University of Concepcion

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Nandita Singh
Editorial Manager
General Editorial Inquiries and Correspondence
Journal of Video Experimental (JoVe)

Dear Ms. Singh:

Please find the attached article submission entitled **"Coculture system to study osteoblast and osteoclast interactions in prostate cancer progression to hormone resistance and metastasis to bone."** This manuscript, as well as supporting figures and materials table, have been revised according to the recommendation of the editor and reviewers. In addition, the relevant protocols to be featured in the video have been highlighted in yellow.

Rebuttal and Responses to Reviewer Comments

Reviewer #1

1. We incorporated the wording recommended into the title. The new title is: **"Coculture system to study osteoblast and osteoclast interactions in prostate cancer progression to hormone resistance and metastasis to bone."**

2. "Rephrase the subtitles 1.2 (line 151) and 2.3 (line 216) specifying the cell types they have used." We agree that the cell lines do not represent osteoblast and osteoclast differentiation. As suggested, we incorporated the use of human derived primary cell culture as an alternative means to represent osteoblast and osteoclast cells. We rephrased subtitles 1.2 and 2.3 to specify cell types used.

3. "I suggest the authors to rephrase this subtitle as "Gene expression profiling." We incorporated the wording recommended for subtitle 5.3.

4. "Please clarify Fig.1 labeling as "Saos-2" and "Thp-1" as they have noted in Fig.2." We incorporated "Saos-2" and "Thp-1" into Fig 1, as recommended.

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Reviewer #2

1. We agree that bone metastasis is only present in metastatic prostate cancer patients. Therefore, we acknowledged in the long abstract and introduction that bone metastasis ***may*** be considered to be an early event in patients with colonized bone metastasis. We also agree that, although the clinical data points to the change in androgen-estrogen ratios affect estrogen receptor function, the specific experiment(s) in vivo remain to be determine, i.e. how the modification of E to T ratio change tumor cell proliferation, migration and bone metastasis using tumor cell heart injection. With regards to the

Gleason score, we had used the Gleason score described in the reference #7, but in acknowledgement of the reviewer's observation, we have changed the text to read, " disseminated cells can be detected in the bone marrow niche in lower Gleason grades of the primary tumor[7,8]" With regards to the reviewer's concern of the introduction being "over-generalized" and containing "a number of incorrect statements," and in addition to changes specified above, the supporting references #3 and 4 have been updated to support the concept that metastasis is an early event, which may be influenced by changing steroid hormone ratios (. Hu, Y., Yu, X., Xu, G., Liu, S. Metastasis: an early event in cancer progression. J Cancer Res Clin Oncol. 143 (5), 745-757 (2017); Tang L. et al. Associations between polymorphisms in genes related to estrogen metabolism and function and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Carcinogenesis. 39 (2), 125-133 (2018)).

2. "The methods outlined are extremely detailed and are written in the text of a protocol rather than an outline of what has been performed:" The format used for the protocol described in the manuscript was in compliance with the requirements of JoVE.

3. "There are a number of spelling errors in the text including "cytometry", misspelled as "cytometry" and on line 191, it should read, "RPMI/DMEM". We corrected "cytometry," and added "RPMI/DMEM" as recommended.

Please contact me if any additional changes are required.

Sincerely,

Sergio A. Onate, Ph.D.