Peer Review File

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Review Comments-reviewer A

Tumor-derived exosomes are involved in the process of tumor metastasis and angiogenesis. MicroRNAs (miRNAs) are the most widely investigated factors in exosomes. In the manuscript "Exosomal miR-93-5p as an important driver of bladder cancer progression", authors successfully revealed the biological role and mechanism of bladder cancer (BLCA)-derived exosomes in tumor progression.

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

(1) What were the characters of tumor-derived exosomes? Please state in the introduction.
(2) In the introduction, it was proposed to add related reference (DOI: 10.21037/atm-20-6456) about the roles of exosome derived miRNAs.
(3) What were the roles of tumor-derived exosmes in the tumor microenvironment? Please state in the introduction.
(4) It was better to list all sequences of used primers and antibodies in a table.
(5) Why to choose female mice? And state clearly the body-weight of mice in the methods.
(6) How to determine the dose of exosomes per mouse? Please state in the methods.
(7) It was better to further test the target gene by rescue experiment.
(8) In the ARRIVE checklist, please supplement the line number.

Comment 1: What were the characters of tumor-derived exosomes? Please state in the introduction.

Reply 1: Cancer cells generally produce more exosomes than normal cell. Importantly, the proportion of cancer suppressor genes and oncogenes in tumor-derived exosomes is out of balance.

Changes in the text: line 93-97

Comment 2: In the introduction, it was proposed to add related reference (DOI: 10.21037/atm-20-6456) about the roles of exosome derived miRNAs.

Reply 2: we added this reference in the paper

Changes in the text: line101-103

Comment 3: What were the roles of tumor-derived exosmes in the tumor microenvironment? Please state in the introduction.

Reply 3: tumor-derived exosmes promotes the growth and metastasis of tumors and inhibit the immune response of the body.

Changes in the text: line 90-97

Comment 4: It was better to list all sequences of used primers and antibodies in a table.

Reply 4: We have listed the used primers in **table1**. There are few antibodies, and the expression in the text is very clear. We do not think it is necessary to relist them.

Comment 5: Why to choose female mice? And state clearly the body-weight of mice in the methods.

Reply 5: Female mice are quiet and easy to manage. And our requirements for hormone levels are not as strict as those for prostate cancer. The body-weight of mice (6–8 weeks) was 18-20g in our research.

Changes in the text: Establishment of tumor xenografts in mice /line 233

Comment 6: How to determine the dose of exosomes per mouse? Please state in the methods.

Reply 6: Based on previous research results (Gupta D, Zickler AM, El Andaloussi S. Dosing extracellular vesicles. Adv Drug Deliv Rev. 2021 Nov;178:113961), we designed the corresponding dose gradient and obtained the maximum injection dose, at which the mice can live normally.

Changes in the text: line 241-243

Comment 7: It was better to further test the target gene by rescue experiment.

Reply 7: We have carried out rescue experiment, see Figure 5(J-L) for details.

Comment 7: In the ARRIVE checklist, please supplement the line number..

Reply 7: We uploaded and modified the checklist as required.

Review Comments-reviewer B

1. **Main text should be structured as #Introduction, #Methods, #Results, #Discussion and #Conclusions; please add "#Conclusions" accordingly.**

Answer: The **conclusions** section is added at the end of the text.

2. **Highlight Box**

In the second item of the highlight box, only 2 points should be indicated: what is known and what is new, but you have 4 points. Please revise.

64 oncogene.

65 **What is konwn and what is new?**

66 The relation of miR-93-5p to exosomes in BLCA is unclear.

67 Our study revealed that exosomal miR-93-5p was a predictive index for the diagnosis

68 of BLCA.

73 In *vitro* experiments showed that miR-93-5p can not only accelerate the proliferation

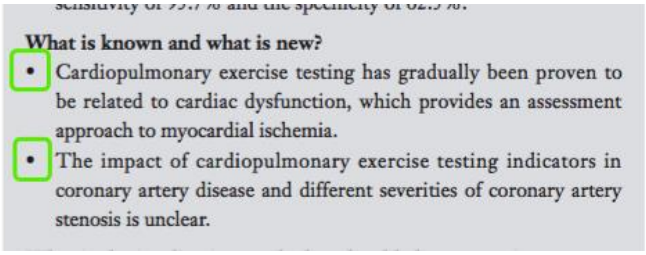
74 and migration of BLCA, but can also promote the angiogenesis of BLCA, and these

75 findings were confirmed by in vivo experiments.

76 Mechanically, phosphatase and tensin homolog (PTEN) may be a vital downstream

77 effector of exosomal miR-93-5p.

Here is an example for your information:



What is known and what is new?

- Cardiopulmonary exercise testing has gradually been proven to be related to cardiac dysfunction, which provides an assessment approach to myocardial ischemia.
- The impact of cardiopulmonary exercise testing indicators in coronary artery disease and different severities of coronary artery stenosis is unclear.

Answer: We have revised this part as required.

3. Ethical Statement

a) For research involving human, please also provide a statement that the participants gave informed consent before taking part (or a statement that it was not required and why).

- *Suggested wording*: *"The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by ethical committee of the Second Affiliated Hospital of Soochow University (ethical code: JD-LK-2019-104-01), and informed consent was taken from all the patients."*

Answer: We have revised this part as required.

b) Please provide the ethical approval ID. If there's no approval number or the number is same as the one of human research "(ethical code: JD-LK-2019-104-01)", please provide the ethical approval file (with affiliation seal) for backup.

"*Animal experiments was approved by ethical committee of the Second Affiliated Hospital of Soochow University (No. ******), in compliance with national or institutional guidelines for the care and use of animals.*"

Answer: We have revised this part as required. And we provide the ethical approval file (with affiliation seal) for you as required.

1) This statement is not needed. We helped to delete it, please confirm.

252 Animal Center of Soochow University, and they were fed in a specific-pathogen-free

253 animal facility. All of the experimental procedures were performed in accordance with

254 protocols approved by the Institutional Animal Care and Research Advisory Committee

255 of Soochow University. According to the minimum number requirements of previous

256 animal experiments, the number of animals = the number of groups + 10. The mice

Answer: There is no problem deleting it

4. **ALL abbreviations used in each table/figure or table/figure description should be defined in a footnote below the corresponding table/figure. Please check carefully and revise.**

Such as: TCGA, BLCA, RPM, …….

Here is an example for your information:

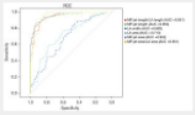


Figure 5 Performances of six indexes generated by the AI segmentation model in classification of moderate-severe *vs.* non-moderate-severe in MR patients based on ROC curves. AI, artificial intelligence; MR, mitral regurgitation; ROC, receiver operating characteristic.

Answer: When abbreviations in the notes appear for the first time, we have marked them with full names, and the unmarked ones such as TCGA, KEGG and GEO have been added to the corresponding notes as required.

5. **Table 2**

It seems that the P value is in the wrong box. Please check and revise.

Table 2 Relationship between miR-93-5p expression and clinicopathologic factors in 25 patients

Parameters	N	Relative miR-93-5p expression	P value
Age (years)			
<60	10	0.0003481±7.043e-005	0.641
>60	15	0.0003042±5.936e-005	
Gender			
Male	23	0.0003243±4.822e-005	0.849
Female	2	0.0002919±9.9e-005	

Answer: We have revised this part as required.

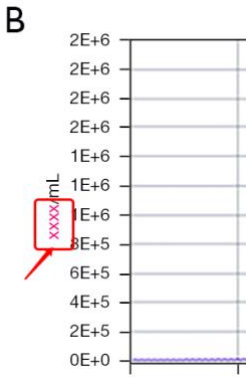
6. **Figure 1**

Please indicate the meaning of *** in figure legend.

Answer: *** means “$p < 0.001$”, and we have added it in this part.

7. **Figure 2**

1) Please indicate the meaning of *** in figure legend.
2) Please check here.



3) Please provide the scale bar in figure 2F or magnification in figure legend 2F.

Answer: 1） *** means "p < 0.001", and we have added it in this part. 2） we revised it in figure 2B. 3) We have provided the scale bar in figure 2F.

8. Figure 3

1) Please indicate the meaning of *, **, *** in figure legend.

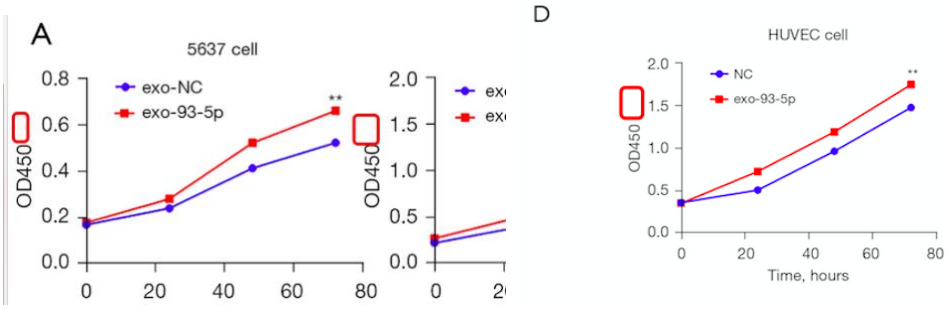
Answer: *p < 0.05, **p < 0.01, and ***p < 0.001. We have added it in this part.

2) Figure 1 is a (A-D) combined picture, no 1F. Please check and revise.

660 Figure 3 The role of BLCA exosomes in cancer cells and angiogenesis. T24 cells, 5637
661 cells, and HUVECs were incubated with the exosomes above (Figure 1F) exosomes
662 transfected with miR-93-5p (exo-93-5p) and exosomes transfected with negative

Answer: This should be Figure 2E, which we have corrected it in the text.

3) figure 3A and 3D: please confirm if here should be "OD 450nm".



Answer: Yes, we have corrected it in the fig3.

4) Please indicate the stanning method of figure 3B, 3C, 3D and 3F figure legends.

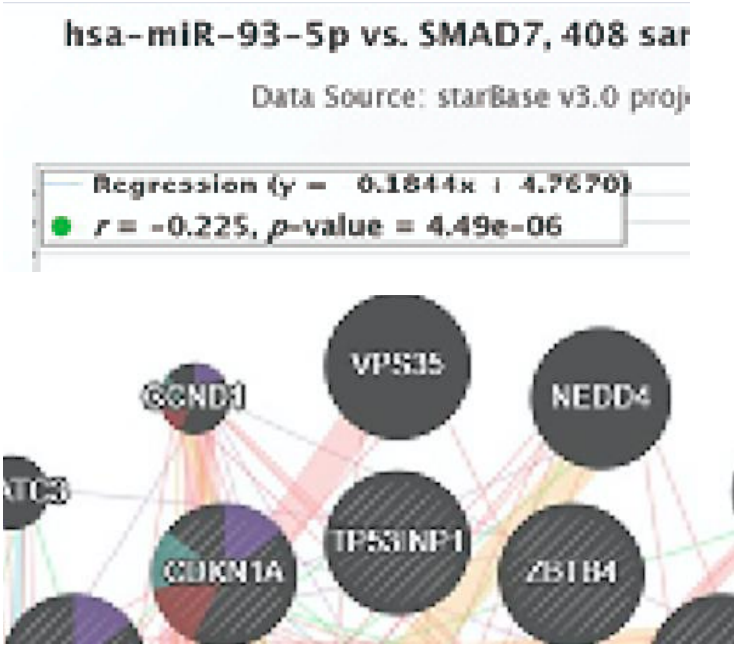
Answer: Fix with methanol for 30min, dye with 0.1% crystal violet solution for 30min, wash with PBS for three times to remove excess dye solution, and dry at room temperature (These contents have been mentioned in the materials and methods section).

5) Please provide the scale bar in figure 3C, 3D, 3F and 3G or magnification in figure legends.

Answer: the magnification of these figure was 100X, which have been added in figure legends.

9. Figure 4B and figure 5A are not clear enough for publication. Please provide a clear

version to us in jpg or tiff format.



Answer: We have revised the two figures.

10. Figure 5

1) Please indicate the meaning of *** in figure legend.

Answer: ***p < 0.001. We have added it in this part.

2) Figure 1 is a (A-D) combined picture, no 1F. Please check and revise.

716 Figure 5 Exosomal miR-93-5p targeted PTEN directly in HUVECs and BLCA cells.
717 (A) Analysis of the function of 18 strongly related genes in GeneMANIA. (B) The
718 expression of PTEN in HUVECs was determined by qRT-PCR, after transfected with
719 the exosomes above (Figure 1F), exosomes transfected with miR-93-5p (exo-93-5p)
720 and exosomes transfected with negative control (exo-NC). (C) The expression of PTEN

Answer: This should be Figure 2E, which we have corrected it in the text.

3) Please indicate the staining method for figure 5J in figure legend.

Answer: Fix with methanol for 30min, dye with 0.1% crystal violet solution for 30min, wash with PBS for three times to remove excess dye solution, and dry at room temperature (These contents have been mentioned in the materials and methods section).

4) Please provide the scale bar in figure 5J or indicate the magnification in figure legend.

Answer: the magnification of these figure was 100X, which have been added in figure legends.

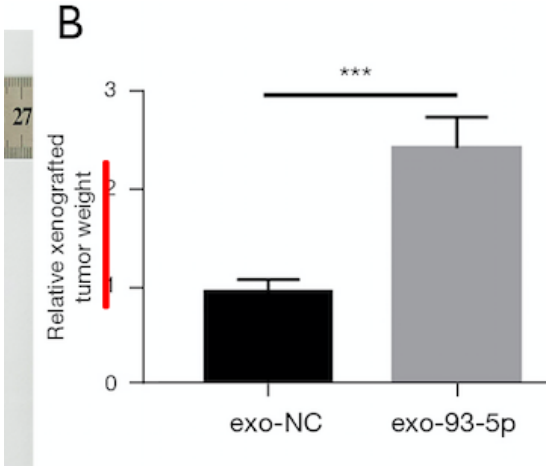
11. Figure 6

1) Please indicate the meaning of "**, ***" in figure legend.

Answer: **p<0.01 and ***p < 0.001. We have added it in this part.

2) Please confirm if a unit is needed for "tumor weight".

Answer: This is the ratio of tumor weight to the control group. So, it has no unit.



3) Please also draw scale bars: 50 μm in figure 6D.

here is an example for your information:

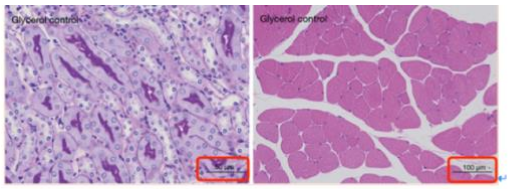


Figure 4 Pathological features of kidney and muscle. Two study groups: (I) Saline Control group (n=8); and (II) Glycerol Control group (n=8). Kidney sections were stained with PAS (50 μm). Muscle sections were stained with H&E (100 μm). PAS, periodic acid-Schiff; H&E, hematoxylin and eosin.

Answer: We have provided the scale bar in figure 6F.

12. Since only one study was cited here, "A study has" would be appropriate.

302 tissues was significantly different. Studies by Armstrong et al. (16) have shown that
303 among these miRNAs, miR-93-5p is expressed significantly differently BLCA
304 exosomes, which may be an important factor in the exertion of BLCA exosomes (Figure

Answer: we have corrected it in the text.

13. ARRIVE reporting checklist

Some items only indicated Section or just Line Number. Please indicate the both detailed Section and Line Number (e.g., Introduction/Line 1-10).

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	Materials and methods
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	line130-137£¬233-235
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	line237-239
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	line238
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly.	line233
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	no exclusions
		c. For each analysis, report the exact value of *n* in each experimental group.	Materials and methods

Here is an example for your information:

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Methods/Line136-137
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods/Line137

Answer: we revised **checklist** as required.