Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-20-1858

Comment 1: This article still suffers from grammatical inconsistency. I would highly recommend checking the text for the use of correct English grammar.

- line 62: is --> are

- line 67: remove 'been' OR change to 'have been identifying'

- Line 78-79: incorrect sentence, missing verb

- Line 80: confirmed

- Line 259-261: missing verb

- Line 262 incorrect grammer change to '...and that is involved in...'

Please make sure these and others will be checked and corrected.

Reply 1: Thank you for your careful inspection. We have revised it have modified our text as advised and revised portion are marked in yellow in the new file (see Page 3, line 62; Page 4, line 67, 77 and 79; Page 13, line 279-281, 281-282).

Changes in the text:

- line 62-63: Currently, Plasma prostate specific antigen (PSA) assays are used globally as the primary screening method for PCa (7,8).

- line 67-68: Considerable research efforts have identified markers associated with the initiation and progression of PCa (12).

- Line 76-78: In addition, prostate-specific G protein-coupled receptor (PSGR) has been identified as a novel specific gene of prostate tissue, with homology to the G protein-coupled odorant receptor gene family (18).

- Line 78-80: Highly-significant, cell-specific overexpression of this receptor has been identified in 67.2% of tumor specimens, when compared to normal tissue (19).

- Line 279-281: PSGR is a novel prostate-specific gene of the G-protein coupled OR family that maps to chromosome 11p15 (18). It is expressed as different transcripts using at least three different polyadenylation signals (25).

- Line 281-282: Xu (19) found that PSGR is overexpressed in PCa cells and suggested the involvement of PSGR in the progression of PCa.

Comment 2: In the introduction, it is stated that the main treatment for metastatic disease is surgery. While this is indeed correct for primary disease, surgery in the metastatic setting is still experimental (and only considered in the oligometastatic setting). So please remove this from the sentence. Metastatic disease is mainly treated with ADT, androgen receptor inhibitors (ARSI) or chemotherapy.

Reply 2: Thank you for your reminding. We have removed "surgery" from the sentence (see Page 3, line 62-64).

Changes in the text: The main treatments for metastatic PCa are medical castration, androgen receptor (AR) blockers, and chemotherapy (9).

Comment 3: RESULTS:

*** I struggle with understanding the word combination "exosome derived PSGR-overexpressing PC3".**

I think you mean 'exosomes derived FROM PSGR-overexpressing PC3'?

However, in figure 1 you show 'characterization of exosomes derived hFOB1.19 cells' which is confusing since I would interpret this as exosomes derived from these osteoblast cells.

Please clarify this combined term since it reads very difficult, prone to misinterpretation of the results. Also provide consistency between fig1 and the text.

Reply 3: Thank you for your reminding. We have revised "exosome derived PSGR-overexpressing PC3" to "exosomes derived from PSGR-overexpressing PC3" (see Page 10, line 212). We have also modified Figure 1 diagram notes (see Page 19, line 418-421).

Changes in the text: To identify the collected exosomes derived from PSGR -overexpressing PC3 cells, TEM analysis was used, which revealed that we had obtained particles with a complete membrane structure (Fig. 1).

Figure 1. Characterization of exosomes derived from PSGR-overexpressing PC3 cells. (A–B) Representative transmission electron microscopy images of exosomes from the PSGR-overexpressing PC3 cells (PC3 $PSGR^{+}$ exosome) group. The scale bar is 100nm (A). The scale bar is 200nm (B).

Comment 4: DISCUSSION:

- Line 231 you write 'other life activities'. This is incredible broad and could mean every single activity in the cell. I would suggest to remove this.

- Line 254: You suggest the use of tumour-derived exosomes (related to the process of bone metastatis for early diagnosis. I strongly disagree with this statement. Since you investigated paracrine effects of exomes, this implicates close contact between tumor cells and bone cells. If this is indeed the case, Pca cells already left their primary tumor (in the prostate) and one can consider this as micrometastatic disease. So I would suggest to describe it as early diagnosis of metastatic of micrometastatic disease, in which you can intensify your treatment since these patients are at an elevated risk of developing overt metastatic disease.

Reply 4: Thank you for your comment. We have removed **'other life activities'** and corrected it in the revised manuscript (see Page 12, line 246-248; Page 13, line 272-274).

Changes in the text: We revealed that DEmRNAs were mainly implicated in cellular responses to interleukin-1 (IL1), chemotaxis, inflammation, and positive regulation of angiogenesis.

Gaining insight into the mechanisms of PCa and the factors surrounding this process of bone metastasis of PCa could provide an opportunity for early diagnosis and therapeutic targeting of PCa.

Comment 5: Experiments: Although you confirmed key finding from the RNA seq with qPCR, no further exploration of the identified pathways was performed. Trying to find a true mechanism would greatly increase the impact of this article. However, if this is not possible, please formulate a (literature-based) hypothesis on the possible mechanism or how these inflammatory pathways might contribute to metastasis development. This can trigger future research.

Reply 5: Thanks for your reminding. We have revised the article based on the literature (see Page 14, line 304-306).

Changes in the text: It is suspected that DElncRNAs regulates the expression of ICAM1, RELB and IL1B in PCa cells through the MAPK pathway, and promotes the adherence of PCa cells to bone via the expression of intercellular adhesion molecules-1 (ICAM1) in osteoblasts.