

Dear prof. Lian-Sheng Ma,

We are now pleased to submit our revised manuscript entitled “Role of exosomes in metastasis and therapeutic resistance in esophageal cancer” for consideration to **World Journal of Gastroenterology**.

We thank you for your email of 31 August 2023 in which you advised us to revise our manuscript (ID:86402) and shared with us the reviewer’s expert comments. We thank you and the reviewers for the insightful suggestions. In this two weeks, we have collected new papers, and revised our manuscript. In summary, we have made the following major changes:

1. We have tried our best to increase the focus on esophageal cancer and deleted the texts considering other cancers in our revised manuscript.

2. We have re-searched the newly published literature on the topic of esophageal cancer and exosome as well as the authoritative literature and organized them into our revised text.

3. We have collated the results reported by our predecessors and added our own insights at the end of each paragraph, including the outlook for the future and issues still to be resolved, etc.

4. We have removed the inappropriate sections of the text and streamlined and added their content to the appropriate locations

5. We have consulted editing professionals, proofread the manuscript, and corrected the grammar or spelling errors.

Our point-to-point responses to the reviewers are listed below. In addition, we attach a copy of the manuscript in which we have highlighted all the changes in yellow as Supplementary Information for your reference.

There still one thing to explain to you. Since our figures are drawn online using www.figuredraw.com or www.biorender.com, platforms for drawing figures, in which the output figures are allowed to publish. So, it is hard to provide decomposable figures (in which all components are movable and editable) in PowerPoint file.

Here, we provide the editable figures by online links to your figures, which are listed below:

Figure 1: https://www.figdraw.com/static/index.html#/paint_canvas?canva=314085

Figure 2: <https://app.biorender.com/illustrations/64f5d4d27f71bd98286a2987>

Figure 3: <https://app.biorender.com/illustrations/642ccd8a431ccce314a41f29>

And, we also organize them into a single PowerPoint file for submission.

We look forward to presenting our improved work to the readers of World Journal of Gastroenterology. Thank you again for your consideration.

Yours faithfully,

Junting

Response to the reviewers:

Response to reviewer 1:

Q1. Specific Comments to Authors: The article deals with a very interesting issue in esophageal cancer regarding the role of exosomes in metastases and therapeutic resistance in esophageal cancer. It is very well structured covering in an understandable way the role of exosomes in metastasis. Also provides all the necessary information for the different signaling pathways that exosomes are involved in therapeutic resistance. Although the surgical treatment is the gold standard therapeutic method in the future may exosomes can act as adjunct to it.

A1. Thank you very much for the comments and your acknowledgement to our work, which will be a source of motivation for us to continue our work in this area in the future. We also provide an outlook on the beneficial role of exosomes for surgery in the main text as following: “Additionally, exosomes can act as carriers to deliver critical components for gene therapy and cancer vaccines. Exogenous exosomes also hold tremendous potential for optimizing the therapeutic outcomes of the surgical treatment of EC.” **(Revised accordingly on MS, Lines 533-535, page 20)**

Response to reviewer 2

Q1. Specific Comments to Authors: Overall the manuscript gives a general overview of the role of exosomes in oesophageal cancer. There are many aspects discussed (probably too many) and I would suggest the author to focus on information specific for oesophageal cancer.

A1. We deeply appreciate your insightful suggestion. We did discuss too many concerning the relation between exosomes and other cancers. Based on your advice, we have made some cuts to ensure that our manuscript is more concise and on topic in our revised manuscript.

For your convenience, we lists the deleted parts as below:

~~“Research revealed that sixty-four lipids, including diacylglycerols and lysophosphatidylethanolamines, were significantly reduced in exosomes secreted by hereditary alpha-tryptasemia patients compared with exosomes derived from normal volunteers [64]~~

~~Endothelial cells in EC are reported to augment tumor metastasis ability and attenuate the survival time of patients through overexpression of the epiregulin (EREG) gene [83].~~

~~TAMs play a key role in blood vessel formation and lymph angiogenesis by upregulating VEGF, secreting growth factors, and transforming into lymphatic endothelial cells, ultimately leading to tumor metastasis [97].~~

~~Wnt/ β -catenin signaling pathway~~

~~The main components of the Wnt signaling pathway include members of the Wnt secretory protein family, transmembrane receptors/coreceptors from the Frizzled family, dishevelled (DVL), glycogen synthase kinase 3 (GSK3), adenomatous polyposis coli (APC), axin, β -catenin, and TFs [108]. β -Catenin activates the canonical Wnt signaling pathway by initiating gene transcription, thereby playing numerous roles in various cellular events, including tumorigenesis and progression, and affecting the prognosis of cancers [109]. MiR-106b-3p is upregulated in ESCC~~

and other types of cancer, and it can play different roles under specific conditions.

PI3K/AKT signaling pathway

Emerging evidence has revealed that exosome interventions targeting the signaling pathway are closely correlated with the development of EMT in various cancers. In EC, miR-19b-3p transferred by exosomes can inhibit the expression of phosphatase and tensin (PTEN), which is associated with EMT, by diminishing the expression of phosphatidylinositol 3-kinase (PI3K/AKT) and augmenting the expression of matrix metalloproteinase-2 (MMP2) and vimentin [112]. Together, these results indicate that the PI3K/AKT signaling pathway influences the metastasis of EC cells.

NF- κ B signaling pathway

In the human body, CSCs and non-CSCs are in a dynamic equilibrium state: single cancer cells can transform into CSCs through EMT [115]. In addition to altering the transition between CSCs and non-CSCs, it is reasonable to hypothesize that ESCC-derived exosomes can affect other processes of tumor metastasis through the NF- κ B signaling pathway.

Antitumor immune cells, such as M1 macrophages, NK cells, and dendritic cells (DCs), can inhibit tumor development or directly kill tumor cells. During the interaction between tumor cells and the TME, protumor cells, such as Tregs, MDSCs, and M2 macrophages, emerge to help tumor cells escape the immune system [123].

Although radiotherapy is less toxic to normal tissues throughout the body than chemotherapy, it still has some serious adverse reactions when it hits normal tissues and affects normal cells surrounding the tumor [151].

As tumor cells are heterogeneous, immunotherapy may not be effective against insensitive tumor cells, or there may be additional interfering targets present in the tumor microenvironment, which can contribute to primary resistance [158].

Application of engineered exosomes that simultaneously deliver the anticancer drug 5-FU and miR-21 inhibitor oligonucleotide (miR-21i) reversed drug resistance and reduced tumor cell growth in colon cancer [189].”

Q2. Specific Comments to Authors: The authors should also add their opinion of what is known and what is the way forward.

A2. We deeply apologize for providing so little input in our previous manuscript and greatly appreciate for your kind reminder. After learning the latest research advances tightly associating with our topic and deep thinking, we have added more personal insights throughout our manuscript.

For your convenience, we show the added sentences as follows:

“We focused on how cells in the TME influence EC development and the role of exosomes in this process (**Figure 2**).” **(Revised accordingly on MS, Lines 156-158, page 6)**

“In addition to being associated with the formation of CAFs, exosomes play an indispensable role in immune dysfunction in patients with EC, which may lead to disease progression and poor response to therapy.” **(Revised accordingly on MS, Lines 195-197, page 7-8)**

“Based on the alterations in cells in the TME, the use of exosomes to intervene in this process to regulate TME is worth studying.” **(Revised accordingly on MS, Lines 222-224, page 8)**

“All these studies demonstrate that when studying the relationship between exosomes and EC, we can focus on changes in the molecules of the relevant signaling pathways, which opens up possibilities for diagnosis and subsequent treatment.” **(Revised accordingly on MS, Lines 266-269, page 10)**

“These studies will bring new promise for immunotherapy of EC.” **(Revised accordingly on MS, Lines 300, page 11)**

“Owing to the complex and unknown mechanisms of chemotherapy resistance, it is difficult to find an effective solution.” **(Revised accordingly on MS, Lines 337-339, page 12)**

“After radiotherapy, EC cells may acquire radiation resistance by secreting specific exosomes, and it is crucial to identify effective interference targets to improve the efficacy of radiotherapy and reduce the recurrence rate.” **(Revised accordingly on MS, Lines 398-401, page 15)**

“In addition to PD-1/PD-L1, the relationship between exosomes and other immunotherapy approaches for EC remains to be explored. Identifying a reliable and universal relationship may help address immunotherapy resistance, although this remains a severe challenge.” **(Revised accordingly on MS, Lines 425-428, page 16)**

“We believe that exosomes can achieve similar regulatory effects in EC, suggesting new approaches for EC treatment.” **(Revised accordingly on MS, Lines 499-500, page 18)**

“In summary, we can expect engineered exosomes to have great potential for use in EC treatment.” **(Revised accordingly on MS, Lines 526-527, page 19)**

“Additionally, exosomes can act as carriers to deliver critical components for gene therapy and cancer vaccines. Exogenous exosomes also hold tremendous potential for optimizing the therapeutic outcomes of the surgical treatment of EC.” **(Revised accordingly on MS, Lines 532-534, page 19-20)**

Q3. Specific Comments to Authors: Be sure to include all recent and relevant literature.

A3. We are truly grateful for your suggestion and sorry for not including all recent and relevant literature in our previous manuscript. We have re-searched the highly cited literature in the field and added them to our main text.

For your convenience, we show the newly included citations as follows:

14 Jia L, Qiu Q, Zhang H, Chu L, Du Y, Zhang J, Zhou C, Liang F, Shi S, Wang S, Qin W, Wang Q, Li F, Wang Q, Li Y, Shen L, Wei Y, Jia J. Concordance between the assessment of A β 42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid. *Alzheimers Dement* 2019; 15(8): 1071-1080 [PMID: 31422798 DOI: 10.1016/j.jalz.2019.05.002]

15 Liu T, Zhang Q, Zhang J, Li C, Miao YR, Lei Q, Li Q, Guo AY. EVmiRNA: a database of miRNA profiling in extracellular vesicles. *Nucleic Acids Res* 2019; 47(D1): D89-d93 [PMID: 30335161 PMCID: PMC6323938 DOI: 10.1093/nar/gky985]

16 Gebara N, Scheel J, Skovronova R, Grange C, Marozio L, Gupta S,

Giorgione V, Caicci F, Benedetto C, Khalil A, Bussolati B. Single extracellular vesicle analysis in human amniotic fluid shows evidence of phenotype alterations in preeclampsia. *J Extracell Vesicles* 2022; 11(5): e12217 [PMID: 35582873 PMCID: PMC9115584 DOI: 10.1002/jev2.12217]

17 He Q, Liu L, Wei J, Jiang J, Rong Z, Chen X, Zhao J, Jiang K. Roles and action mechanisms of bile acid-induced gastric intestinal metaplasia: a review. *Cell Death Discov* 2022; 8(1): 158 [PMID: 35379788 PMCID: PMC8979943 DOI: 10.1038/s41420-022-00962-1]

18 Chen B, Sang Y, Song X, Zhang D, Wang L, Zhao W, Liang Y, Zhang N, Yang Q. Exosomal miR-500a-5p derived from cancer-associated fibroblasts promotes breast cancer cell proliferation and metastasis through targeting USP28. *Theranostics* 2021; 11(8): 3932-3947 [PMID: 33664871 PMCID: PMC7914354 DOI: 10.7150/thno.53412]

19 Zhang S, Chuah SJ, Lai RC, Hui JHP, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. *Biomaterials* 2018; 156: 16-27 [PMID: 29182933 DOI: 10.1016/j.biomaterials.2017.11.028]

20 Jiang TY, Shi YY, Cui XW, Pan YF, Lin YK, Feng XF, Ding ZW, Yang C, Tan YX, Dong LW, Wang HY. PTEN Deficiency Facilitates Exosome Secretion and Metastasis in Cholangiocarcinoma by Impairing TFEB-mediated Lysosome Biogenesis. *Gastroenterology* 2023; 164(3): 424-438 [PMID: 36436593 DOI: 10.1053/j.gastro.2022.11.025]

21 Lin Z, Wu Y, Xu Y, Li G, Li Z, Liu T. Mesenchymal stem cell-derived exosomes in cancer therapy resistance: recent advances and therapeutic potential. *Mol Cancer* 2022; 21(1): 179 [PMID: 36100944 PMCID: PMC9468526 DOI: 10.1186/s12943-022-01650-5]

22 Hu N, Cai Z, Jiang X, Wang C, Tang T, Xu T, Chen H, Li X, Du X, Cui W. Hypoxia-pretreated ADSC-derived exosome-embedded hydrogels promote angiogenesis and accelerate diabetic wound healing. *Acta Biomater* 2023; 157: 175-186 [PMID: 36503078 DOI: 10.1016/j.actbio.2022.11.057]

23 Hu Z, Chen G, Zhao Y, Gao H, Li L, Yin Y, Jiang J, Wang L, Mang Y, Gao Y, Zhang S, Ran J, Li L. Exosome-derived circCCAR1 promotes CD8+ T-cell dysfunction and anti-PD1 resistance in hepatocellular carcinoma. *Mol Cancer* 2023; 22(1): 55 [PMID: 36932387 PMID: PMC10024440 DOI: 10.1186/s12943-023-01759-1]

24 Zhang Q, Deng T, Zhang H, Zuo D, Zhu Q, Bai M, Liu R, Ning T, Zhang L, Yu Z, Zhang H, Ba Y. Adipocyte-Derived Exosomal MTTP Suppresses Ferroptosis and Promotes Chemoresistance in Colorectal Cancer. *Adv Sci (Weinh)* 2022; 9(28): e2203357 [PMID: 35978266 PMID: PMC9534973 DOI: 10.1002/advs.202203357]

25 Tenchov R, Sasso JM, Wang X, Liaw WS, Chen CA, Zhou QA. Exosomes—Nature's Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics. *ACS Nano* 2022; 16(11): 17802-17846 [PMID: 36354238 PMID: PMC9706680 DOI: 10.1021/acsnano.2c08774]

26 Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, Tao Y, He Z, Chen C, Jiang Y. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther* 2020; 5(1): 145 [PMID: 32759948 PMID: PMC7406508 DOI: 10.1038/s41392-020-00261-0]

29 Wang X, Huang J, Chen W, Li G, Li Z, Lei J. The updated role of exosomal proteins in the diagnosis, prognosis, and treatment of cancer. *Exp Mol Med* 2022; 54(9): 1390-1400 [PMID: 36138197 PMID: PMC9535014 DOI: 10.1038/s12276-022-00855-4]

30 He J, Ren W, Wang W, Han W, Jiang L, Zhang D, Guo M. Exosomal targeting and its potential clinical application. *Drug Deliv Transl Res* 2022; 12(10): 2385-2402 [PMID: 34973131 PMID: PMC9458566 DOI: 10.1007/s13346-021-01087-1]

35 Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, Liebler DC, Ping J, Liu Q, Evans R, Fissell WH, Patton JG, Rome LH, Burnette DT, Coffey RJ. Reassessment of Exosome Composition. *Cell* 2019; 177(2): 428-445.e418 [PMID: 30951670 PMID: PMC6664447 DOI: 10.1016/j.cell.2019.02.029]

37 Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, Lin LH, Lin YH, Liu JK, Liu YC, Lunde R, Shen WT. Exosome Processing and Characterization Approaches for Research and Technology Development. *Adv Sci (Weinh)* 2022; 9(15): e2103222 [PMID: 35332686 PMCID: PMC9130923 DOI: 10.1002/advs.202103222]

39 He L, Zhu D, Wang J, Wu X. A highly efficient method for isolating urinary exosomes. *Int J Mol Med* 2019; 43(1): 83-90 [PMID: 30365060 PMCID: PMC6257847 DOI: 10.3892/ijmm.2018.3944]

40 Gao H, Zhong S, Dangayach R, Chen Y. Understanding and Designing a High-Performance Ultrafiltration Membrane Using Machine Learning. *Environ Sci Technol* 2023 [PMID: 36790106 DOI: 10.1021/acs.est.2c05404]

41 Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, Du L. Review on Strategies and Technologies for Exosome Isolation and Purification. *Front Bioeng Biotechnol* 2021; 9: 811971 [PMID: 35071216 PMCID: PMC8766409 DOI: 10.3389/fbioe.2021.811971]

45 Jin MZ, Jin WL. The updated landscape of tumor microenvironment and drug repurposing. *Signal Transduct Target Ther* 2020; 5(1): 166 [PMID: 32843638 PMCID: PMC7447642 DOI: 10.1038/s41392-020-00280-x]

46 Novoa Díaz MB, Martín MJ, Gentili C. Tumor microenvironment involvement in colorectal cancer progression via Wnt/ β -catenin pathway: Providing understanding of the complex mechanisms of chemoresistance. *World J Gastroenterol* 2022; 28(26): 3027-3046 [PMID: 36051330 PMCID: PMC9331520 DOI: 10.3748/wjg.v28.i26.3027]

47 Fokas E, Engenhardt-Cabillic R, Daniilidis K, Rose F, An HX. Metastasis: the seed and soil theory gains identity. *Cancer Metastasis Rev* 2007; 26(3-4): 705-715 [PMID: 17786535 DOI: 10.1007/s10555-007-9088-5]

52 Singleton DC, Macann A, Wilson WR. Therapeutic targeting of the hypoxic tumour microenvironment. *Nat Rev Clin Oncol* 2021; 18(12): 751-772 [PMID: 34326502 DOI: 10.1038/s41571-021-00539-4]

56 He G, Peng X, Wei S, Yang S, Li X, Huang M, Tang S, Jin H, Liu J, Zhang

S, Zheng H, Fan Q, Liu J, Yang L, Li H. Exosomes in the hypoxic TME: from release, uptake and biofunctions to clinical applications. Mol Cancer 2022; 21(1): 19 [PMID: 35039054 PMCID: PMC8762953 DOI: 10.1186/s12943-021-01440-5]”

Response to reviewer 3

Q1. Specific Comments to Authors: Remove exosome biogenesis and uptake from theoretical aspect. Just cite Figure 1 instead of theory

A1. Graciously thanks for your kind notice. We have completely deleted the two parts of exosome biogenesis and uptake following your suggestion. To ensure the integrity of the content, we use three words to briefly introduce exosome biogenesis and uptake in the main text and put the **figure 1** at the end of the sentence in our revised manuscript. And we sincerely hope that our rectifications are appropriate and can meet your requirements.

For your convenience, we show the revised sentences and deleted parts as follows:

Revised sentences:

“Exosomes are phospholipid bilayer-encapsulated nanosized vesicles formed via the endocytic pathway [25]. There are multiple ways in which exosomes are transported from donors to recipient cells (**Figure 1**).” (**Revised accordingly on MS, Lines 97-99, page 4**)

Deleted parts:

~~“Biogenesis of exosomes~~

~~Exosomes were first discovered by Eberhard G. Trams and R.M. Johnston during the culture of sheep reticulocytes and were known as “cellular dust” that was present during the maturation of reticulocytes to erythrocytes [36]. Although exosomes have long been assumed to be “junk”, accumulating evidence since 1996 has revealed that exosomes may function in intercellular signaling and cell to cell communication [37-39]. Exosomes are phospholipid bilayer-encapsulated nanosized vesicles formed by the endocytic pathway [40]. First, the cytoplasmic membrane invaginates inwardly~~

to form the early endosome structure [41]. Then, during the process of transition from early endosomes to late endosomes, which will further mature into multivesicular bodies (MVBs), the endosomal plasma membrane invaginates, and specific cytosolic contents, such as a range of DNA sequences, RNAs, and proteins, are absorbed to generate intraluminal vesicles (ILVs) [42]. The endosomal sorting complex required for transport (ESCRT) pathway and the ESCRT-independent pathway are reported to participate in the above process [43, 44]. The ESCRT machinery is mainly composed of four protein complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III), which have help from chromatin modifying protein 4C, vesicle trafficking 1, ALG-2 interacting protein X, tumor susceptibility gene 101, signal transducing adaptor molecule 1, and vacuolar protein sortin 4B [20]. Heat shock protein 60 (HSP60), HSP70, HSP90, and tetraspanins (such as CD9, CD63, and CD82) are reported to participate in the ESCRT-independent pathway [45]. Finally, there are three types of outcomes for MVBs. The first is captured by the trans-Golgi network (TGN) [44]. The second is fusion with autophagosomes or lysosomes, which causes their degradation [46]. The third is fusion with the plasma membrane, which causes ILVs releases. ILVs released into the extracellular environment are often referred to as exosomes [47, 48].

Exosomes uptake

It has been widely established that the mechanism of exosome uptake is similar to that of viral uptake, but differences between them must be considered [49]. There are multiple ways for exosomes to be transported from donor cells to recipient cells: endocytosis, macropinocytosis, direct fusion, direct binding of ligands to receptors, and phagocytosis [49, 50]. The uptake process also depends on the microenvironment pH [51]. Shreds of evidence shows that exosome uptake is an energy-consuming process dependent on the proteins and glycoproteins on the membrane [52]. Endocytosis can be divided into clathrin-mediated endocytosis and clathrin-independent endocytosis, including caveolin-mediated uptake and lipid raft-mediated internalization [52, 53]. The uptake process of exosomes derived from the human gastric normal cell line HFE-145 into gastric epithelial cells was

~~potentiated by clathrin and micropinocytosis but inhibited by caveolin, indicating that different molecules may play different roles in particular situations [54].~~

Q2. Specific Comments to Authors: Grammatical errors have to be rectified

A2. Greatly appreciate for the reminding. We are very sorry for our incorrect grammar and have sought professional help in touching up our manuscript.