Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-940

Reviewer A

In this study, the author's goal was to evaluate differentially expresses mRNAs and miRNAs to develop a network associated with exosomes in ovarian cancer. Overall, this was an interesting manuscript that relates to an important topic, the carcinogenesis of ovarian cancer.

General

Comment 1: While I appreciate the effort and contribution made by the authors after thoroughly reviewing the manuscript my major comment regards the interpretation of the results pertaining to FYCO1. While the findings pertaining to FYCO1 are intriguing and provide the rationale for further studies into the role of FYCO1 in the pathogenesis of ovarian cancer, I have some reservations about the strength of the interpretations given the available evidence. It appears that the conclusions drawn may be somewhat overstated, considering the limitations and scope of the study. Examples of the statements that I am referring to include "FYCO1 may hold promise as a therapeutic strategy for treating ovarian cancer" in the introduction and "Therefore, FYCO1 may be a reliable diagnostic gene 294 for predicting ovarian carcinogenesis, while its role in prognostic survival remains to be investigated." To improve the overall rigor and credibility of your work, I would suggest adopting a more cautious tone in discussing the implications of the results. This could involve acknowledging the potential uncertainties and limitations inherent in the study design, sample size, or methodology. Additionally, it may be helpful to consider alternative explanations or factors that could influence the observed outcomes.

Reply 1: We appreciate the reviewer's thoughtful and constructive feedback regarding the interpretation of our results, particularly in relation to FYCO1. We acknowledge the importance of maintaining a balanced and cautious tone when discussing the implications of our findings. We have revised the manuscript to reflect a more measured approach in our statements about FYCO1 (see page 4, highlight box and page 15, line 283-284). We recognize the limitations of this study, including the acquisition and validation of clinical samples, and discuss these potential uncertainties in the Discussion section. We understand the need to consider alternative factors that could influence our results and have addressed this in our revised manuscript. We believe these changes enhance the rigor and credibility of our work.

Other specific comments are included below.

Introduction

Comment 2: Authors state that "with the widespread use of oral contraceptives and HPV vaccination, the incidence of OV has declined, 55 but it remains the seventh most common cancer in women worldwide". HPV vaccination does not reduce OV risk, please remove this statement, or provide supporting evidence that there is a strong association between HPV vaccination and OV risk.

Reply 2: We appreciate the reviewer's comment and have re-evaluated the statement in question. Upon further examination, we find that there is no strong evidence to support a direct association between HPV vaccination and a reduced risk of ovarian cancer. As a result, we have removed the statement from the manuscript to ensure accuracy and clarity. (see Page 4, line 54-55)

Methods

Comment 3: Please provide further rationale for the target genes selected.

Reply 3: Thank you for your comment. Firstly, these target genes were obtained by using public databases, specifically, the miRDB, miRTarBase, and TargetScan databases. They were computationally generated and not manually selected by us. Then, through the construction of the exosomal miRNA-mRNA network, we identified FYCO1 and PURA as two target genes. In subsequent validation, we found that FYCO1 may have diagnostic and prognostic value in OV.

Discussion

Comment 4: Individuals at risk for ovarian cancer include anyone with ovaries. Please consider updating the terminology of "OV is a significant threat to the health of middle-aged and older women and is the leading cause of death among gynecological tumors" to something more inclusive of the true population at risk.

Reply 4: We appreciate the reviewer's input and recognize the importance of inclusive terminology. The description has been revised to "Ovarian cancer is more prevalent in middle-aged and older women. It poses a significant threat to the health of this demographic and is the leading cause of death among gynecological tumors" (see Page 15, line 273-274).

Comment 5: Overall, the first paragraph of the discussion could likely be shortened to reduce the text and make the aim of the paper clearer.

Reply 5: We have carefully considered your suggestion and made revisions to the first paragraph of the discussion to improve clarity and conciseness (see Page 15, line 273-284).

Comment 6: Please restructure to discuss specifically what results are contradictory "However, we found that high expression of the FYCO1 gene may lead to lower OS in OV, 293 which seems to contradict the results of Fig. 5."
Reply 6: Thank you for your valuable feedback. We have restructured the description you mentioned. More information and detailed explanation were added to the discussion (see page 16, line 291-297). We appreciate your guidance in improving the clarity of our paper.

Reviewer B
The article "Construction of an exosome-associated miRNA-mRNA regulatory network and validates FYCO1 and miR-17-5p as potential biomarkers associated with ovarian cancer", by Li Chen et al., explored the key association between differentially expressed microRNAs from ovarian cancer patients' exosomes and their mRNAs targets. The dual approach proposed by the Authors is based on i) in silico study through bioinformatical methods, as a first step and ii) wet, in vitro analysis, with an experimental evidence provided regarding the inhibition by miR-17-5p of its target gene FYCO1.
The Authors reach their stated goal of constructing a miRNA-mRNA network associated with exosomes in ovarian cancer, with experimental validation of one key target gene.
Of note, a good use of the bioinformatical software currently available and the promising results concerning miR-17-5p make this article interesting and worth publishing. New ideas and opportunities for ovarian cancer diagnosis and treatment may come from this work. However, some major and minor revisions need to be pointed out.
This Reviewer recommends resubmission with revisions, as listed below.

Comments to the authors:
Major comments:
Comment 1: Title: the title is not clear and probably needs to be corrected. Should it be as follows? Construction of an exosome-associated miRNA-mRNA regulatory network and validates validation of FYCO1 and miR-17-5p as potential biomarkers associated with ovarian cancer.
Reply 1: Thank you for your comments. We have modified our article title as you advised (see Page 1, line 2-3).

Comment 2: Section "Introduction", line 111, sentence: "In this paper, we speculate that miRNAs carried by exosomes secreted from OV tissues may

regulate the expression of other mRNAs in OV tissues and thus participate in the development of oogenesis". -Here, the term "oogenesis" causes some confusion, as the article is mainly focused on ovarian cancer. Are the authors interested in finding a relation between oogenesis and ovarian cancer (i.e. incessant ovulation theory)? If so, they need to tell more and expand the concept.
Reply 2: We have confirmed "Oogenesis" here was an editing error. Now, it has been corrected it to "OV." (see Page 7, line 114)

Comment 3: Section "Methods", line 121, paragraph "Data resources and preprocessing": Is the research on GEOPROFILE made with additional filters/criteria other than "ovarian cancer" and "exosomes"? Are exosomes from biofluids excluded? Authors need to clarify.
Reply 3: We conducted our search using only two key terms, "ovarian cancer" and "exosomes," without applying any additional filters or criteria. In our description, we have specified that our samples were all solid tissue samples, primarily sourced from OV cell lines, normal human ovarian surface epithelial cell lines, OV surface epithelium, ovarian surface epithelium, OV tissues, and normal peritoneal tissues. Biofluid samples were not included in this study. We have added this clarification to address this question (see Page 8, line 127).

Comment 4: Section "Methods", line 179, paragraph "Luciferase reporter assay": the Authors should specify the level of cell confluency at co-transfection time. In addition, this paragraph should provide more information or references concerning plasmid construction. Specifically, does miR-17-5p have just one target binding sites on 3'UTR (the one reported in fig. 7A)? Is it predicted or was it validated?
Reply 4: Thank you for your valuable feedback. In our experiments, cells were co-transfected with re-constructed plasmids when they reached approximately 80% confluency which has been specified now in our revised manuscript (see Page 10, line 184-185). The plasmid construction was referred to two reference which has been added in our manuscript (see Page 11, line 188-189). Actually, miR-17-5p has two target binding sites on 3'UTR, the other target binding site positioned at 3619-3626 of FYCO1 3' UTR. The binding site of miR-17-5p was validated instead of predicted.

Comment 5: Section "Results", line 208, paragraph "Identification of DEMs and DEGs": How data were normalized? Which method has been applied?
Reply 5: The data were normalized using the *DESeq2 R* package. We have made the necessary modifications and removed the corresponding description from

section "Results," paragraph "Identification of DEMs and DEGs (see Page 12 line 213)," and moved it to the section "Method," paragraph "Screening of DEMs and DEGs."(see Page 8, line 143,)

Comment 6: Figure 4 (B): The KEGG analysis results should be presented with another type of graph or with a table. The present graph really does not make it easy to connect genes and pathways.
Reply 6: Thank you for your suggestion. In fact, during the KEGG analysis of DEGs, we not only generated the circos plot (as shown in Figure 4B) but also created bar plots and bubble plots for KEGG analysis. However, the latter two do not provide details on gene-pathway connections, and the information displayed is not as rich as in the circos plot. When submitting our manuscript, we provided high-resolution vector graphics. By zooming in on the images, you can read the detailed information within the figure, making the connections between genes and pathways clearer. We hope this response meets your requirements for Figure 4B.

Comment 7: Figure 5 (B): Are the red and grey boxplots referred to ovarian cancer patients and healthy subjects? The name of Groups is missing.
Reply 7: Yes, the red and grey boxplots refer to ovarian cancer patients and healthy subjects, respectively. We have now added the group names in Figure 5B. Please find the newly uploaded Figure 5 in the submission system (named as “Figure 5_Revised”).

Minor comments
Comment 8: Through the Abstract, there is an occasional use OV for ovarian cancer. The Authors should be consistent in the use of the abbreviation, which we suggest be OC and not OV.
Reply 8: Thank you for your advice. We have replaced all instances of "OV" with "OC" in the manuscript.

Comment 9: Section “Methods”, line 185: it should be reporter gene (instead of report gene).
Reply 9: We have modified the text as advised (see Page 11, line 189).

Comment 10: Section “Methods”, line 192: it should be containing (instead of consisting of).
Reply 10: We have modified the text as advised (see Page 11, line 196).

Comment 11: Section “Discussion”, line 280: exosomes
Reply 11: We have modified the word as advised (see Page 15, line 278).

Comment 12: Section “Discussion”, line 280-283: the sentence is not clear, please rephrase.
Reply 12: We have rephrased the sentence as suggested (see Page 15, line 279-283).

Comment 13: Legend of Figure 1, sentence: “Flow chart of the analysis performed in this
study. The RNAs were analysed to obtain DEMs and DEGs along the colon cancer progression.” Is this about colon cancer or ovarian cancer?
Reply 13: It was our editing mistake. This is about ovarian cancer. We have revised the related description in the legend of Figure 1 and reviewed our manuscript to prevent similar errors (see Page 27, line 632).

Comment 14: Figures 2-3, for the sake of clarity, Y axes should have the same scale for Volplot of DEGs.
Reply 14: Thank you for your suggestion. The Y-axes in Figure 2A and 3A are auto-generated to ensure a clearer distribution of data points in the figures, avoiding excessive dispersion or concentration. Because samples in different datasets may vary in their source, type, and time of detection, it can lead to differences in absolute values, but it does not affect the relative differences in DEMs and DEGs results. Therefore, we believe it is not necessary to set the Y-axis ranges consistently. We hope this explanation satisfies your inquiry.

Comment 15: Some abbreviations are not explained, as an example: “NC” for negative control.
Reply 15: We have added the explanation of "NC" in our manuscript (see Page 10, line 188, and Page 28, line 664). In addition, we have reviewed all abbreviations in the manuscript and provided comprehensive explanations. (see Page 10, line 188. Page 28, line 655).