Author’s response to reviews

Title: CAISMOV24, a new human low-grade serous ovarian carcinoma cell line.

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Author’s response to reviews:

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Original article title: “Usefulness of copy number variation (CNV) to compare a new epithelial ovarian carcinoma cell line to its original serous ovarian carcinoma”.

New article title: "CAISMOV24, a new human low-grade serous ovarian carcinoma cell line".

We thank the reviewers for their thoughtful review of our manuscript. We understand this takes time to review manuscripts thoroughly and we appreciate the reviewers’ insightful comments for improvements. We have addressed the reviewers’ comments below, and felt that this process has significantly improved our manuscript.

Reviewer reports: Kwong-Kwok Wong, Ph.D. (Reviewer 1):

This study reported the characterization of a newly established low-grade ovarian serous carcinoma cell lines from ascites of a 60-year-old patient. Cytogenetic and comparative
hybridization using the Affymetric Cytoscan HD platform microarray were performed. The cell line appeared to have a slow doubling time of ~71 hours, and expressed TGF-beta1, CD73, CA125 and HE4. The total number of CNVs has slightly increased from 28 to 37 from primary malignant cells to established cell line CAISMOV24. Extensive genomic alterations were also detected in chromosomes 2,3,5,7, 8, 12, 13,14, 19, and X.

The use of CNV is one of the established techniques to compare original carcinoma and established cell lines or PDX models. Thus, the use of CNV in this study is not novel. However, since a limited number of established low-grade ovarian serous carcinoma cell lines is available, a newly established low-grade ovarian serous carcinoma cell line (CAISOMV24) will be desirable.

Reviewer 1 comment 1: Additional characterization of this cell line will be important for investigators in the field. Exome sequencing and tumorigencity of the cell line in vivo are important basic characterization.

Response to comment 1: Indeed, as mentioned by the reviewer, additional characterization of CAISMOV24 will allow a wider usage of this cell line. Right now, we are putting efforts on the characterization of several parameters related to the in vitro tridimensional growth of CAISMOV24. We are also sharing the cell line with other collaborator researchers for evaluation of CAISMOV24 cell line in animal models for ovarian cancer studies. However, we were able to include in the new version of our manuscript additional genetic characterization of CAISMOV24 cell line, which support its low-grade serous type.

Reviewer 1 comment 2: It is not very clear if the cell line was derived from ascites of post-treatment patients or before treatment.

Response to comment 2: Yes, the cell line was derived from epithelial ovarian cancer cells present in ascites of a post-treatment patient. We have modified the text, item “Patient and ascites sampling” in Methods section, to make it clearer. The new text is highlighted in yellow and mentioned bellow:

“Relapse occurred one year later, when ascites fluid originally used to initiate the in vitro culture of EOC cells was collected by sterile aspiration at the time of a laparotomy, in May 2011”.

Reviewer 1 comment 3: An H&E of the original tumor showing low-grade histology will strength the authenticity that the cell line is derived from a low-grade serous carcinoma.
Response to comment 3: As recommended, a new figure, showing an H&E staining of the original tumor was included in the manuscript.

Kylie Louise Gorringe (Reviewer 2):

The authors describe a new cancer cell line derived from a low-grade serous ovarian carcinoma (LGSC). Similar cell lines are only rarely available in the literature, so description of any novel cell line of this subtype are welcome. The authors should state whether the cell line is available to be used by other researchers, as it would be a valuable resource for in vitro studies of LGSC.

However, the description requires some modification:

We thank the reviewer for your thoughtful review of our manuscript. Regarding the availability of CAISMMOV24, we are currently contacting Rio de Janeiro Cell Bank BCRJ (Banco de células do Rio de Janeiro, bcrj.org.br/) and ATCC - The Global Bioresource Center to make CAISMMOV24 available for other researchers.

Reviewer 2 comment 1. Pathological description. The authors should include an H&E image of the original tumour to show the features that have classified this case as LGSC.

Response to comment 1: As recommended, a new figure, showing an H&E staining of the original tumor was included in the manuscript.

Reviewer 2 comment 2. Mutations are also important in LGSC, and while an exome analysis would be excellent, at the very least sequencing should be done for KRAS (codons 12/13 and 61) and TP53 (at least exons 4-9), with BRAF (V600E) and NRAS (codon 61) assessed if the case turns out to be KRAS wild-type. TP53 mutation screening is essential to ensure that this case is not high-grade serous. Lack of a CN change over TP53 is not necessarily indicative of lack of mutation.

Response to comment 2: We agree that mutation screening of specific genes add important information to the histopathological result to define a serous carcinoma as high- or low-grade. We also agree that lack of a copy number change over TP53 is not necessarily indicative of lack of mutation. As recommended, we were able to include in the new version of our manuscript additional genetic characterization of CAISMMOV24 cell line. Thus, CAISMMOV24 cell line was screened for typical mutations found in serous ovarian carcinoma. Additionally, TruSight RNA Pan-Cancer Panel (Illumina, Inc., USA) was employed for transcriptome analysis of 1,385 genes.
and 21,043 exons regions implicated in hotspot cancer pathways. As a result CAISMOV24 cell line was characterized as a low-grade serous carcinoma, since the cell line harbored KRAS mutation with wild TP53. The sections “Methods”, “Results” and “Discussion” of the manuscript were modified to include new findings, and all text changes was labeled in yellow.

Reviewer 2 comment 3. The authors conflate copy number change with mutation - the existence of COSMIC mutated genes in copy number regions is of little relevance as these are distinct processes. It would be better to compare the copy number profiles to other LGSC in the literature. In addition, referring to other "type I" profiles is inappropriate since endometrioid/clear cell/mucinous histotypes have a different etiology to LGSC and this subtype is underrepresented in COSMIC and other databases. Instead, the authors should read and reference recent exome and other molecular analyses of LGSC (Jones et al, J Pathol. 2012 Feb; 226(3): 413-420. Hunter et al., Oncotarget. 2015 Nov 10;6(35):37663-77, Boyd et al., Gynecol Oncol. 2013 Sep;130(3):560-4, Emmanuel et al., Clin Cancer Res. 2014 Dec 15;20(24):6618-30., McIntyre et al Histopathology. 2017 Feb;70(3):347-358) for a more appropriate point of comparison. Discuss how the current cell line fits with the molecular profiles (including copy number) of true LGSC.

Response to comment 3: We apologize for our text in “Discussion” section, which linked, erroneously, copy number alterations with gene mutations; we agree that these are distinct processes. We just wanted to mention that our findings support what was previously suggested by Huang et al [2012], that CNV profiles can allow a better characterization among different histotypes of epithelial ovarian cancer, since they could harbor histotype-specific gene mutations. The text was altered (highlighted in yellow). In addition, references to other “type I” ovarian cancer histotypes were replaced by some of the references indicated by the reviewer.

Reviewer 2 comment 4. The authors should also put their cell line in context of other LGSC cell lines e.g. Fernandez at al., Am J Cancer Res. 2016 Oct 1;6(10):2235-2251.

Response to comment 4: As recommended the reference by Fernandez at al. 2016 was included in “Discussion” section of the new version of our manuscript.

Reviewer 2 comment 5. The authors should report STR profiles of the cell line to ensure it is unique (compare to DSMZ STR profile website database https://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.ht).

Response to comment 5: As recommended, STR profile of CAISMOV24 was determined and compared to that STR profiles available at DSMZ database. Table 1 A and B summarizes the
main findings regarding STR analysis. Comparisons of STR profiles between CAISMOV24 and other 3,274 human cell lines did not match EV values greater than 0.9, confirming the uniqueness of CAISMOV24 cell line.

Reviewer 2 comment 6. Discuss in more detail why TGF-b1 and CD73 are expressed in the cell line when not in the primary. Why would these get re-expressed in culture? Is this a common event in cell line development?

Response to comment 6: In fact, this is an interesting question. We are trying to approach this issue in new ascites samples that we have collected currently. Up- and down-regulation of surface markers such as EpCAM, PVR, CD44 and CD24 on epithelial ovarian carcinoma cells has been described in literature more often. However, to our knowledge, alterations of expression of TGF-β1 and CD73 on these cells are not well explored.

Reviewer 2 comment 7. Table 1. Please do not use a decimal point "." to separate thousands from hundreds, either leave a space or put a comma. I interpret 242.771 to be 242 kb, not 242 Mb. Rather than the cytoband start, please include the full cytoband interval, and/or the basepair start and stop points (which would be more accurate).

Response to comment 7: Regarding using decimal point in the CNVs size, you are right in your interpretation. To correct this in the manuscript, we have changed the points “.” to commas “,” in table 1. We agree that showing the genome coordinate for the CNVs it is a very important point. To address this in the manuscript, we have included in the Table 1 a column informing the cytoband interval for each CNV and a note in the footer of Table 1 stating that these coordinates are referring to assembly human genome hg19 assemble.

Reviewer 2 comment 8. Figure 1C, what are the shaded areas? There are no units shown on the graph. In the results, stating a proliferation index of 3.94 (no units given) is meaningless to me - is this fast? Slow? How does it compare to other cell lines? To other passages?

Response to comment 8: As mentioned in the manuscript, cell division in cultures was assessed by a method based on detection of a proliferation tracker dye (VPD450) through flow cytometry. As viable cells divide, the VPD450 dye is distributed uniformly between daughter cells, i.e. to each of the new cell generations, retaining approximately half of the VPD450 fluorescence intensity of its parent cell. The shaded areas in figure 1C represent each of these cell generations, and they resulted from analysis using FlowJo Proliferation Platform. Proliferation index was calculated as total number of divisions divided by the number of cells that went into division. The proliferation index only takes into account the cells that underwent at least one division. In
the case of CAISMOV24 cells, most of the cells (or even 100%) underwent at least one cell division. Thus, the mean proliferation index of CAISMOV24 cells resulted in 3.94±0.94 times. CAISMOV24 cell line has a slow doubling time, which has been very regular along of the whole period it has being maintained in our laboratory.

Reviewer 2 comment 10. In Figure 3, remove the methods from the figure legend. The figure is low resolution with text near the arrows unreadable. Recommend replacing these with arrows &/or arrowheads and placing the text for what they are in the legend.

Response to comment 10: The figure was modified as recommended. Now is figure 4.

Reviewer 2 comment 11. More explanation of Figure 3 is needed - what are the black and grey lines? Are either of these representative of LOH? Because I can't see in this figure the regions of copy number neutral LOH listed in table 1 (chr 16, chr2)

Response to comment 11: The black and grey lines were produced by the staining technique for condensed chromosomes Giemsa-banding or G-banding, widely used in cytogenetics to produce visible karyotypes. They are not representative of LOH, since in the context of our array comparative genomic hybridization, LOH does not represent genomic structural loss. We must apologize for not making our LOH findings more clear. Searches of the genomic copy number can identify LOH due to hemizygous deletions. However, LOH can also occur independently of copy number change, where one chromosome or chromosomal region has been duplicated and its homologue has been deleted. In the cases where LOH occurs without copy change, it is usually termed copy neutral LOH. We have failed to explain adequately that. Once there weren’t copy number change in the LOHs (chr 16 and chr2) you will not be able to identify these events through the karyotype, just through the genotyping. To clarify this, we have changed the term “LOH” to “Copy neutral-LOH” (cnLOH). Beside, we have added as supplementary data the genotyping of those cnLOH segments.