Peer Review File

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Reviewer A

The paper titled "Tumor-derived exosomal miR-103a-3p promotes vascular permeability and proliferation by targeting ZO-1 and ACOX-1 in nasopharyngeal carcinoma" is interesting. The data demonstrate that exosomal miR-103a-3p can facilitate the development of NPC by regulating the crosstalk between NPC cells and HUVECs. Exosomal miR-103a-3p could potentially serve as a therapeutic target for NPC. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: Nasopharyngeal carcinoma-related exosomes may also induce epithelial-mesenchymal transition, thus promoting tumor metastasis and chemoradioresistance. What role does miR-103a-3p play in this process? It is recommended to add relevant content.

Reply 1: Thanks for the reviewer's suggestions very much. Considering the reviewer's suggestion, we have performed additional experiments to further explored the effect of exosomal miR-103a-3p in the progression of EMT and re-written this part.

Changes in the text:

Abstract. Page 2 Line 40. ", and the epithelial-mesenchymal transition (EMT) progression" was added.

Results. Page 14 Line 260-269. "Tumor cell migration is often associated with the occurrence of the epithelial-mesenchymal transition (EMT) (28). Then the effect of exosomal miR-103a-3p on EMT of NPC cells was further explored. We found that the EMT construction was verified by the observation of EMT-like cell morphology (a spindle-shaped and fibroblast-like morphology) after co-cultured with miR-103a-3p overexpressed exosomes (Supplementary Figure 1A). The Western blot assay results showed that miR-103a-3p overexpressed exosomes increased the expression levels of N-cadherin and Vimentin proteins, while decreased the expression level of E-cadherin protein in CNE2 cells, whereas miR-103a-3p down-regulated exosomes led to the opposite results (Supplementary Figure 1B). Cellular immunofluorescence data also confirmed the result above (Supplementary Figure 1C). These findings further approve the stimulation of exosomal miR-103a-3p on NCP cells metastasis by EMT." was added.

Discussion. Page 21 Line 394-395. ", and EMT progression (Supplementary Figure 1A-C)" was added.

Figure. Supplementary Figure 1 was added.

Figure legend. Page 30 Line 636-642. "**Supplementary Figure 1. The effect of exosomal miR-103a-3p on cell morphology and the expression of EMT-related proteins.** (A) The morphological change of CNE2 cells after different exosomes treatment by an inverted microscope. Scale bar: 100 μm. (B) Western blots of the epithelial marker E-cadherin, and the mesenchymal markers (N-cadherin, Vimentin) in CNE2 cells with exosomes treatment. (C) Cellular immunofluorescence staining showed that exosomal miR-103a-3p affected cellular expression of EMT markers (Green: E-cadherin; Blue: hoechst)." was added.

Comment 2: The description of some methods in this study is too simplistic, please describe in detail.

Reply 2: Thanks for the reviewer's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: The most part of "Methods" had been modified as suggested **(see Page 8-11 Line 140-206).**

Comment 3: It is recommended to specify the number of patients in the method, which may make the entire research method more rigorous and comprehensive.

Reply 3: Thanks for the reviewer's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: The number of patients had been added as suggested **(see Page 7 Line 115-118):** "Tissues samples from pathologically confirmed 7 cases of NPC and 8 cases of normal donors were collected at the Affiliated Hospital of Nantong University following ethics committee approval (IRB number: 2018-L052). Serum samples from NPC patients (n=5) and normal donors (n=5) above were further used."

Comment 4: It is recommended to increase the expression and clinical application of exosomal microRNA in NPC in the discussion.

Reply 4: Thanks for the reviewer's suggestions very much. We have re-written those parts according to the reviewer's suggestion.

Changes in the text: "The expression and clinical application of exosomal microRNA in NPC" had been added in the discussion as suggested in:

Page 20 Line 370-377: "Exosomes are prevalent in many bodily fluids, including as breast milk, urine, saliva, blood, and amniotic fluid. As a result, exosomes can also be used as a non-invasive or minimally invasive method for the early identification of a variety of diseases. Furthermore, miRNAs packed to exosomes should be protected and more stable in bodily fluids than naked RNA, which is more likely to breakdown. Exosomes from patients with NPC have been found to have dysregulated miRNA expressions which control the expression of genes involved in cell division, differentiation, and stress response. It has been shown that exosomes released by NPC cells in vivo include loaded EBV-miR-BARTs that are stable enough to spread from tumor tissue into the peripheral circulation. Exosomes are prevalent in many bodily fluids, including as breast milk, urine, saliva, blood, and amniotic fluid. As a result, exosomes can also be used as a non-invasive or minimally invasive method for the early identification of a variety of diseases. Furthermore, miRNAs packed to exosomes should be protected and more stable in bodily fluids than naked RNA, which is more likely to breakdown. Exosomes from patients with NPC have been found to have dysregulated miRNA expressions which control the expression of genes involved in cell division, differentiation, and stress response. It has been shown that exosomes released by NPC cells in vivo include loaded EBV-miR-BARTs that are stable enough to spread from tumor tissue into the peripheral circulation."

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Mesenchymal stem cell-derived exosomes regulate the

polarization and inflammatory response of macrophages via miR-21-5p to promote repair after myocardial reperfusion injury, PMID: 34532460", "CircHIPK2 promotes proliferation of nasopharyngeal carcinoma by down-regulating HIPK2, PMID: 35966290". It is recommended to quote the articles.

Reply 5: Thanks for the reviewer's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: According to your suggestions, we have re-written those parts **(see Page 4 Line 58);** We cited the articles mentioned above **(see Page 4 Line 59 as reference 4 and Page 5 Line 65-66, Line 67-69 as reference 8).** We have rewritten the introduction section based on the reviewer's suggestions.

Comment 6: What are the relevant characteristics of the tumor microenvironment of NPC? What is the correlation between exosomal miR-103a-3p and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply 6: Thanks for the reviewer's suggestions very much. According to your suggestions, we have re-written the discussion.

Changes in the text: "The relevant characteristics of the tumor microenvironment of NPC" was added in:

Page 18-19 Line 347-352: "It is becoming more obvious that the tumor microenvironment (TME) is important for driving treatment resistance, metastasis, and tumor growth. The complex network of tumor-associated cells and noncellular elements that makes up the TME of NPC promotes the growth of tumors. Exosomes secreted by stromal cells as well as NPC cells are the key mediators of cell-to-cell communication in the tumor microenvironment, which facilitate the NPC aggravation. They are regarded as one of the most important mechanisms for determining the various TME features."

Page 21 Line 410-411: "Metabolic reprogramming of tumor cells is emerging as a critical feature of the tumor microenvironment influencing tumor growth, metastasis, and response to therapy."

"The correlation between miR-103a-3p and the tumor microenvironment" was added **(see Page 20 Line 377-385):** "As an oncomiR, miR-103a-3p has been previously reported overexpressed in exosomes multiple cancers, including liver cancer, thyroid cancer, lung cancer, colorectal cancer, cervical cancer, glioblastoma. The levels of serum exosome-derived miR-103a-3p, and miR-4505 were significantly different among patients with multiple myeloma (MM). Exosomal miR-103a-3p derived from cancer-associated fibroblast (CAF) promoted non-small cell lung cancer (NSCLC) cells cisplatin resistance by suppressing apoptosis. This study also discovered a sizable increase of miR-103a-3p in NPC tissues and exosomes generated from NPC cells (Figure 1E-J). We then proposed that exosomal miR-103a3p could be used as a prognostic marker and a potential therapeutic target."

"The possible goals of future drug development" was added **(see Page 19 Line 356-369):** "As exosomes containing nucleic acid medicines have low immunogenicity and good biocompatibility, exosomes have entered the new industrial therapeutic frontiers as both drug delivery systems and intrinsic therapeutic agents. Because endogenous RNase prevents

miRNAs from being degraded, miRNAs in exosomes are more stable. Exosomes containing miRNAs therefore hold significant promise for the clinical therapy of many types of cancer as NPC. Exosomes have been loaded with let-7a miRNA to target EGFR-expressing xenograft breast cancer tissue and inhibit tumor development in vivo. AntagomiR-BART10-5p and antagomiR-18a, two iRGD-tagged exosomal antagomiRs, had a significant anti-angiogenesis and anti-tumor therapeutic impact on NPC when administered in vivo. Currently, more than 20 miRNA-based medicines are undergoing clinical trials. While no miRNA drug has been approved thus far, biotechnology companies such as Regulus Therapeutics, Synlogic, and Miragen are making progress toward developing miRNA-related drugs. The limited targeting ability of exosome-based tumor medication delivery systems severely limits their real applicability. Exosomes are still in their early stages of clinical application; more research will help identify time- and money-efficient nanotechnologies for exosome synthesis on a big scale."

Reviewer B

1. Please provide the full name of "mRNA" "ZO-1" "ATP" "FAO" "SFM" "EGF" "RPMI" "DMEM" … in the main text. Please **check through your article** to make sure **all the abbreviated terms** have been defined when they **FIRST** appear in the Abstract and the main text.

Reply 5: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had provided the full names of the abbreviated terms as advised in the in the Abstract and the main text.

2. Please double check the full name of "HNSC", "RT-qPCR" in the manuscript.

neck squamous cell carcinoma (HNSC) cohort analysis revealed that miR-103a-3p was highly increased.

quantitative reverse transcription polymerase chain reaction (RT-qPCR) analyses of fresh NPC tissues

Reply 6: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had changed to the correct abbreviation in Page 13 Line 229: head and neck squamous cell carcinoma (HNSC); Page 13 Line 234: Reverse transcription quantitative polymerase chain reaction (RT-qPCR).

3. Please check if any reference should be added since you mention "studies".

"Clinical studies have shown that nearly all NPC patients in advanced stages have invasive growth at the base of the skull and that around 70% of patients with NPC develop metastasis to the neck lymph nodes -0(30)."

Reply 7: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had added two new references as advised: "Jiang D,Li Y,Cao J, et al. Cell Division Cycle-Associated Genes Are Potential Immune Regulators in Nasopharyngeal

Carcinoma. Front Oncol. 2022;12:779175." "Du M,Hu X,Jiang X, et al. LncRNA EPB41L4A-AS2 represses Nasopharyngeal Carcinoma Metastasis by binding to YBX1 in the Nucleus and Sponging MiR-107 in the Cytoplasm. Int J Biol Sci. 2021;17 (8):1963-1978." in Page 19 Line 355.

4. Figures

- **All abbreviations** in figures and legends should be explained. "NPC" "qRT-PCR" "CM" "RPM" "HR" in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

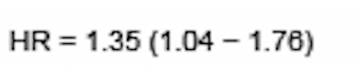
Changes in the text: We had provided the full names of the "NPC" "qRT-PCR" "CM" "RPM" "HR" abbreviated terms as advised in the in the Figure 1 legend in Page 28 Line 591, 594, 597-599, 604: nasopharyngeal carcinoma (NPC); Reads per million mapped reads (RPM); Hazard Ratio (HR): the ratio of two risk rates. HR=1.35(1.04-1.76) (HR>1), it means that the experimental group (study subjects) is a risk factor relative to the control group. P=0.024. (E-F) Reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay; conditioned medium (CM).

- Please indicate the meaning of "****" in Figure 1 legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the meaning of "****" in Figure 1 legend as advised in Page 28 Line 605: "****: $P < 0.001$; ***: $0.001 < P < 0.01$;".

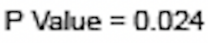
- It is suggested to indicate how data are presented in Figure 1D.



Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the meaning of Hazard Ratio (HR) in Page 28 Line 597-599: "the ratio of two risk rates. HR-1.35(1.04-1.76) (HR>1), it means that the experimental group (study subjects) is a risk factor relative to the control group."

- Please revise the P value in Figure 1D. [If P value≥0.01, report the specific P value to 2 decimal places, e.g., "P=0.01" "P=0.06" "P=0.10" "P=0.90".]

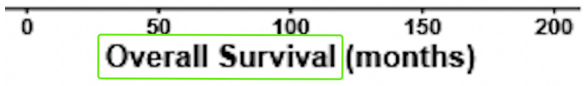


Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had revised the P value in Figure 1D as advised in Page 28 Line

599: “*P*=0.026.”

- Please double check the x-axis in Figure 1D.



Reply: Thanks for the editor’s suggestions very much and we confirmed that the x-axis in Figure 1D was correct.

- Please indicate the magnification in Figure 1G legend.

Reply: Thanks for the editor’s suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the magnification in Figure 1G legend in Page 28 Line 602: at ×25.0 K magnification.

- Figure 1G and 1H are not clear enough.

Reply: Thanks for the editor’s suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had uploaded clear images again Figure 1G and 1H.

- Please double check the word in Figure 1I-J legend.

(I-J) qRT -qPCR analysis of the miR-103a-3p expression levels in exosomes which

Reply: Thanks for the editor’s suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the word in Figure 1I-J legend in Page 28 Line 603: RT-qPCR.

- Figures should be cited **consecutively** in the text and numbered in the order in which they are discussed. Therefore, Figure 1B should be cited before Figure 1C, unless Figure 1 is cited as a whole before. Please check through and revise.

Reply: Thanks for the editor’s suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the text and cited consecutively in the text and numbered in the order in Page 13 Line 228-231: “Based on either unpaired (Figure 1A) or paired (Figure 1B) analysis, the Cancer Genome Atlas (TCGA) head and neck squamous cell carcinoma (HNSC) cohort analysis revealed that miR-103a-3p was highly increased. Violin plot also showed miR-103a-3p level was significantly higher in HNSC (Figure

1C)".

- Please double check Figure 2B-C legend.

transfected CNE2 cells. (B-C) qRT-PCR analysis for exosomal miR-103a-3p in cells transfected with miR-103a-3p mimic (B) and inhibitor (C). (D) qRT-PCR analysis for miR-103a-3p expression in CNE2

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the text in Page 28 Line 609-610: "(B-C) RT-qPCR analysis of miR-103a-3p expression levels in transfected CNE2 cells (B) and exosomes (C)."

- Please indicate the staining method in Figure 2E legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the text in Page 29 Line 612: "Crystal violet staining of transwell assays and statistical analysis of CNE2 cells treated with exosomes with different miR-103a-3p level."

- Please check the meaning of "***" in Figure 2 legend.

, ***, $P< 0.00$.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the meaning of "***" in Figure 2 legend in Page 29 Line 618: "***, $P< 0.001$".

- Please indicate the meaning of "ns" in Figure 3 legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the meaning of "ns" in Figure 3 legend in Page 29 Line 627: "ns.: non-significant."

- Please indicate the staining method and magnification in Figure 3B, 3D legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it. We have consulted a large number of literature and experimental guidelines and found that cloning experiments mainly rely on observing the formation of clones with

the naked eye, without a clear magnification factor. We had just indicated the staining method in Figure 3B legend. We indicated the staining method and magnification in Figure 3D legend.

Changes in the text: We had modified the text as advised in Page 29 Line 621-622, 623-624: "(B-C) Colony formation capacities in exosomes-cultured CNE2 cells were assessed by crystal violet staining quantification". "(D) Representative fluorescence images of EdU staining, nuclei: Hoechst (blue), and proliferating cell: EdU + (red). Magnification = ×20."

- Please indicate the meaning of "ns" in Figure 4 legend.

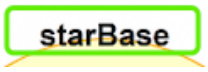
Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the meaning of "ns" in Figure 4 legend in Page 30 Line 646: "ns.: non-significant."

- Please unify the database in Figure 4A, its legend and the text.

(A) miRDB, miRTarBase and TargetScan were used to estimate the potential targets of miR-103a-3p.

"Using TargetScan, starBase and miRanda databases, putative targets of miR-103a-3p were identified (Figure 4A)."

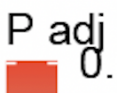


Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had unified the database in Figure 4A in Page 16 Line 297: "Using miRDB, StarBase and TargetScan databases, putative targets of miR-103a-3p were identified (Figure 4A)"; We had unified the database in Figure 4A legend in Page 30 Line 630: "(A) miRDB, StarBase and TargetScan were used to estimate the potential targets of miR-103a-3p.".

- Please unify the description in Figure 4B and its legend.

"The color of the dots corresponded to the range of the q-value"



Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

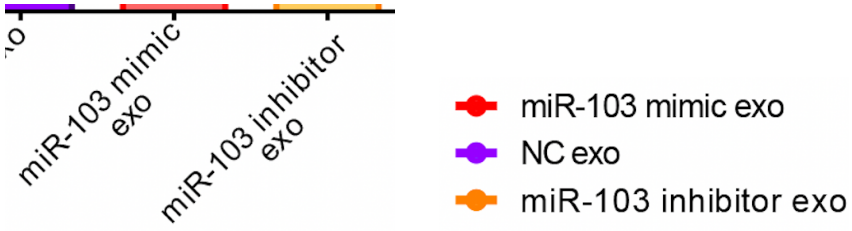
Changes in the text: We had unified the description in Figure 4B and its legend in Page 16 Line 302-303, in Page 30 Line 631-632.: "The color of the dots corresponded to the range of the q-value".

- Please add description for the y-axis in Figure 4C.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had added description for the y-axis in Figure 4C. "The number of genes".

- Please double check the description in the x-axis of Figure 4E and the labels in Figure 4H, whether "miR-103" should be "miR-103a-3p".



Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had modified the x-axis of Figure 4E and the labels in Figure 4H.

- Please add description for the x-axis in Figure 4H.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had added description for the x-axis of Figure 4H.

- Please indicate the meaning of the arrows in Figure 4I legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the meaning of the arrows in Figure 4I legend in Page 30 Line 640: "Arrow indicated the integrity damage of tight connection".

- Please check whether "RNA" should be "mRNA" in the y-axis of Figure 4L.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had changed "RNA" to "mRNA" in the y-axis of Figure 4L.

- Please indicate the meaning of "ns" in Figure 5 legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

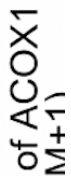
Changes in the text: We had indicated the meaning of "ns" in Figure 5 legend in Page 31 Line 656-657: "ns.: non-significant."

- Please indicate the magnification in Figure 5A legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had indicated the magnification in Figure 5A legend in Page 31 Line 650: Magnification = ×40.

- It is suggested to revise "ACOX1" to "ACOX-1" in the y-axis of Figure 5C and 5H.



Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.

Changes in the text: We had revised "ACOX1" to "ACOX-1" in the y-axis of Figure 5C and 5H.

5. Supplementary

- **All abbreviations** in the Supplementary Figure 1 and legend should be explained.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.
Changes in the text: We had explained abbreviation of EMT in Page 31 Line 661: the epithelial-mesenchymal transition (EMT).

- Please indicate the staining method in Supplementary Figure 1A legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for not indicate clearly. We observed the morphology of cells using a light microscope without any staining.

Changes in the text: We had modified the Supplementary Figure 1A legend in Page 31 Line 662: "The morphology of CNE2 cells after different exosomes treatment using a light microscope."

- Please indicate the magnification in Supplementary Figure 1C legend.

Reply: Thanks for the editor's suggestions very much and we are very sorry for our negligence of it.
Changes in the text: We had indicated the magnification in Supplementary Figure 1C legend in Page 31 Line 666: "Magnification = ×40".