18th December, 2013
The Editor,
Baishideng Publishing Group Co., Limited
Flat C, 23/F, Lucky Plaza,
315-321 Lockhart Road
Wan Chai, Hong Kong,
China

Dear Editor,

ANSWERING REVIEWERS COMMENTS-
Title: The role of β-microseminoprotein: from prostate cancer initiation to recurrence- a mini-review. (7190-review.doc)

Authors: *Nishi Karunasinghe, Karen Bishop , Pamela Murray, Yuanye Xu , Megan Goudie, Lance Ng, Shuotun Zhu, Dug Yeo Han, Lynnette R Ferguson, Jonathan Masters, Benji Benjamin and Michael Holmes*

Name of Journal: *World Journal of Clinical Urology*

ESPS Manuscript NO: 7190

We appreciate the constructive comments made by the reviewers. The manuscript has been revised and improved according to their suggestions. The details of revisions and our replies to the comments are summarized below. We have also submitted the edited document in Word format **(file name: 7190-review.doc)** with changes made to the document highlighted for easy reference.

Thank you again for considering our manuscript for publication in the *World Journal of Clinical Urology*.

Sincerely yours,

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*Reply to the review by **reviewer 02446005***

***Reviewer comment**-The title of this MS is appealing as the topic is important and maybe it is time for writing a review article. However, this MS is very confusuing as written and needs to be extensively revised before any further consideration. here are some examples (of many others) of sentences that are not clear:*

Reply-We have thoroughly gone through the manuscript and have made revisions suggested as follows.

***Reviewer comment 1.** The abstract is too long and poorly focalized.*

Reply-We have shortened the abstract as suggested.

***Reviewer comment 2.** In the introduction, it is first introduced PSP94 and then the AUthors start to talk about MSMB and the reading is not clear. Only later (page 5, MSMB variants) it becomes clear that two proteins (PSP94 and PSP57) exist.*

Reply-We have now made it clearer that PSP94 protein is also referred to as β-microseminoprotein (MSMB) and is coded by *MSMB* gene. We have referred to this protein as MSMB from page 4 onwards under the heading-

Characteristics of β-microseminoprotein

β-microseminoprotein (MSMB) coded by *MSMB* gene and is synthesized by..

***Reviewer comment 3.** page 6: the sentence: The ration of variant carrying exons 2,3, and 4.....exons 2 and 4. is not clear and should be re-written.*

Reply-We have changed the text as follows.

Ohnuma et al 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 although both variants are found in lower proportions compared to normal prostate tissue.

***Reviewer comment 4.** page 7: the sentence: Protein encoded by the MSMB gene is found to be trimethylated in histone H3 on Lys27 in androgen- refractory... is not clear at all.*

Reply-Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. H3 is the most post- translationally modified histone and histone H3 on Lys27 is a particular repressive histone mark[1]. We have now included Tsai et al reference to the text to inform more details of this histone mark. We have also changed the text to read as-

Protein encoded by the MSMB gene is found to be carrying gene repressive histone marks [1] of trimethylated histone H3 on Lys27 in androgen-refractory, but…

***Reviewer comment 5.** page 9: when talking about PAP try to be clearer. The sence is not*

clear in this paragraph

Reply-We have made a slight modification to the sentences that mentions PAP with a better readability as follows.

MSMB bound to prostatic acid phosphatase (PAP) secreted by the prostate gland has been recorded from human seminal plasma [2]. According to Kuciel et al [3] 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 [4]

Reviewer comment 6. *page 10: the paragraph on MSMB expression in advance prostate cancer is also poorly focused. I suggest to initate the paragraph first talking about the epxression of the protein, its relation with Gleason score and then about what happens to the protein after ADT.*

Reply-We have attended to the reviewer's suggestion.

Reviewer comment *-In summary in many of its parts this MS appears lika a list of previous published papers resulting quite boring. Also the concepts should be linked in some way.*

Reply-As this is a mini review paper on MSMB we had to review and present previous work to get them in to the flow of our presentation.

Reviewer comment *-In addition I think that results on prostate cancer cell lines should be discussed in a different way respect to those obtained in real tissues and the AUthors should give their own comments about these results, like for instance some insights n what should be done to further improve results obtained so far.*

Reply-We have made a comparison of our data on the gene expression (PCA3 and PCA3 score) of cells captured from urine and the urinary and serum measurements of MSMB with MSMB measurements made on tissue micro arrays by Dahlman et al [5]. The results from our studies and Dahlman et al studies follow similar trends of MSMB after androgen deprivation therapy (ADT). We have now added that fact to the text as follows.

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 is similar to observations made by Dahlman et al with prostate cancer tissue sections.

1 Tsai HC, Baylin SB. Cancer epigenetics: linking basic biology to clinical medicine. *Cell Res* 2011; **21**(3): 502-517 [PMID: 21321605 PMCID: 3193419 DOI: 10.1038/cr.2011.24]

2 Anklesaria JH, Jagtap DD, Pathak BR, Kadam KM, Joseph S, Mahale SD. Prostate Secretory Protein of 94 amino acids (PSP94) binds to prostatic acid phosphatase (PAP) in human seminal plasma. *PLoS ONE* 2013; **8**(3): e58631 [PMID: 23469287 DOI: 10.1371/journal.pone.0058631]

3 Kuciel R, Bakalova A, Mazurkiewicz A, Bilska A, W. O. Is the subunit of prostatic phosphatase active? Reversible denaturation of prostatic acid phosphatase. *Biochem Int* 1990; **22**(2): 329-334 [PMID: 2090098]

4 Wojciak P, Mazurkiewicz A, Bakalova A, Kuciel R. Equilibrium unfolding of dimeric human prostatic acid phosphatase involves an inactive monomeric intermediate. *Int J Biol Macromol*

2003; **32**(1-2): 43-54 [PMID: 12719131 DOI: 10.1016/S0141-8130(03)00024-2]
5 Dahlman A, Edsjo A, Hallden C, Persson JL, Fine SW, Lilja H, Gerald W, Bjartell A. Effect of androgen deprivation therapy on the expression of prostate cancer biomarkers MSMB and MSMB-binding protein CRISP3. *Prostate Cancer Prostatic Dis* 2010; **13**(4): 369-375 [PMID: 20680031 DOI: 10.1038/pcan.2010.25]

Reply to the review by -Reviewer 00646368

We wish to thank the reviewer for considering our manuscript as 'a timely survey of the literature on MSMB gene and its involvement in prostate cancer'. We have attended to the reviewers comments as follows.

Reviewer comment 1*. The authors abruptly introduced their own unpublished study on relative expression of PCA3 and PSA genes in cells captured from urine of patients undergone various treatments. The relevance of this discussion to the article needs to be made clearer.*

Reply-Many thanks for highlighting the above. We have changed the text to make it more obvious that we have reported our measurements of MSMB and PCA3 mRNA to show that our own data has also shown a pattern between the levels of MSMB and PCA3 mRNA, as a supplement to the report by Chen et al 2013.

Reviewer comment 2*. Although their own unpublished studies on the relative MSMB levels measured from urine and serum of prostate cancer patients undergone various treatments should be relevant and interesting to read, the author's interpretation of the reduction of MSMB with PCA3 in treated patient urines as potential escaped cells from treatment was too speculative at best. Without detailed experimental support data, this can be misleading.*

Reply-We have referred to the work carried out by Nilsson et al [1]and Dijkstra et al [2] in working with cells captured from urine of prostate cancer patients in evaluating gene expression. We have added the fact to support our procedure.

Reviewer comment 3. *Another point is that although MSMB gene can be a valid tool to be used in conjunction with PCA3 test in a urine-based detection assay, the concept of MSMB itself as a marker of prostate cancer needs to be elaborated or verified. For example, it would be more difficult to demonstrate that a reduction of marker indicates increased risk of prostate cancer than to show that an increased expression of a marker indicates increased risk of prostate cancer. The failure of detection of a marker could be simply the failure of the test.*

Reply-We agree with the referee regarding the danger of associating a reduction of a marker with prostate cancer risk. However, our work with both MSMB and PCA3 associates the presence of the marker with treatment efficacy and not the absence or lowered levels of the marker. We have included that in our text as follows.
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.

Reviewer comment 4. *Because there are a number of aspects of MSMB that are quite important for prostate cancer research, it would be good to summarize its involvement in prostate cancer in a table.*

Reply-We have now provided a summary of functions of MSMB in a new table (new Table 1). That makes it easier for a quick read of various functions of MSMB as well. We wish to thank the referee for this idea.

1 Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, Widmark A. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br J Cancer* 2009; **100**(10): 1603-1607 [PMID: 19401683 DOI: 6605058 [pii] 10.1038/sj.bjc.6605058]

2 Dijkstra S, Birker IL, Smit FP, Leyten GH, de Reijke TM, van Oort IM, Mulders PF, Jannink SA, Schalken JA. Prostate cancer biomarker profiles in urinary sediments and exosomes. *J Urol* 2013 [PMID: 24211598 DOI: 10.1016/j.juro.2013.11.001]