Author's response to reviews

Title: Exosome derived from epigallocatechin gallate treated breast cancer cells suppresses tumor growth by inhibiting tumor-associated macrophage infiltration and M2 polarization

Authors:

Ji-Young Jang Dr. (jjy93@hanmail.net)
Jong-Kuen Lee Mr. (fz2jk@naver.com)
Yoon-Kyung Jeon Prof. (junarplus@chol.com)
Chul-Woo Kim Prof. (cwkim@snu.ac.kr)

Version: 5 Date: 10 July 2013

Author's response to reviews: see over
Thank you for comments of reviewers and receive a lot of help. I did best for complementary experiment. I marked with red color for modification.

The authors have clearly shown by qPCR the modulation of the expression levels of IL6, TGFb and TNFa in macrophages and of CSF1 and CCL2 in 4T1 tumor cells after treatment with EGCG: I think the data should be confirmed at the protein level, at least for the main findings, in order to be convincing that the observed gene modulation has biological effects. This data was asked in the previous revision (Major point #4).

: Add the Fig. 2C [western-blot (IL-6, TGF-b/TNF-a), p10, p13-p], p12, p29

As already commented in the first revision, the scheme depicted in the last figure is not clear and should be revised. My suggestions are the following: delete repetitions (‘target of miR-16:…’), indicate ‘tumor exosomes’, indicate that down-regulation of CCL2 and CSF1 occur in tumor cells and that regulation of TNFa, IL6 and TGFb occur in TAM.

: Edit the Fig. 5

The new abstract needs rewriting, as the working hypothesis and the aim of the study is not clearly stated.

: Edit the abstract p2

No data are provided that miR-16 carried by 4T1 exosomes are responsible of the changes observed in macrophages. Does miR-16 treatment in macrophage modulate IL6, TGFb, TNFa?

: Add the supplementary data 2, p14~15

The experiments with miR-16 inhibitor lack controls to verify the levels of miR-16 are reduced by the treatment and are not when the scrambled oligo was used.

: Add the supplementary data 1, p14

Minor Essential Revisions

pg 7, paragraph ‘Exosomes isolation and purification’: clarifications required have been provided, correct µg into g specify how the tested exosomal markers resulted in the 4T1 exosomes; calexin should be changed into calnexin: edit p6, p7
pg 8, paragraph ‘quantification of miR-16 by qPCR’: please specify the ‘different’ exposure times with EGCG specify the quantity of miR-16 inhibitor and miR scrambled used to transfect the cells: edit p7

anti-mouse CD163 mAb (ab74604) is indicated presumably by mistake, since it isa mouse mAb specific for human CD163: edit p10, CD163 antibody (Novus: NBP1-95135)

for better clarity Figure 1G should be moved after Figure 4C and it should be named Figure 4D: edit change Fig. 1G to Fig. 4D, p14

In Figure 1D and Figure 2A, the expression levels of CCL2 mRNA are not correctly normalized and 4T1 treated with PBS control should be used as calibrator: edit the Fig 1D, 2A

Figure 1F: correct negative p value: edit the Fig 1F

The numbers in Figure 4A and 4B presumably report the quantification of the bands by imaging analysis, but the authors should specify it in the figure legend. : p30

Abstract . line 13 please reformulate, impossible to understand: edit the p2

page 13, line 2. 'exibited' , maybe you wanted to say ' suggest'? : edit the p12

page 14 line 10 'which shoud be 'whose', : edit the p13

why didi you use mRNA for miRNA : edit the p13

page 18 line 2-3 , reformulate , not clear : edit the p17

Reference : remove ref24 and 25