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## Peer Review File

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### Reviewer A

The prognosis of esophageal squamous cell carcinoma (ESCC) is improved by neoadjuvant chemoradiotherapy (nCRT), especially for patients with pathologic complete response (pCR). Despite the efforts to predict treatment response using multimodality, no molecule has proven to be a strong biomarker. In the manuscript “Use of exosome transcriptome-based analysis to identify novel biomarkers in patients with locally advanced esophageal squamous cell carcinoma undergoing neoadjuvant chemoradiotherapy”, authors profiled the expression of exosome transcriptome that could predict pCR in ESCC before and after nCRT.

Couple questions are required to be answered before it will be accepted.

(1) Why to focus on the exosome from blood, not the other-derived exosome in the study?

Please state in the introduction.

Reply: The exosomes secreted by tumor cells consist of many molecules closely related to the characteristics of tumor. Analysis of exosomes is benefit to the diagnose of tumor and predict the prognosis of tumor. Previous studies have shown that the exosomes secreted by tumors can enter into circulatory system and be detected in the blood. Therefore, separating the exosomes in the blood could realize the non-invasive way to identify the characteristics of tumors at an early stage and then to find potential therapeutic targets. However, the exosomes in the tumor microenvironment need to obtain tumor samples to detect, which has no advantage over conventional pathological diagnosis.

Changes in the text: see page 4, line 92-98.

(2) In the introduction, it was proposed to add related reference (DOI: 10.21037/atm-21-4198) about the functions of exosome.

Reply: We added the ref. according to your suggestion.

Changes in the text: see page 4, line 95.

(3) What were the roles of neoadjuvant chemoradiotherapy in esophageal squamous cell carcinoma? Please state in the introduction.

Reply: Neoadjuvant chemoradiotherapy (CRT) followed by surgery is the recommended treatment for locally advanced esophageal cancer by the NCCN guideline, which is associated with prolonged overall survival compared to surgery alone. We stated it in the introduction.

Changes in the text: see page 3, line 77-80.

(4) Why to divide into 3 groups? Please state in the methods.

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Reply: Previous study showed that roughly 40% of patients with ESCC have a pathologically complete response (pCR) in the resection specimen after nCRT. For these patients surgical resection might not be necessary. Hence, an active surveillance strategy has been proposed in which patients will undergo frequent clinical response evaluations. We conducted a second response to increase the diagnostic accuracy, which results to the third group.

Changes in the text: see page 5, line 128-133.

It was better to provide representative images of isolated exosome.

Reply: The following figure 1 is the representative image of TEM; and figure 2 is the representative image of protein analysis.

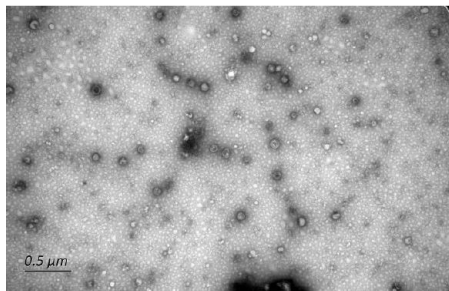


figure 1

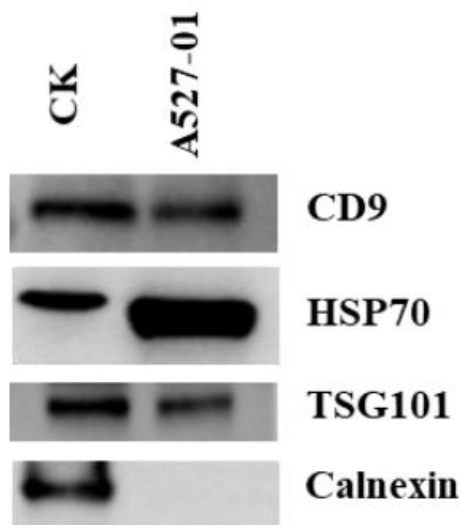


figure 2

(5) It was better to validate the representative genes by experiments.

Reply: Thank you for your comments. This is very important to confirm the results founded in our study. Therefore, we will conduct the next research plan to validate the representative genes by *in vitro* and *in vivo* experiments.

Changes in the text: see page 12, line 367-369.

(6) What will be the next research plan following the study? Please state in the discussion.

Reply: The next research plan is to validate the representative genes by *in vitro* and *in vivo*

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experiments. We added it in the discussion.

Changes in the text: see page 12, line 367-369.

## **Reviewer B**

### **1. Reporting Checklist**

The article follows the MDAR checklist for reporting standards. Please revise your manuscript according to the attached checklist.

**Reply: We revised it.**

### **2. Statistical Analysis**

Original Article should include a part with a subtitle named “Statistical Analysis” in “Method” section. Or declare in the point-by-point response that it is not applicable, and explain the reason.

Reply: This present study is a description of the differentially expressed genes related to exosomes, not involving statistical analysis for significance. Therefore, no Statistical Analysis was presented in the article.

### **3. Ethical Statement**

**For research involving human**, the article must include

1) a statement that ethical approval was obtained (or a statement that it was not required and why), including the name of the ethics committee(s) or institutional review board(s), the number/ID of the approval(s),

Reply: This study was approved by the institution review committee of Shanghai Chest Hospital (No. KS2160). We have presented it in the Methods.

2) and a statement that the participants gave informed consent before taking part (or a statement that it was not required and why).

Reply: This has been presented in the Methods section and the part of “Ethical Statement” in the section of Footnote.

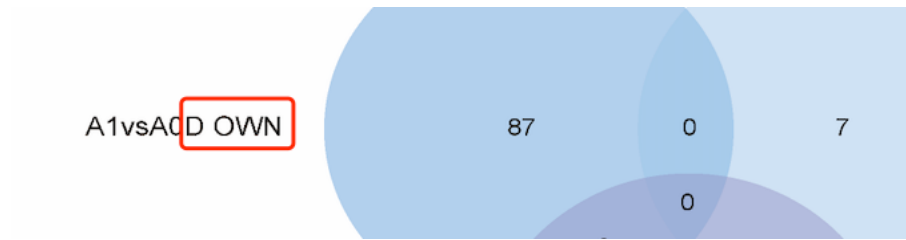
3) Authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013), available at:  
<https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf>

Reply: Yes, we revised it.

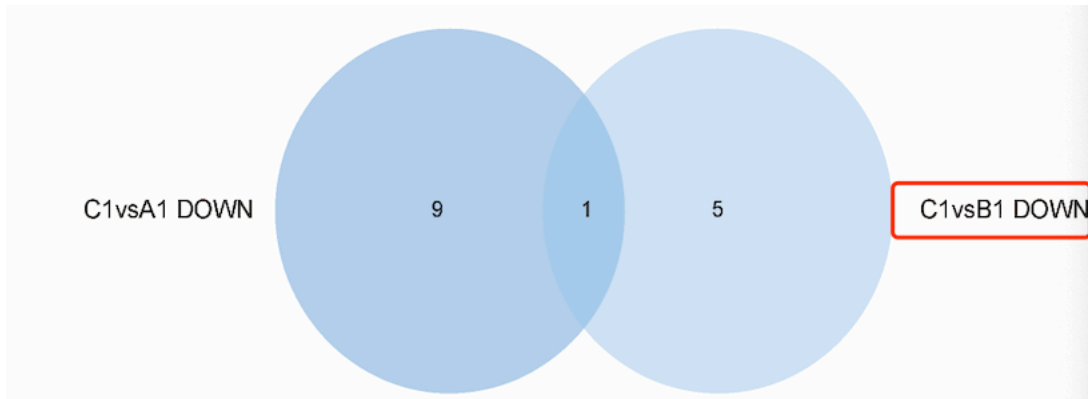
### **4. Figure**

#### **Figure 5**

a) In 5B, please revise “D OWN” to “DOWN”.



b) Please check if the figure matches with the legend.

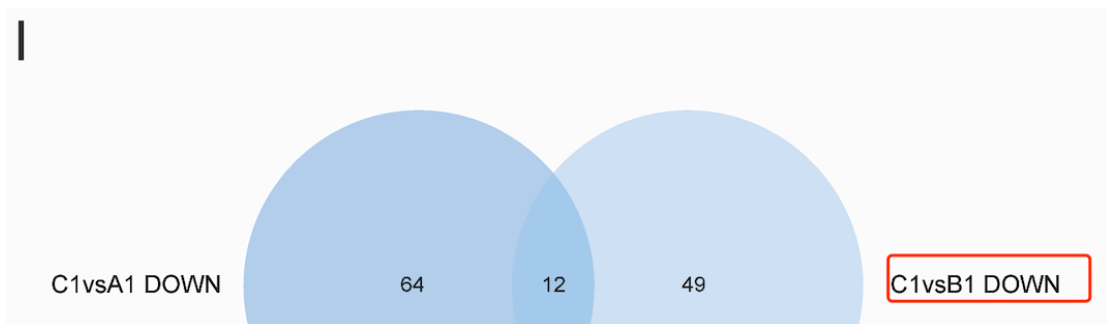


the C1-A1 upregulation and C1-B1 upregulation sets; Lower: The intersection of the C1-A1 downregulation and C1-B1 upregulation sets. (H) The key miRNA-mRNA

Reply: We revised it. Please see the attached files.

**Figure 6I**

Please check if the figure matches with the legend.



sets. (H) The volcano plots of DE lncRNAs in the comparison of C1 vs. B1. (I) The intersection of the C1-A1 downregulation and C1-B1 upregulation sets.

Reply: We revised it.