Reviewer's report

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

Version: 4 Date: 27 May 2013

Reviewer: monica rodolfo

Reviewer's report:

The revised manuscript is better focused and more clear and most of the requested modifications have been accomplished. However, some requests were not fulfilled, and the English form still needs improvement.

Major Compulsory Revisions
1. 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).

2. 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.

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

4. 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? 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.

Minor Essential Revisions
1. 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; calnexin should be changed into calnexin

2. pg 8, paragraph ‘quantification of miR-16 by qPCR’: please specify the ‘different’ exposure times with EGCG

3. specify the quantity of miR-16 inhibitor and miR scrambled used to transfect the cells
5. anti-mouse CD163 mAb (ab74604) is indicated presumably by mistake, since it is a mouse mAb specific for human CD163

2. for better clarity Figure 1G should be moved after Figure 4C and it should be named Figure 4D

3. 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

4. Figure 1F: correct negative p value

5. 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.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests