Peer Review File

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Reviewer A

Comment 1: There is too much enumeration of the individual facts from previous reports. There is much overlap of similar outcomes and the sentences, too. Those make the review miscellaneous and hard to understand. The authors are required to summarize the contents and show comprehensible figure(s), which explains what is transported through exosomes from which cell to which cell, and how it leads to the vascular calcification.

Reply 1: We apologize for the overlap of similar outcomes and the sentences, and the poor language, which make the review miscellaneous and hard to understand. We have deleted some of the sentences that have repeated meaning in the review, and we have sent the manuscript to the English editing service for English language editing, so as to make the review easier to understand. In addition, we also add some figures to show the mechanisms that how exosomes regulate VC. We really hope that the language level has been substantially improved.

Changes in the text: see the highlighted in yellow and the figures

Comment 2: Compared to coronary artery with calcification 1,2), carotid calcified plaques have been described clinically stable and frequently found in asymptomatic patients with carotid stenosis 3,4). The difference between the coronary and carotid plaques has been discussed elsewhere but not fully elucidated. A report said there might be double cerebrovascular symptomatic peaks according to calcium scores in

calcified carotid plaques 5). How the researches with the exosomes approach this issue? What happens to the asymptomatic calcified carotid arteries if the therapeutic actions are achieved to suppress calcification through regulating exosome secretion as the authors described? The authors are advised to add the discussion regarding this matter.

1) Detrano R et al. N Engl J Med. 2008;358:1336-45.

2) Ehara S et al. Circulation. 2004;110:3424-9.

3) Nandalur KR et al. AJR Am J sRoentgenol. 2006;186:547-52.

4) de Weert TT et al. AJNR Am J Neuroradiol. 2009;30:177–84

5) Katano H et al. J Stroke Cerebrovasc Dis. 2015;24:1341-50.

Reply 2: After literature search and further analyzed, several studies have reported that miRNA profiles were significantly altered in exosomes from VSMCs associated with coronary artery calcification, however, the role of exosomes in the formation of carotid calcified plaque has not been extensively studied. Considering that there might be double cerebrovascular symptomatic peaks according to calcium scores in calcified carotid plaques, reducing calcification through exosomes and other means may increase the risk of developing calcification symptoms and may not be appropriate for all VC patients.

Changes in the text: Page 8/line19-Page9/line 20

Review B

Comment 1: Similar reviews have been published fairly recently (see J Cell Mol Med 2018;22:4024-4033, Clin Chim Acta 2019;499:118-122). The content of this review manuscript does not come at a timely moment and may need more information to

make it informative for readers.

Reply 1: We agree with this suggestion. In fact, current studies have found that the role of exosomes in VC is indeed limited to several aspects, including promoting the formation of extracellular mineral deposits, inducing phenotypic conversion of VSMCs, transporting microRNA between cells, regulating autophagy and oxidative stress. Comparing to the review before, we arrange our manuscript in a similar structure, however, we cited lots of the latest reports to enrich each part of our review (highlighted in yellow in reference), and want to provide the latest researched to our readers.

Changes in the text: see the highlighted in yellow

Comment 2: In the introduction, the authors literally described the potential mechanisms of VC (esp. the 2nd paragraph), but the content is really superficial and should be enriched substantially. In the 1st paragraph, the authors described "the underlying mechanisms of VC remain unclear", which is not valid in light of the progress made during recent 5 years. There is a treatment clinically available at this moment (ex. sodium thiosulfate) although popularity needs enhancement. The entire introduction section needs careful rewording.

Reply 1: We have added some information about the potential mechanisms of VC in the introduction (see in the 2nd paragraph). In addition, we found some treatments are clinically available, such as sodium thiosulfate and SNF472, and more information has been enriched in introduction (see in the 1st paragraph).

Changes in the text: Page3/line 10-Page 4/line 4; Page 4/line 11-Page 5/line 15

Comment 3: If the authors aimed to use a systematic approach to present this topic, they should adopt a more standardized strategy such as providing the literature search algorithm and selection steps, with numbers of reports screened and retained and synthesized illustrated using a figure. Moreover, the keyword combinations are few and ought to miss reports with resemblance (ex. aortic calcification? Coronary calcification? Etc.)

Reply 3: Thanks for your suggestions about the literatures search in the review. We categorize this paper as a narrative review and a figure on the flowchart of study identification and selection procedure may not be necessary. Further, we check several narrative reviews published in ATM, and method sections of these articles were also not illustrated with figures 1), 2). As for the keyword combinations, we added some key words related to the exosomes in VC, so as to include the literature as comprehensively as possible. Search terms such as “aortic calcification” and “coronary calcification”, whose search results have been included in the search terms “calcification”.

Changes in the text: Page 6/line 17-Page 6/line 19

1) Benedetto, U., et al., *A narrative review of the interpretation of guidelines for the treatment of infective endocarditis.* Annals of Translational Medicine, 2020. **8**(23).

2) Mihos, C.G. and F. Nappi, *A narrative review of echocardiography in infective*

endocarditis of the right heart. Annals of Translational Medicine, 2020. **8**(23).

Comment 4: For section 3.1, the 1st paragraph is repetitive of the 3rd paragraph of the introduction section, and can be removed.

Reply 4: We agree with this suggestion and have modified these sections as appropriate. For the 3rd paragraph of the introduction section, we briefly introduce exosomes and their role in VC, while in the 1st paragraph, section 3.1, we present a more detailed description of the characteristics of exosomes and their possible roles in VC in combination with clinical practice. We removed some of the introduction section and added more in the discussion section, in order to make the paragraph properly detailed and concise.

Changes in the text: Page 5/line 16-Page 6/line9; Page 7/line 2-Page 9, line 20

Comment 5: For exosomes' role in VC, several aspects are minimally covered, such as the precipitators and factors that promote inter-cellular crosstalk. More is needed to enrich this manuscript.

Reply 5: We apologized for the minimally covered aspects for exosomes' roles in VC. For the role in promoting inter-cellular crosstalk, we think that exosomes can promote inter-cellular crosstalk through the cargos including miRNA and proteins, more details have been added in section 3.2.3 and the 1st paragraph in section 3.2.4. For other aspects, we also added some information form the latest reports, in order to provide more information to readers.

Change in the text: Page 17/line1-11; Page 18/line 5-Page 19/line 15; Page 19/line 18-Page 20/line 9.

Comment 6: The English contains many mis-spellings and should be polished.

Reply 6: We apologized for the poor language of our manuscript. We worked on the manuscript for a long time and the repeated addition and removal of sentences and sections obviously led to poor readability. We have now worked on both language and readability and have also sent to English editing service for language corrections. We really hope that the language level has been substantially improved.

Changes in the text: see the highlighted in yellow