identity, indicating that PSP94 could have an inherent fungicidal property.

12.4. Tumor suppression

PSP94 is secreted by the acinar epithelial cells of the prostate (150–152). In BPH, there is a

simple micronodular hyperplasia, which evolves into a macroscopic nodular enlargement that gradually gains clinical proportions (153). However, the molecular mechanisms underlying development of BPH are still not clear (154). PSP94 being a major secretory protein of the prostatic epithelium (151), the elevated levels of sPSP94 in BPH can be attributed to progressive proliferation of the hyperplastic epithelial cells of the prostate (155). On the contrary, PCa develops through early and late precancerous histologic modifications (153). Differential levels of *EZH2*, which causes gene silencing, were shown to be causally related to the PSP94 transcript levels (93). The gene for PSP94, *MSMB* is highly expressed in normal and benign tissue and can inhibit MMP9 secretion, promoting CD44 shedding, which leads to apoptosis (156,157). *MSMB* is epigenetically silenced by *EZH2* in PCa (93). Thus, loss of *MSMB* during tumorigenesis leads to less apoptosis and as a result an increase in cell growth. The gradual silencing of the gene is evident from several other gene expression profiling and in-situ hybridization studies which have demonstrated that expression of the *MSMB* gradually decreases from primary PCa to highly invasive, androgen independent state (158). Dahlman *et al.* (2010), found high levels of *EZH2* and low *MSMB* expression in metastatic lesions (88). On the contrary, in BPH, *EZH2* is not overexpressed as shown by a recent study and the levels are statistically lower than those in PCa (159). This fact would aid in the maintenance of high sPSP94 levels seen in BPH. Conceivably, PSP94 is suggested to function as a tumor suppressor protein which holds considerable diagnostic as well as prognostic potential.

This anti-tumor activity of this protein has been evaluated since many years. A study on the potential mechanism of action and effect of PSP94 on the growth of androgen-independent human PCa cells (PC3) was undertaken by Garde *et al.* (160). They observed that PSP94 inhibited the growth of PC3 cells in a dose and time dependent manner and suggested that PSP94 may represent a novel, apoptosis-based, antitumor agent for treatment of hormone-refractory human PCa. It has been hypothesized that the suppression of PSP94 is necessary for the progression of PCa from a low-invasive to an androgen-refractory state (93). This could be explained by the ability of PSP94 to inhibit the growth of some cancer cells in xenografted thymic mice (160) and *in vitro* in cell lines such as LNCaP or WPE1-NB26 (161).

Several studies have demonstrated the clinical utility of the varied expression of PSP94 as a prognostic marker, wherein the levels of PSP94 progressively decrease as PCa advances from a hormone dependent to independent state in metastatic PCa (162–164). PSP94 reduces PCa growth and experimental metastases to the skeleton in rat models (165). In addition, in animals receiving PSP94 treatment,

a significant dose-dependent decrease in primary tumor volume was observed along with a decrease in plasma calcium and parathyroid hormone-related protein (PTHrP) production (166). Plausibly, reduction of PSP94 transcript levels in prostatic intraepithelial neoplasia (PIN) is an early event and probably a causal factor in the development of PCa (48). A synthetic peptide corresponding to the N-terminus amino acids 31 to 45 of PSP94 (named PCK3145), was also shown to have tumor suppression properties (166). Thus, it is proposed that the increased risk of PCa in individuals with low PSP94 levels may be due to their reduced capacity to inhibit the growth of cancer cells.

13. PSP94 AS A PROSTATE TUMOR MARKER

The PSP94 mRNA and protein expression was shown to be reduced in malignant prostatic epithelium compared to benign epithelium and healthy prostate tissue (15,48,150,164). Hence, PSP94 was proposed to be a useful biomarker for PCa diagnosis (167). Further, monitoring the expression of PSP94 in androgen-independent conditions was proven to be advantageous during androgen ablation therapy of PCa patients (163). At the tissue level, the distribution of PSP94 has been examined in needle biopsy samples of PCa patients to comprehend the utility of this protein as a prognostic indicator. Multivariate analysis of different parameters like age, clinical stage, histological grade, serum PAP, PSP94 immunoreactivity and type of initial treatment show that difference in the PSP94 immunoreactivity was a significant and independent prognostic indicator of PCa (168). Similarly, along with tissue levels, PSP94 levels in the urine show significant decrease in malignant cases as compared to benign cases. Likewise, PSP94 was proposed as a biomarker of PCa risk, diagnosis and prognosis (158). PSP94 has also been proposed to be a strong independent tissue marker for decreased risk of PCa recurrence after radical prostatectomy (169).

Fusion transcripts of *MSMB* along with an adjacent PCa candidate gene, nuclear receptor coactivator 4, *NCOA4* (*ARA70*), known to be androgen receptive in nature (170) were found to be downregulated in PCa. This transcript correlated with the *MSMB* expression though this was not associated with prognosis (89). The *MSMB-NCOA4* fusion transcript could pave the path for new markers and new therapeutic targets for PCa (170). Recent research focuses on developing multiarray models of screening markers for PCa. Many reports involving PSP94 as a complementing marker have yielded encouraging results. A quadriplex model of urine mRNA levels of *PCA3* (prostate cancer antigen 3), *AMACR* (alpha-methylacyl-CoA racemase), *TRPM8* (transient receptor potential cation channel, subfamily M, member 8) and *MSMB* could be implemented as a sPSA or urine PCA3 adjunct test in patients having

PSA levels in the diagnostic gray zone (171). Also, MALDI MS profiling of urine samples after digital rectal examination (DRE) of BPH and PCa patients, demonstrated that on combining PSP94 levels to PSA in a logistic regression model, PSP94 could improve the accuracy of the sPSA test (172).

14. DEVELOPMENT OF DIAGNOSTIC ASSAYS FOR PSP94

PSP94 has been evaluated as a possible tumor marker. A radioimmunoassay (RIA) was first developed by Vaze *et al.* for the detection of PSP94 levels in seminal plasma and urine (173). Von der Krammer *et al.* determined the serum concentration of PSP94 in PCa patients, prior to any surgical intervention; however, no significant differences were observed when these serum levels were compared with those of control healthy men and BPH patients (174). Further, a simple and sensitive ELISA for measuring PSP94 in urine and seminal plasma was developed by Jose *et al.*, in 1992 (175). They obtained a very high correlation between PSP94 values determined by ELISA and RIA for urine samples and a moderate correlation for semen samples. A two-site binding ELISA using two polyclonal antibodies against PSP94 was developed by Anahí Franchi *et al.* (147). They showed that PSP94 showed a significant rise in levels in sub-fertile patients as compared to fertile controls. Nam *et al.* studied the PSP94 levels in a case-control study of 1,212 men with no previous history of PCa and who underwent a prostate biopsy because of an increased PSA or an abnormal DRE (167). The median PSP94 levels in cases was significantly lower (2.6.0 ng/ml) than among controls (3.4.0 ng/ml). Low sPSP94 levels were found to increase the probability of detecting PCa at biopsy. These total PSP94 levels were also able to identify patients with high grade disease among a subset of patients in whom PSA was least informative. Notably, PSP94 was found to be useful as a serological protein marker for PCa diagnosis and to distinguish patients with aggressive forms of PCa (167).

In one study, 185 patients with localized PCa were followed up for about 4 years for biochemical relapse; where total PSP94, free PSP94 and PSPBP were quantified in the serum using ELISA (112). Another immunoassay was developed by Valtonen-André *et al.* to measure PSP94 in serum and seminal plasma (100). This specific, competitive, europium-based immunoassay found a correlation between PSP94, PSA and Zn²⁺ present in the seminal plasma as well as between PSP94 found in the serum and seminal plasma of 205 healthy young men. Hence, even though PSP94 is not secreted solely by the prostate, PSP94 in serum can be used as a marker of prostate secretion. Further, this assay was used in another study to measure PSP94 levels in a

sample of 500 prostate cancer-free men from four racial/ethnic populations. They observed significant differences in PSP94 levels between populations, with PSP94 levels highest in African Americans and lowest in Japanese Americans (70). In another study by the same group, plasma levels of PSP94, as measured by the assay, were significantly lower in PCa subjects than in cancer-free control subjects in all racial and ethnic groups studied. Further, men with lower PSP94 levels were at greater risk of developing PCa (69).

Since sPSP94 is shown to have diagnostic and prognostic utility in PCa, it would have been worth investigating the potential of PSP94 in improving existing diagnosis of PCa. Towards this goal, sandwich ELISA was developed and validated in our laboratory to measure sPSP94 concentrations (50) using polyclonal antibodies raised in rabbit as well as rat. Similar to earlier studies, we observed that the sPSP94 concentrations were significantly lower in patients with PCa as compared to BPH, suggesting the differential expression of PSP94. Additionally, the PSP94 mRNA significantly correlated positively with the sPSP94 levels in BPH and PCa patients, and hence the sPSP94 levels are indicator of the prostatic expression of the protein (64).

15. UTILITY OF sPSP94 IN ASSOCIATION WITH sPSA FOR DIFFERENTIAL DIAGNOSIS OF PCa AND BPH

A recent nested case-control study, carried out in multiple populations, reported significantly high sPSA levels in patients with PCa as compared to healthy men (69). Further, the sPSA levels were also found to be highest in PCa than in healthy men (72) or BPH patients (176), irrespective of the racial diversity. The combined results of three separate recent Indian studies also show the sPSA levels to be highest in PCa, intermediate in BPH and lowest in healthy conditions (50,177,178). Since sPSP94 and sPSA are predominantly secreted by the prostate, their interrelationship in order to enhance the differential diagnosis between PCa and BPH was evaluated by our group (50). It was observed that, the sPSA increases, whereas sPSP94 decreases significantly in PCa patients, as compared to BPH patients. This inverse relationship was studied and the ratio of sPSP94/sPSA was analyzed. Remarkably, this ratio was statistically different among all the three study groups; highest in healthy, intermediate in benign and lowest in malignant and showed the least overlap as compared to the individual markers (Figure 4). Further, a more marked demarcation between benign and malignant cases was seen with the sPSP94/sPSA ratio as compared to sPSA or sPSP94 alone.

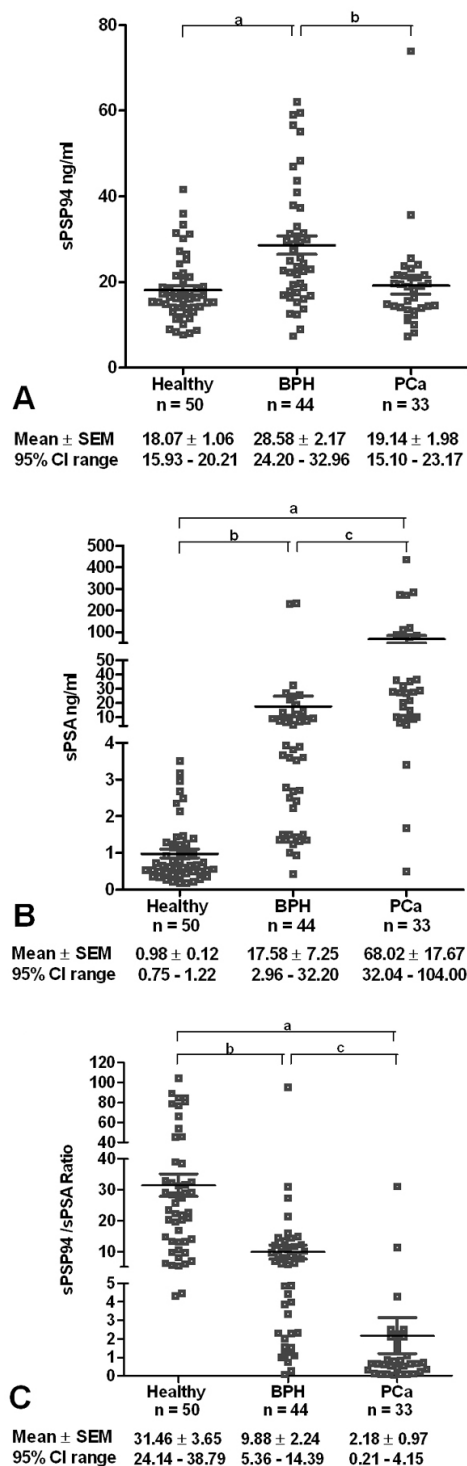


Figure 4. Scatterplots showing A, sPSP94 (ng/ml); B, sPSA (ng/ml); C, sPSP94/sPSA Ratio. One-way ANOVA test for analysis of variance with KruskalWallis correction for non-parametric test was carried out. P less than 0.05 was considered statistically significant. Values significantly different between healthy, BPH or PCa groups are denoted by letters (a, b or c) accompanying brackets connecting the significantly different groups. Values are mean \pm SEM with 95 percent CI. CI= Confidence Interval. Reproduced with permission from Elsevier (50).

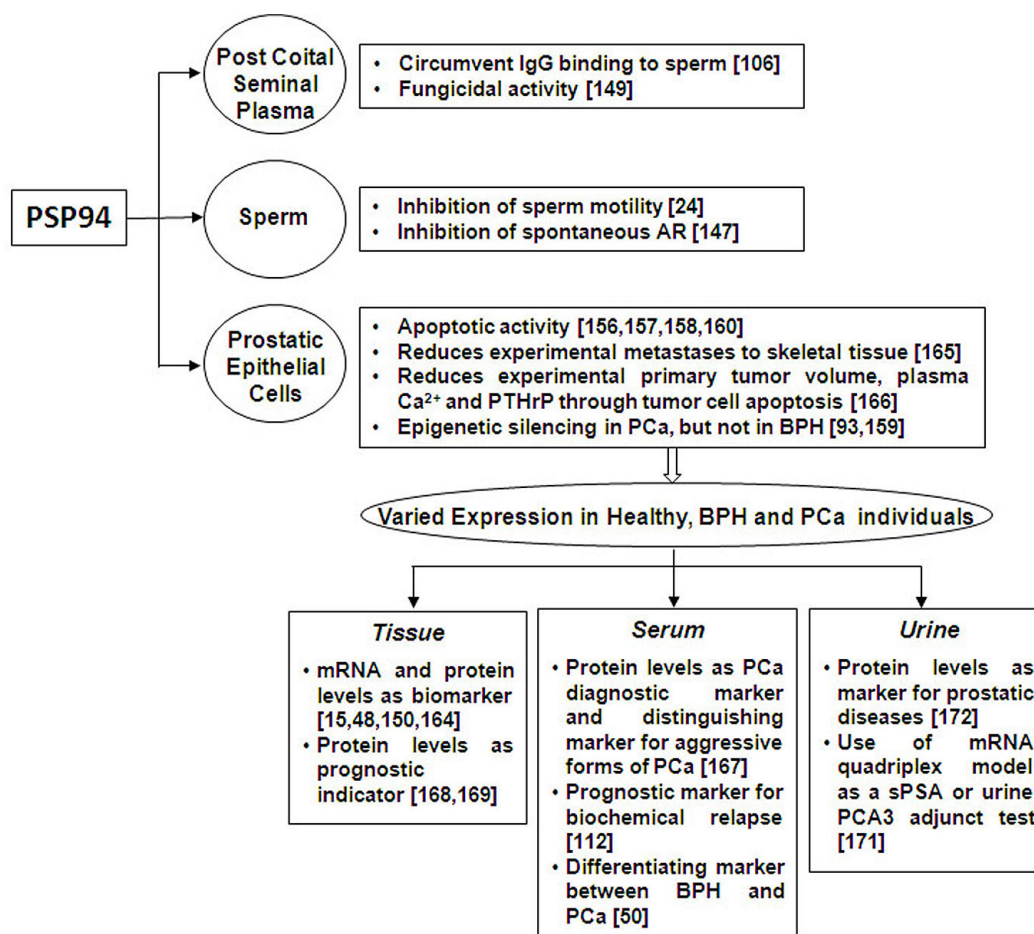


Figure 5. Probable functions of PSP94 in various milieus and its clinico diagnostic potential.

Additionally, both sPSP94 and sPSP94/sPSA ratio had a significant predictive ability for BPH as well as PCa. The sPSP94/sPSA also had the highest AUC for discriminating BPH from PCa patients and offered the best net benefit for identifying PCa in patients opting for prostate biopsy. Further, the sPSP94/sPSA ratio showed maximum reduction in unnecessary biopsies on comparing with sPSA or sPSP94, across all biopsy-threshold probabilities above 10 percent. Interestingly, the ratio sPSP94/sPSA at a cutoff value of less than 2.93 had an identical sensitivity (90.91 percent) to sPSA (cut-off greater than 4 ng/ml) but had increased specificity, and could decrease the false positivity by 13.63 percent in our study cohort (50). Many groups have earlier studied the advantages of considering ratios of proteins present in the serum like the free/total PSA ratio (179), intact-free PSA/free PSA ratio (180), chromogranin A/PSA ratio (181) and bound/free PSP94 (112) for bettering the existing diagnostic and prognostic tests for PCa. The clinical utility of the sPSP94/sPSA ratio needs to be validated further with more number of samples.

16. CONCLUSION

PSP94 has been postulated to be a rapidly evolving protein across species, which exists as a monomer and could form homo- or heterodimers. Interestingly, it is present in highest concentrations in the seminal plasma and binds with other proteins like PSPBP, IgG, CRISP-3, CRISP-2 and PAP in the serum and semen. This emphasizes that PSP94 is a regulatory protein. Though PSP94 is well characterized structurally, its exact function has not been unequivocally established. Postulated functions of PSP94 include immunoglobulin binding, sperm coating, apoptosis, tumor suppression and antifungal action (Figure 5). On the other hand, several researchers have demonstrated the clinical potential of PSP94 levels in various milieus. Essentially, PSP94 is observed to be under tight control with the allele at -57 position driving the inherent protein levels in the prostate and also being the causal variant for PCa. Differential splicing to its isoform, *MSMB2*, ensures maintenance of optimal physiological levels of PSP94 in the prostate. Furthermore, PSP94 is

epigenetically silenced by EZH2 in PCa to curtail the tumor suppressing property of the protein. Thus, multi-level control of PSP94 protein levels seems to be instrumental in the pathophysiology of the prostate.

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