Author's response to reviews

Title: Influence of Monolayer, Spheroid, and Tumor Growth Conditions on Chromosome 3 Gene Expression in Tumorigenic Epithelial Ovarian Cancer Cell Lines.

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Author's response to reviews: see over
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Dr. Melissa Norton,
Editor-in-Chief, BioMed Central
BMC Medical Genomics

Dear Editor,

We have submitted electronically the second revision of the manuscript entitled “Influence of Monolayer, Spheroid, and Tumor Growth Conditions on Chromosome 3 Gene Expression in Tumorigenic Epithelial Ovarian Cancer Cell Lines” by Neal Cody, Magdalena Zietarska, Ali Filali-Mouhim, Diane M. Provencher, Anne-Marie Mes-Masson, Patricia N. Tonin for consideration in the journal BMC Medical Genomics. [This manuscript was original submitted to BMC Cancer and then upon suggestion by the editors transferred to BMC Medical Genomics during the review process.]

This manuscript was initially reviewed by four reviewers and has been returned for a second review as one of the reviewers, Dr. Wei Hu still expresses concerns about the manuscript. In the first review Dr. Hu questioned our use of two of the three ovarian cell lines, OV-90 and TOV-21G, in our analysis as they exhibit deletions at chromosome 3p24. We responded as follows to this review:

This reviewer questioned why we used OV-90 and TOV-21G cell lines in this study as both cell lines “exhibit deletions at chromosome 3p24”. All of the cell lines used in this study were generated in our research group and we have studied them extensively for over 10 years. While OV-90 exhibits loss of heterozygosity of 3p arm, TOV-21G does not. We are not aware of the deletions specifically occurring at 3p24 in either of these cells lines. Notable is that whole genome SNP analysis does not support this statement (unpublished data). Noteworthy is that the loss of heterozygosity of 3p in OV-90 does not significantly impact on the global patterns of gene expression of this chromosome as assayed by Affymetrix expression microarray as demonstrated by our group in Manderson EN, et al., 2002. Expression profiles of 290 ESTs mapped to chromosome 3 in human epithelial ovarian cancer cell lines using DNA expression oligonucleotide microarrays. Genome Res 12:112-121. However, it is not clear to us why this is important for this study. We are applying Affymetrix expression analyses as a ‘tool’ to assay whether or not growth conditions significantly influence transcriptome. The focus on chromosome 3 is largely because our interest in this chromosome and its genes in ovarian cancer. In addition, the main focus is on the magnitude and extent of transcriptional differences and as such comparative analyses are made with transcriptomes generated with each alternative growth condition for each EOC cell line. However, we have pointed out in the revised manuscript the unique genetic attribute of the EOC cell lines to emphasize this point.

In the second review, Dr. Wei has now suggests that we should test the influence of various growth conditions with more ovarian cancer cell lines since the three cell lines used in the study represent three different histological ovarian cancer subtypes. The rationale for testing more cell lines because the ones we used are different histopathological subtypes is not clear to us. Testing more cell lines will not alter the outcome of the present study for the cell lines tested. The fact
that our results show that modest differences in gene expression were observed in the study of three vastly different cell lines would suggest that gross differences in gene expression do not occur as a result of alternative growth conditions, which leaves open the opportunity to study a large number of genes using ovarian cell line model systems regardless of histopathological subtype. Once again we iterate that we have used whole genome transcriptome analyses to assay for the variability of gene expression in cell lines grown in very different in vitro and in vivo contexts as tool to study such variation and in the present study used as an example chromosome 3 as focal point of discussion. The cell lines under study were derived from different ovarian cancers and exhibit very different molecular genetic properties, all of which are discussed at length in the Discussion section of the manuscript. To extend this study to other ovarian cancer cell lines, would be interesting and important to investigators using these other cell lines in their molecular genetic studies, but which ones? Not all ovarian cancer cell lines exhibit in vitro (3-D spheroid formation) and in vivo (xenografts at subcutaneous and intraperitoneal formation with ascites in some cases) tumorigenic properties that are amenable to such studies.

With the reference to ATCC, Dr. Wei has now clarified for us as to why he thinks that OV-90 and TOV-21G exhibit a deletion specifically involving chromosome 3p24. Drs. Anne-Marie Mes-Masson and Diane Provencher, both co-authors of this manuscript developed all of the ovarian cancer cell lines used in the present study. In collaboration with them, we have extensively studied these cell lines and incorporated them into numerous studies which are also cited in the present manuscript. The initial study describing their development and in vitro and in vivo tumorigenic properties was published in Provencher DM, Lounis H, Champoux L, Tetrault M, Manderson EN, Wang JC, Eydoux P, Savoie R, Tonin PN, Mes-Masson AM: Characterization of four novel epithelial ovarian cancer cell lines. In Vitro Cell Dev Biol Anim 2000, 36(6):357-361. This manuscript also included a karyotype analysis of the cell lines. Specifically, OV-90 and TOV-112D exhibited complex karyotypic anomalies consistent with those typically seen in the majority of epithelial ovarian cancers, whereas TOV-21G exhibited an atypical diploid karyotype with trisomy 10 as the only gross abnormality (as also described in Wang J-C, Mes-Masson A-M, Tonin PN, Provencher D, Eydoux P. 2000. Trisomy of chromosome 10 in two cases of ovarian epithelial carcinoma. Cancer Genet Cytogenet 118: 65-68). Karyotype analyses demonstrated evidence of an unique chromosome 3 abnormality in OV-90 comprised of a chromosome 22 derived homogeneously staining region replacing the 3p arm but not affecting the 3q arm, where the chromosome 22 amplicon was investigated in Arcand SL, Mes-Masson AM, Provencher D, Hudson TJ, Tonin PN: Gene expression microarray analysis and genome databases facilitate the characterization of a chromosome 22 derived homogeneously staining region. Mol Carcinog 2004, 41(1):17-38. OV-90 has also emerged as an interesting in vitro model with the potential for identifying and testing chromosome 3 tumor suppressor genes because of extensive loss of heterozygosity of the 3p arm (Lounis H, Mes-Masson AM, Dion F, Bradley WE, Seymour RJ, Provencher D, Tonin PN: Mapping of chromosome 3p deletions in human epithelial ovarian tumors. Oncogene 1998, 17(18):2359-2365), and the recent demonstration of suppression of tumorigenicity in chromosome 3 fragment transfer experiments attributable to functional complementation of 3p genes (Cody NA, Ouellet V, Manderson EN, Quinn MC, Filali-Mouhim A, Tellis P, Zietarska M, Provencher DM, Mes-Masson AM, Chevrette M et al: Transfer of chromosome 3 fragments suppresses tumorigenicity of an ovarian cancer cell line monoallelic for chromosome 3p. Oncogene 2007, 26(4):618-632.). The ovarian cancer cell line TOV-21G has

To clarify the molecular genetic properties of these cell lines, including their chromosome 3 content, we have included all of the above information in the Background section of the manuscript and added a separate section in the Materials and Methods section describing the origin of the cell lines. This will complement the description provided in the Discussion section which was added in the previous revision.

As for the ATCC annotation, Dr. Mes-Masson who is the depositor of these ovarian cancer cell lines in this repository is in communication with ATCC to rectify the discrepancy noted in the karyotypes of OV-90 and TOV-21G appearing on their website.

The second point made by this reviewer is that there may be discrepancy between mRNA expression and protein function and further validation study is needed for supporting the conclusion. Once again we reiterate that we agree with this statement as made in the initial response as shown below:

We agree with this reviewer that further work is required to determine how differences at the transcriptional level translate at the protein level. It is not feasible for us at the present time to address this question using a comparable large-scale proteomics approach. We agree that future studies of specific genes could be addressed in this way. Indeed the application of 3D spheroid models has identified a gene modified by microenvironment using Affymetrix GeneChip analyses, which was then validated by immunohistochemistry as published by our group in Zietarska M, et al., 2007. Molecular description of a 3D in vitro model for the study of epithelial ovarian cancer (EOC). Mol Carcinog 46(10):872-85. We have clarified this in our Discussion section.

To this response, we again reiterate that purpose of this present study was to use Affymetrix technology to address the extent and magnitude of differences occurring as a result of growth conditions as applied to a cell line model for ovarian cancer. If overall there were significant differences in the transcriptome, then it would be very difficult to use cell line as models to study molecular pathways, particularly involving studies which initially address changes in gene transcription. On the other hand as differences in genes expression at the mRNA level are expected, the fact that the number of differences are small suggest that one could study some genes in all growth conditions without concern for significant alterations in gene expression profiles. We do agree that such studies can be conducted on a per gene basis should a reliable antibody be available for a specific gene under investigation and that this should be incorporated into studies involving in vitro models, as numerous studies (from our own group and others) have done. However, we are not aware of any feasible large scale proteomic analyses involving immunohistochemistry based approaches that could be applied to properly address this question.
in the manner in which we have done with the transcriptome analyses employing Affymetrix GeneChips. Our conclusions are based only on global transcriptome analyses. However, we have modified the conclusion in the Abstract section to specifically emphasize that the chromosome 3 transcriptome appears to be modestly influenced by growth conditions of ovarian cancer cell line models least any reader misinterpret our conclusions from our transcriptome analyses. This conclusion complements the Discussion section and Conclusion which were already modified in the first revision of the manuscript and which have discussed the merits of further analyses involving proteomic studies.

Additional changes:

We have clarified the figure legend for Figure 3 as suggested by reviewer, Dr. Jennifer Byrne.

The reviewer Dr. Julie A DeLoia has accepted the changes to first revision, and reviewer, Dr. Eliana Bignotti’s discretionary changes from the initial review have already been incorporated into the first revision.

In addition, we have added that the expression microarray data will be deposited in Gene Expression Omnibus.

We hope that we have satisfactorily addressed the reviewer’s comments and that this manuscript is found suitable for publication in BMC Medical Genomics.

Respectfully submitted by,

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