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Role of β-microseminoprotein from prostate cancer initiation to recurrence: A mini-review

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Abstract

Medline/Pubmed articles relevant to this topic were considered using the search terms β-microseminoprotein, MSMB, prostate secretory protein of 94 amino acids and PSP94. Full articles were retrieved when the abstract was considered relevant. In addition, other data related to this topic including our own are discussed. Summary of findings-β-microseminoprotein (MSMB) is increasingly being considered as a marker for prostate cancer, as reduced levels have been associated with the disease. Here we review various aspects of this protein including its biological and physiological variants, binding proteins and immune modulation; its importance as a marker for biochemical recurrence of prostate cancer; prostate cancer related splice variants and its therapeutic utility. Two of the most important properties of MSMB are related to anticancer functions and immune modulation. Predominant expression of two (short and full-length) splice variants of MSMB has been observed from normal prostate and several other tissues. In benign prostate hyperplasia the short isoform is dominant, constituting 98% of this isoform, whereas in prostate cancer 96% constitute the full-length isoform. The MSMB promoter single nucleotide polymorphism rs10993994 with the C allele functions as an activated cyclic adenosine monophosphate response element binding protein binding site. This C variant of rs10993994 could be responsible for the production of splice variants under variable conditions. MSMB has binding motifs to a few known proteins including immunoglobulin G and several Cysteine-rich secretory proteins family proteins. MSMB bound to these proteins is considered as immune modulating. Use of MSMB as a urinary marker for detecting aggressive prostate cancers that could resist radiation and surgical treatments, seems possible, but needs further investigation. The ratio of MSMB splice variants could also be a possible approach in understanding prostate cancers, with higher ratios indicating severe disease.

Key words: β-microseminoprotein; Anticancer properties; Immune modulation; Splice variants; Promoter single nucleotide polymorphism rs10993994; Biochemical recurrence

Core tip: Potential exists to use the ratio of MSMB full-

length and short splice variants as a predictor of prostate cancer recurrence particularly after radiation therapy or surgical procedure or both. Control of the level of full-length splice variant in proportion to the short isoform could also carry a therapeutic use in controlling biochemical recurrence of the disease.

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INTRODUCTION

With the widespread use of prostate specific antigen (PSA) as a screening tool for prostate health, there is an associated prostate cancer stage shift in the diagnosed population and many patients carrying indolent disease are increasingly captured through these assessments[1]. For this and other reasons there is an immediate need to differentiate between indolent disease and aggressive forms of cancer (*i.e.*, that will proceed to biochemical and clinical recurrence after primary treatment). The utility of various markers for use in this differentiation have been widely discussed. These include clinical markers, bone markers, markers of proteomics, tissue DNA and protein/RNA microarray, identification of microRNA, tumor stem cells, and pathological markers including tumor immunohistochemistry[2-9]. Amongst protein markers with suggested utility for differentiating aggressive cancers, is prostate secretory protein of 94 amino acids (PSP94); also known as β-microseminoprotein. Here we review and discuss involvement and utility of this protein from prostate cancer initiation to recurrence after treatment.

RESEARCH

This review is based on Medline/Pubmed articles relevant to this topic searched through the terms β-microseminoprotein, MSMB, prostate secretory protein of 94 amino acids and PSP94. Full articles written in English were retrieved when the abstract was considered relevant. The bibliographies of retrieved papers were subsequently searched and relevant articles were considered to be presented in this review. In addition, other data related to this topic including our own are discussed.

CHARACTERISTICS OF β-MICROSEMINOPROTEIN

β-microseminoprotein (MSMB) coded by *MSMB* gene and is synthesized by epithelial cells in the prostate gland similar to that of PSA. It is not specific to the prostate gland as noted by Weiber *et al*[10]. MSMB produced in the prostate gland is secreted into the seminal plasma and is the second most abundant protein in seminal plasma after PSA[11]. Both PSA and MSMB are generally contained in prostatic ducts and thought to leak into the blood circulation system in detectable levels. However, abnormal levels in blood show that secretion of these proteins can be erratic[12]. Liang *et al*[13] have estimated the molecular weight of MSMB as 16 kDa, using sodium dodecyl sulphate polyacrylamide gel electrophoresis under reducing conditions and 27 kDa under non-reducing conditions. The elution profile of MSMB using gel-filtration chromatography has revealed that MSMB is a dimeric protein molecule under high pH and a monomer under low pH[14]. MSMB has been shown to have five disulphide bonds and the reduction of these bonds have shown changes in the secondary and tertiary structure of the molecule[15].

Table 1 A summary of recorded functions of β-microseminoprotein

Growth inhibition and apoptosis control of prostate epithelium	[16]
Decrease of tumor growth, hypercalcemia and skeletal metastases	[17]
Decrease tumor vessel density	[19]
Anti-vascular endothelial growth factor effects	[19]
Inhibit the vascular endothelial growth factor binding to tyrosine kinase receptor Flk-1/KDR expressed by endothelial cells	[19]
Inhibits the secretion of a matrix metalloproteinase	[20]
Controls anchorage-independent colony growth of prostate cancer	[21]
Interaction with IgG and modulating immune function	[57]
Interaction with CRISP-3 and modulating ion channel regulation	[43]
Interaction with CRISP-3 to inhibit effects on innate immunity	[43]
A mediator of natural killer cells and an activator of neutrophils	[53]
A pH and Ca^{2+} dependent candidacidal activity	[23]

IgG: Immunoglobulin G; Flk-1/KDR: Fetal liver kinase 1/Kinase insert domain receptor; CRISP-3: Cysteine-rich secretory proteins.

MSMB is reportedly involved in a variety of functions including those modulating prostate cancer (Table 1). MSMB is believed to have growth inhibitory and apoptosis properties in prostate epithelium[16]. Treatment with MSMB appears to decrease tumor growth, hypercalcemia and skeletal metastases associated with induced malignancy in rat models. These *in vivo* studies have been performed with MatLyLu rat prostate cancer cells transfected with full-length cDNA encoding parathyroid hormone-related protein, which is known to be the major pathogenetic factor for malignancy-associated hypercalcemia/bone metastasis[17]. Using synthetic peptides corresponding to various regions of MSMB it has been shown that these decreases are attributed to amino acids 31-45 of the MSMB protein[18]. According to Lamy *et al*[19] the synthetic peptide PCK3145 corresponding to amino acids 31-45 of the MSMB protein is reported to decrease markers related to tumor vessel density in an *in vivo* model of rat prostate cancer. These studies have further shown anti-vascular endothelial growth factor (VEGF) effects in *in vitro* models[19]. This *in vitro* study has also shown a possibility for PCK3145 to inhibit the VEGF binding to tyrosine kinase receptor Flk-1/KDR expressed by endothelial cells. MSMB also inhibits the secretion of a matrix metalloproteinase that is implicated in tumor metasta-

sis[20]. Suppression of MSMB expression in immortalized (previously normal) prostate epithelial cells has shown a significant increase in anchorage-independent colony growth[21], a property associated with tumorigenicity[22]. This feature was unique to prostate epithelial cells as breast epithelial cells from the same study have shown no such changes in tumorigenicity by MSMB suppression. In addition MSMB is reported to have a pH and Ca^{2+} dependent candidacidal activity localised to amino acid region 66-76[23].

MSMB VARIANTS

According to records in Ensembl[24], human MSMB has six splice variants of which two are protein coding[25]. A full-length (long) and a short isoform have been reported for human MSMB mRNA with the full-length isoform and the short isoform translating to MSMB of 94 and 57 amino acids (also known as PSP57) respectively[26,27]. Using reverse transcriptase polymerase chain reaction and Southern blotting with primers corresponding to the human prostate *MSMB* gene, it has been shown that endometrium, myometrium, ovary, breast, placenta and the human endometrial cancer cell lines carry this long variant[26]. Human endometrial tissues additionally recorded the short isoform while human ovary, breast, placenta and endometrial cancer cell lines, show only the full-length isoform[26]. Xuan *et al*[27] record that exon 3 is deleted in the short isoform while showing a frame-shift at exon 4. The two isoforms possess identical exons 1 and 2 including identical secretion signal peptide[25,27]. According to Xuan *et al*[27] the short isoform is localised in the nuclear fraction of prostate cancer cell lines. Predominant expression of these two splice variants have been observed from normal prostate, urinary bladder, lung, and stomach tissues[25]. Ohnuma *et al*[25] have further shown that the prostate cancer cell line LNCaP-FGC, derived from the subclavicular lymph node, carry proportionately higher amounts of the long variant compared to the short variant. Both variants are found in lower proportions compared to normal prostate tissue[25]. However, in prostate cancer cell lines DU145 and PC3 derived from brain and bone metastasis respectively, both variants were barely detected[25]. A study by Pomerantz *et al*[21] also indicates that tumor cells have a relatively higher expression of the long variant compared to the short variant while both isoforms are expressed in significantly lower levels in prostate cancer tumors compared to normal prostate tissue. Harries *et al*[28] analysed the mRNA expression of these MSMB isoforms in patients with benign prostate hyperplasia (BPH) and prostate cancer. In BPH the short isoform was predominant (98%), whereas in prostate cancer the full-length isoform was predominant (96%)[28].

Fusion transcripts between MSMB and nuclear receptor coactivator 4 (NCOA4) have been reported including multiple androgen response elements in the MSMB/NCOA4 region. The presence of the fusion transcript indicates that these fusion genes could be subjected to androgen receptor regulation[29]. Genome-wide association studies on prostate cancer have identified MSMB promoter variant rs10993994:C >T as producing one of the largest effects on relative disease risk, with about 25% per allele increase in the relative risk of the disease[30,31]. This risk allele is also found to be linked to a significant lower level of MSMB in both normal and prostate tumor tissue[21,32]. It is also reported that this single nucleotide polymorphism (SNP) variation also influences the expression of five NCOA4 splice variants with the risk allele being associated with higher levels of NCOA4 variant expression[21].

Using a series of truncated *MSMB* promoter constructs it has been shown that deletion from -27 to -58 eliminates *MSMB* promoter activity, showing that the region around rs10993994, located at -57, is critical for MSMB promoter activation[29]. Protein encoded by the *MSMB* gene is found to be carrying a gene repressive histone mark[33] of trimethylated histone H3 on Lys27 in androgen-refractory, but not in androgen-sensitive prostate cancer cells[34]. Lou *et al*[35] cloned DNA fragments containing SNP rs10993994 into the pGL3 vector and subsequently measured the promoter activities in the transfectants in 293T (human primary embryonal kidney cell line 293 carrying a plasmid containing the temperature sensitive mutant of SV-40 large T-antigen), PC3, and MCF7 (human breast cancer cell line). The transcriptional activities of the *MSMB* promoter fragments with allele C at rs10993994 were higher than those of fragments with allele T at this position.

MSMB promoter activity increase has been attributed to the presence of a possible cyclic adenosine monophosphate response element binding protein (CREB) binding site[36] in the gene sequence carrying allele C[35]. Lou *et al*[35] have further confirmed that the addition of anti-CREB antibody shows consistent reduction of the intensity of the oligonucleotide-protein complex. Lonz *et al*[37] note that CREB is among a few genes that support expression of multiple splice variants of a multitude of genes. Lonz *et al*[37] also report CREB phosphorylation in neurons with hypoxia and oxidative stress. Nicotine exposure has also shown CREB phosphorylation in rat prefrontal cortex[38]. Tobacco specific carcinogens have also been shown to activate CREB protein in human adenocarcinomas of the lungs, pancreas, and breast[39]. It is possible that CREB binding in the area around rs10993994 allele C is partially responsible for producing variation in the amounts of splice variants transcribed from the *MSMB* gene. Over 55% of prostate cancer patients from our New Zealand cohort have been tobacco smokers compared to 38% of healthy men or 45% of patients with benign disease[40]. The rs10993994 T allele frequency in this prostate cancer cohort stands at 43% whereas the 36%-39% of benign and healthy cohorts carry the T allele frequency[40]. Considered together, our prostate cancer cohort may have a higher tendency of producing activated CREB but difficulties with delivering the correct MSMB splice variants due to a relatively higher representation of MSMB rs10993994 T variant.

MSMB BINDING PROTEINS

MSMB has binding motifs to a few known proteins. Binding of MSMB to certain serum proteins via disulphide bonds has been shown to be very stable[12]. Its binding to immunoglobulin G (IgG) earns the name immunoglobulin binding factor and is thought that this binding occurs through the fragment crystallisable portion (Fc factor) of IgG[41]. Jagtap *et al*[15] has shown that the reduction of disulphide bonds in the MSMB molecule does not affect its IgG binding ability[15]. The Cysteine-rich secretory protein (CRISP) family including CRISP-2 and -3 binds to MSMB[42,43]. The structure of CRISP-1 is also considered as having a possible binding capability to MSMB[42]. With multi-dimentional NMR it has been shown that only one strand of the dimeric MSMB molecule binds with CRISP-3[44]. Udby *et al*[42] also suggests that CRISP-3 binds to dimeric MSMB in seminal plasma where the concentration of MSMB is several fold higher compared to that of CRISP-3. The amino acids Y(3), F(4), P(56) and the C-terminal β-strand of MSMB are essential for interacting with CRISP-3 and this interaction is reported to have inhibitory effects on ion channel regulation[43]. MSMB homologues from various animal species carry conserved cysteine residues[45]. It is suggested that both MSMB and CRISPs are evolutionarily conserved across various animal species and their interaction may play an important physiological role[43]. A 50-300 fold increase in CRISP-3 mRNA expression has been shown with Gleason grade 6 prostate epithelial cells compared to normal cells[46]. The N-terminous region of CRISP-3 proteins share similarity to pathogenesis related proteins of group 1 expressed in plants in response to pathogenic insults[47,48]. CRISP-3 proteins are also present in neutrophils[49,50]. Therefore, considered together Breed suggests CRISP-3 as having a role in the innate immune system[43]. Therefore increased levels of CRISP-3 in parallel to decreased levels of MSMB could be related to boosting innate immunity in prostate cancer cells. An increase in PSP94 binding protein (considered as another CRISP protein) has shown a reduced risk of prostate cancer recurrence following radical prostatectomy[51].

MSMB has shown to interact with monoclonal natural killer-associated antibody anti-Leu 11b[52] and polyclonal anti-FcγRⅢ (anti-Fcγ receptor Ⅲ) antibodies, but not with other anti-FcγR antibodies[13]. Monoclonal anti-FcγRⅢ antibodies have been shown to activate neutrophils[53]. The aforementioned indicates that MSMB is a mediator of natural killer cells and an activator of neutrophils. According to Liang *et al*[13] the 16 kDa form of MSMB binds human IgG and murine anti-Leu-11b antibody; whereas the 27 kDa form is inactive. MSMB bound to prostatic acid phosphatase (PAP) secreted by the prostate gland has been recorded from human seminal plasma[54]. According to Kuciel *et al*[55] this PAP protein is also dimeric and is dissociated to its monomers at low pH. The activity of this PAP protein is also affected when it is in its monomeric form[56]. According to Mori *et al*[57], MSMB derived from human seminal plasma and cervical mucus gets converted to its monomeric form in the female reproductive tract and interacts with IgG in preventing immune activation against allogeneic sperms. Therefore, the modulation of antibodies could be an additional role of MSMB.

STABILITY AVAILABILITY AND ACTION OF IGGS

Due to MSMB binding ability to IgGs to prevent a downstream impact of IgG activated immunity, it is important to consider the general attributes of stability, availability and action of IgGs as well. Using hybrid bispecific antibodies it has been shown that the efficient binding of the Fc factor of IgGs activate human FcγRs, macrophages, dendritic cells, and natural killer cells[58-61]. Binding of the Fc factor of IgG to the neonatal Fc receptor (FcRn) has been shown to regulate the homeostatic half-life of IgG[62]. The binding of IgG to FcRn is also pH dependent where lower pH enhances binding and physiological pH causes no binding or release[63-65]. A comparison of the concentration of anti-tetanus toxoid specific IgG in serum and fluids secreted from the prostate gland and seminal vesicles of rats has shown that the latter two tissues have only 0.3% and 0.1% respectively of the titres in serum[66]. This study has also reported the mRNA levels of FcRn in different tissues of the male reproductive tract where ductuli efferentes has shown the highest levels, while prostate and testis have shown comparatively lower levels. This indicates that the prostate gland has limited levels of IgG and in addition, lower levels of FcRn that controls the half-life of IgG. It has been suggested that these lower levels could be related to the avoidance of autoimmunity against spermatozoa[67]. Therefore, in a normal prostate the IgG binding nature of MSMB could support the control of free IgG and its downstream effects. In the event of prostate cancer development this mechanism may need reversing to produce IgG activated immunity through the support of circulating IgG. However, immunoreactivity experiments have shown local synthesis of IgG distinguished from the depositions from circulating IgG in prostate cancer cells and significantly higher levels in prostate cancer compared to benign prostate hyperplasia[68]. In a study by Liu *et al*[68] it was shown that levels of γ chain specific IgG is positively correlated to Gleason score and histological grade.

MSMB EXPRESSION IN ADVANCE PROSTATE CANCER

High staining intensities of MSMB in prostate biopsy samples have been correlated with a Gleason score of < 7 at radical prostatectomy[69]. Whitaker *et al*[7] have shown that the decrease in MSMB level is associated with the histopathological grade of prostate cancer. Their studies have shown that the tissue areas with Gleason grade 4 show no expression while grade 3 tissues have shown reduced expression of MSMB. Consistent with this, ex-

pression of MSMB is lost or lowered in metastatic prostate cancer compared to primary disease[70,71]. A human polycomb repressive complex protein Enhancer of Zeste Homolog 2 (EZH2), shows increased expression in metastatic prostate cancer and is correlated with a loss of the tumor suppressor protein MSMB[70,71]. Beke *et al*[34] tested methylation and acetylation levels of the *MSMB* gene in prostate cancer cell lines (LNCaP and PC3) representing features of advanced prostate cancer. The *MSMB* gene was shown to be carrying gene repressive histone marks[33] with highly trimethylated histone H3 on Lys27 in PC3 and showed very little activity of the MSMB protein, whereas LNCaP prostate cancer cell line that expresses considerable levels of MSMB protein, is only mildly trimethylated at this histone position[34]. Further, Beke *et al*[34] showed the involvement of CpG methylation in the promoter region of the *MSMB* gene in both PC3 and LNCaP and deacetylation in Lys 9 of Histone H3 in PC3, thus repressing MSMB levels.

Patients with advanced prostate cancer are generally treated with androgen deprivation therapies (ADT). MSMB expression levels have shown a significant decrease in patients undergoing ADTs[72]. Dahlman *et al* [72] also showed that although prostate derived CRISP-3 was not affected by short-term ADTs, the level was high in castration-resistant prostate cancer (CRPC) and metastases[72].

MSMB AS A PROSTATE CANCER MARKER

MSMB protein is increasingly being studied as a prostate cancer diagnostic and prognostic marker due to high levels recorded from normal or benign prostate tissue, while lower or undetectable levels are recorded from prostate cancer tissue[7]. Haiman *et al*[73] has shown that with increasing serum MSMB levels there is a progressive reduction of odds ratio of prostate cancer risk when compared in men in the highest decile, with men in the lowest decile of MSMB, regardless of *MSMB* rs10993994 genotype or ethnicity. These odds ratios further increased when the MSMB level was adjusted by serum PSA levels. Reduced MSMB levels have also been reported in invasive neoplasms of the breast and lower levels were associated with reduced survival rates[74].

However, contradictory evidence on the value of MSMB level as a prognostic marker is also recorded in literature[8,9,75]. Girvan *et al*[9] reports that the presence of MSMB in the highest Gleason area as an indicator of risk for biochemical recurrence and disease progression. Dahlman *et al*[8] have recorded the intensity of MSMB in tumor sections in a scale between 0-255. They have reported that the sections with MSMB intensity above 155 are at a lower risk of biochemical recurrence[8]. Another approach of considering the ratio of bound to free MSMB has been considered by Bauman *et al*[75]. Here they report that those with a higher ratio may indicate worse outcome post-radiotherapy. Higher levels of MSMB have been associated with idiopathic male infertility[76,77]. Meanwhile infertile men have been shown to have an increased risk of subsequently developing high-grade prostate cancer[78].

The above contradictory data related to the action of MSMB in prostate cancer etiology, recurrence and metastasis needs explanation. One possible reason could be a reduction of MSMB to make way for IgG induced immunity against developing cancer cells. In the event of prostate cancer initiation, it is required to up-regulate IgG activated immunity. Hemstreet *et al*[79] have shown the induction of IgG responses in men with prostate cancer when immunotherapy is used with autoantigens for prostate cancer. A review by Helo *et al*[80] discusses the utility of the human monoclonal antibody of the IgG2 subtype as a useful therapy to prevent skeletal events related to androgen deprivation therapy on prostate cancer patients. Therefore, a decrease in MSMB could be a protective mechanism to support up-regulation of circulating IgG induced immune responses. Therefore, the prediction of biochemical recurrence associated with retained MSMB activity in the highest Gleason area[9] could be representing tumor areas that have suppressed IgG levels leading to impaired IgG induced immunity. However, as reported by Harries *et al*[28] it is also possible that the tumor environment attempts to overcome the action of IgG induced immunity by producing the longer isoform of MSMB in place of depleting shorter isoform[28]. This could be a possible reason for the presence of MSMB in high Gleason areas[9]. It is also possible that high Gleason areas are producing fusion transcripts between MSMB-NCOA4[29] with multiple androgen response elements, and thus being subjected to androgen receptor regulation. Local production of tumor derived local IgG is also reported by Liu *et al*[68] as discussed before. The above led us to believe that aggressive tumor environments could produce long MSMB isoforms to counteract the hosts immune function; similarly the tumor environment could produce its own immunity by way of tumor specific IgGs for its survival as discussed by Liu *et al*[68].

MSMB AND PCA3

A genome-wide association study involving 1371 men, has shown a significant association of the prostate cancer antigen 3 (PCA3) level with MSMB promoter rs10993994 variant allele T[81]. This non coding RNA marker PCA3 is over expressed in prostate cancer and considered as having great potential as a clinical biomarker for predicting prostate biopsy outcome in subjects with elevated PSA (> 2.5 ng/mL)[82-85].

Our own unpublished data shows trends between urinary MSMB and PCA3 mRNA level variability with various prostate cancer treatments. We measured the PCA3 mRNA expression and PCA3 score[86] and the urinary and serum MSMB levels of a limited number of prostate cancer patients. It has been shown that prostate

Table 2 Relative expression of *PCA3* and *PSA* genes in cells captured from urine of patients undergone various treatments

Treatment type	Relative *PCA3* gene expression	Relative *PSA* gene expression	PCA3 score[1]
RP and RT	0	0	0
RP and RT	3.91	6.45	0.61
RP and RT	0	0	0
RP and RT	0	0	0
RP and RT	5.03	4.67	1.08
RT	0	0	0
RT	0	0	0
RT	4.76	0	-
RT	4.24	4.23	1
RT	4.94	1.86	2.66
ADT and RT	0	0	0
ADT and RT	0	0	0
ADT and RT	0	3.72	-
ADT and RT	0	0	0
ADT and RT	0	0	0
ADT and RT	0	0	0
RP + RT and ADT	0	7.39	0
ADT	0	0	0
ADT	4.39	5.47	0.8
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
RP and NFT	2.23	0	-
RP and NFT	7.97	2.83	2.82
RP and NFT	3.04	3.22	0.94
RP and NFT	0.39	0.34	1.15
RP and NFT	6.56	3.53	1.86
RP and NFT	6.05	4.67	1.3
RP and NFT	0	0	0
RP and NFT	2.33	1.53	1.52
AS	6.02	7.2	0.84
AS	2.83	2.14	1.32
AS	5.42	Not available	-
AS	3.18	2.93	1.09
AS	6.53	8.13	0.8

[1]PCA3 score is the ratio between relative PCA3 and relative *PSA* gene expression. PSA: Prostate specific antigen; *PCA3*: Prostate cancer gene 3; RP: Radical prostatectomy; RT: Radiation therapy; NFT: No further treatment; AS: Active Surveillance; ADT: Androgen deprivation therapy.

cancer cells from urine of prostate cancer patients can be captured and used in the subsequent analysis of gene expression[87,88]. Therefore, with urine samples collected from prostate cancer patients, we were able to capture prostate cells and measure the PCA3 and PSA mRNA expression levels and to estimate the PCA3 score for these patients (Table 2). For these assays urine collections were carried out without prostatic massage. Urine samples were transferred to the laboratory on ice on the same day of collection. A total of 20-40 mL was centrifuged at 300 *g* for 5 min at 4 ℃ and the supernatant was removed. RNA was extracted from the cell pellet using an RNeasy mini kit from Qiagen and cDNA conversions were carried out using QuantiTect Reverse Transcription kit from Qiagen. Gene expression assays were carried out in triplicate using TaqMan gene expression analysis using ABI 7900 real-time polymerase chain reaction machine from Applied Biosystems; *PCA3* gene expression was assayed using primers for assay ID Hs01371939_g1; *PSA* gene expression was assayed using primers for assay ID Hs02576345_m1 and the house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was assayed using primers for assay ID Hs02758991_g1. The PCA3 and PSA mRNA expression levels presented in Table 2 are standardised to the house-keeping gene, *GAPDH*. The level of PCA3 scores recorded among our patient cohort was < 5 for all patient groups including those under active surveillance. PCA3 scores > 100 are generally considered as associated with positive biopsies when urine is collected from patients after a prostatic massage[86]. A smaller proportion of our patients who have undergone radiation therapy or prostatectomy followed by radiation therapy, showed a PCA3 score < 3. The majority of patients who have undergone prostatectomy only, also showed a PCA3 score < 3. ADT on its own or combined with other treatments has resulted in a majority of patients with a PCA3 score of zero. However, a few of the patients who have undergone ADT still expressed PSA. The majority of patients that have recorded PCA3 expression also recorded a PSA expression. However, one patient each that have undergone radiation therapy, and prostatectomy have recorded a PCA3 expression while recording no expression of PSA.

We also measured the urinary and serum MSMB levels of a limited number of prostate cancer patients who had undergone various treatments. MSMB levels were measured using Cusabio Human Beta-microseminoprotein (MSMB/PRSP) ELISA kits (CSB-E17126h)[89]. Urinary MSMB levels in those who had undergone ADT or ADT and radiation therapy showed significantly lower urinary levels compared to those undergoing active surveillance (Table 3), as expected. Serum MSMB expression was also significantly lower among those on ADTs and radiation therapy compared to those on active surveillance (Table 3). The above data indicates that treatment with ADT suppresses the urinary and serum MSMB levels as well as PCA3 mRNA expression in cells captured from urine. The reduced levels of MSMB with ADT treatment are similar to observations made by Dahlman *et al*[72] with prostate cancer tissue sections[72]. The ratio of urinary MSMB and serum MSMB was significantly higher among those who had radiation therapy compared to those on active surveillance (Table 3). It is too early to know the association between biochemical recurrence of prostate cancer of this cohort of patients with increased urinary MSMB levels or PCA3 mRNA levels or PCA3 scores. However, for urine and cells captured in urine from patients who had undergone radiation therapy and surgical treatments to present MSMB or PCA3 mRNA expression respectively, could indicate a possibility of the presence of prostate cells or prostate cancer cells that have escaped treatment. Hypoxic regions are known to resist radiation therapy treatment[90]. Hypoxic tumor regions in theory could have very limited immune response related to IgG as the acidic environment favours monomeric MSMB protein that keeps free

Table 3 Relative β-microseminoprotein levels measured from urine and serum of prostate cancer patients undergone various treatments

	Treatment type (number of patients)	Mean (range)	Estimate (95%CI)	*P*
[Urine MSMB]:[Serum MSMB]	ADT (8)	1.397 (0.163-119.5)	0.396 (0.085-1.852)	0.227
	ADT and RT (9)	4.100 (0.989-50.50)	1.162 (0.19-7.099)	0.866
	RT (5)	25.00 (10.32-45.88)	7.089 (1.16-43.322)	0.035
	AS (5)	3.529 (0.746-70.32)	1	
[Urine MSMB]	ADT (8)	1.908 (0.639-80.88)	0.172 (0.04-0.741)	0.020
	ADT and RT (9)	1.677 (0.579-35.73)	0.151 (0.027-0.839)	0.032
	RT (5)	43.12 (16.73-83.51)	3.879 (0.698-21.57)	0.116
	AS (5)	11.11 (2.186-160.3)	1	
[Serum MSMB]	ADT (8)	1.366 (0.560-25.92)	0.434 (0.145-1.301)	0.129
	ADT and RT (9)	0.409 (0.081-0.720)	0.13 (0.036-0.471)	0.003
	RT (5)	1.723 (0.730-8.093)	0.547 (0.151-1.986)	0.343
	AS (5)	3.149 (0.624-10.21)	1	

Log-transformed β-microseminoprotein measurements were used in the analysis and the results presented above were determined by using the exponential (antilog) function. Generalised linear model was carried out to investigate MSMB level variability among four different treatments. All analyses were carried out using statistical package R[99] and SAS (V9.1 SAS Institute, Cary, NC, United States). ADT: Androgen deprivation therapy; RT: Radiation therapy; AS: Active surveillance.

IgG under control. Besides, under hypoxic conditions CREB protein could be activated[37] and could bind to and cause the *MSMB* gene to produce a splice variant if the gene variant C of rs10993994 is present, while allele T will produce impaired variants. As such, a possibility exists for the utility of these markers in identifying prostate cancers that have resisted radiation therapy or possible metastasis before or after radiation therapy or surgical procedures. However, further investigations with sufficient patient numbers with prospective monitoring for biochemical recurrence is required to confirm the utility of these markers.

MSMB RELATED THERAPIES

Very little information is available in the literature on MSMB related therapies for the treatment of prostate cancer. An MSMB derived peptide, PCK3145, containing amino acids 31-45 of the MSMB molecule, has been used as a therapeutic agent on prostate cancer patients with metastatic hormone refractory prostate cancer[20]. Treatment with this peptide has shown reduction of matrix metaloprotinase-9 (MMP-9) in *in vitro* experiments[91]. This therapy has also shown reduced levels of MMP-9 in patients with higher baseline MMP-9 levels[92]. Daigneault *et al*[93] reveals the clinical utility of this peptide, the use of which can be monitored with various serum markers including levels of follicle stimulating hormone and MMP-9[93].

Moreover, MSMB or related proteins could have future directions in therapeutic use for prostate cancer. Among them is the control of the level of long splice variants in proportion to the short isoform. Tumor specific IgGs also could be considered as therapeutic targets for the control of biochemical recurrence of prostate cancer.

MSMB AND CHOLESTEROL

Several epidemiologic studies report that low levels of high-density lipoprotein levels are associated with the risk and prognostic factor of prostate cancer[94-97]. Recently, Pommier *et al*[98] have shown with mouse models that cholesterol homeostasis regulated by Liver X Receptors (LXR) are involved in maintaining MSMB levels. They have shown that in LXR knockout mice fed high cholesterol diets; there is an increased expression of the EZH2 that in turn down regulates MSMB expression levels. Further studies are needed for evaluating the link between cholesterol levels in prostate cancer patients and their association with MSMB levels.

CONCLUSION

The major properties of MSMB are related to anticancer functions and immune modulation. Anticancer properties have been mapped to amino acid region 31-45. However, in the event of prostate cancer, increased expression of a longer splice variant transcribed additionally by the 3rd exon is reported for this protein. The MSMB promoter SNP rs10993994 with the C allele polymorphism, functions as an activated CREB binding site. This could be responsible for the production of required splice variants under variable conditions ranging from normal to hypoxic and oxidative stress/hypercholesterol/tobacco smoke exposure scenarios. MSMB or PCA3 as urinary markers for detecting aggressive prostate cancers that have resisted radiation and surgical treatments, seems possible, but needs further investigation. MSMB, as a marker, will be useful if the levels are measured in surgical samples from the highest Gleason area of the tumor where the presence of MSMB is indicative of a higher tendency for biochemical recurrence. MSMB level decline below 155 units out of a maximum score of 255 units in the overall tumor is also considered as a factor predicting outcome after radical prostatectomy. Gene expression levels of MSMB in prostate cancer cells, if measured as a ratio of exon 3 region against the expression of exons 1, 2 or 4, could also be a possible approach in understanding prostate cancer severity with higher ratios indicating severe

disease. MSMB with amino acid region 31-45 has been trialled as a therapeutic against prostate cancer. Targeted therapies against long splice variants of MSMB carrying additional amino amino acids coded through exon 3 could also be useful in prostate cancer treatment.

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