Peer Review File

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Reviewer Comments

This manuscript describes nicely the excretion of exosomes coming from M2 macrophages and the uptake of into A459 tumor cells. M2 tumor-associated macrophages are described as promoting metastasis in patients with lung cancer. The presence of these M2 TAMs are high in metastases of non-small cell lung cancer. The primary tumor harbors less of these TAMs, where they are expected to be high and being involved in migration and invasiveness. Is there a cut-off of M2 TAMs in the primary tumor or is the mechanism by exosomes different in the primary tumor? Or is their presence only to accelerate tumor growth as in colorectal cancer?

Reply: Thank you so much for your advice. The presence of M2 TAM is to accelerate NSCLC growth and metastasis. The alveolar M2 TAM density has been reported to be associated with NSCLC differentiation, invasive size, pathological stage and poor prognosis. Our study suggest high expression of M2 TAM promote NSCLC metastasis.

So, whether M2 TAMs promotes metastasis is hard to say from the cell models, their presence is higher in metastases than in the primary tumor, but in the mouse model (figure 7) I would like to see a growth curve of the tumors and the number of metastases.

Reply: Thank you so much for your advice. We have added the bioluminescence signal of the tumors and the number of metastases in mouse model.

Another question is about RASSF4, a tumor suppressor in the development of lung cancer. The RASSF concentration is supposed to be low in this tumor and still miR-155 and miR-196a-5p interact with this RASSF4. Could the authors indicate why modulating an already very low RASSF4 concentration is effective in NSCLC?

Reply: Thank you so much for your advice. RASSF4 plays an important role in tumor inhibition, and low expression of RASSF4 is associated with poor prognosis in lung cancer. The expression of RASSF4 is low in lung cancer, but it still do not reach the lowest point. Our study indicates that miR-155 and miR-196a-5p further inhibit RASSF4 expression to accelerate NSCLC metastasis.

Small remarks:

Abstract: CCK-8 first time full text.

Reply: Thank you so much for your advice. We have expanded in Abstract (see Page 2, line 33).

Line 39: typo of p: pPotential binding etc.

Reply: Thank you so much for your advice. We are so sorry for our typo. We have modified our text as advised (see Page 2, line 38).

Line 113: The project was informed consent, the project required informed consent? **Reply:** Thank you so much for your advice. The project required informed consent.

Line 174+184: Typo: ..., A549 cells (1 x 104 cells/well) should be (1 x 104 cells/well). See also line 235 1x 109 ifu?

Reply: Thank you so much for your advice. We are so sorry for our typo. We have modified our text as advised (see Page 6, line 166; Page 7, line 176; Page 8, line 227).

Line 297: Typo: G4869 must be GW4869.

Reply: Thank you so much for your advice. We are so sorry for our typo. We have modified our text as advised (see Page 10, line 289).

Line 417: typo: inaicated is indicated.

Reply: Thank you so much for your advice. We are so sorry for our typo. We have modified our text as advised (see Page 14, line 409).

Line 429: fascinated is facilitated?

Reply: Thank you so much for your advice. We are so sorry for our incorrect description. We have modified our text as advised (see Page 15, line 421).

Figure 3: it would be nice to include the scale bar into the figure A. **Reply:** Thank you so much for your advice. We have added the scale bar in Figure 3A.

Figure 4A and B: quality of figures is poor and I cannot read properly the information. **Reply:** Thank you so much for your advice. We have divided Figure 4 to improve the image quality.

Figure 7: add a new figure that shows the significantly increased number of metastases in mice. **Reply:** Thank you so much for your advice. We have added a new figure to increase number of metastases in mice.