Reviewer’s report

Title: Exosome derived from epigallocatechin-3-gallate treated breast cancer cells suppresses tumor growth through the inhibition of infiltration of tumor associated macrophages and M2 polarization

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Reviewer: monica rodolfo

Reviewer’s report:

The manuscript by Jang and colleagues presents novel data about the mechanisms underlying the antitumoral activity of EGCG in the murine breast tumor model 4T1. In particular, they report results indicating that EGCG treatment determines a decrease of TAM numbers through down-modulation of CSF-1 and CCL2, and supporting the skewing of TAM to M1 producing TNFa through up-regulation of miR-16 expression.

Although the data presented appear original, scientifically sounding and of interest in the context of the mechanisms modulating tumor microenvironment, the manuscript requires several major modifications.

First of all, the paper is poorly written. It is possible that a draft and not the final version has been submitted by mistake: in fact, there are some incomplete and unclear periods (for example at pg 4 in the second text period, at pg 5 line 8-10, at pg11 line 7-9, pg 12 line 6-10, 13 line 12), and inconsistencies between figures, legends and text (fig 1, 2, 3, 4). In detail: in the Abstract the Methods are not described; the Introduction and the Discussion sections and the bibliography are too long and largely unfocused; the Material and Methods are incomplete in some parts (see below); the Figures details are poorly defined (see below).

The experiments and the methods used appear generally adequate, although some points need to be clarified, which are the following.

1. the experiments utilizing tumor derived exosomes need clarification about the methods used (cell culture conditions, the use of exosome-depleted FCS, the controls performed on exosome purification) and how they were performed.

2. the second paragraph of the Results (pg11) is particularly unclear and requires rewriting.

3. the experiment with the miR-16 inhibitor (fig 2) should be detailed and explained. In addition, the authors should evaluate TGF#, IL6, TNF# mRNA levels in TAM treated with exosomes isolated from 4T1 cells silenced for miR-16 expression in order to demonstrate the role of miR-16 in promoting M1 polarization of macrophages.

4. the experiments in which macrophages are stimulated with tumor-derived exosomes should be detailed in the Methods section (how TAM stimulation with exosomes is performed, specifying the number of stimulated TAM and the
quantity of exosomes). In addition, the Authors should confirm at the protein level
the modulation of CSF-1, CCL2, TGF#, IL6 and TNF# showed at the mRNA level
by qPCR data.

5. the use of anti-human CD68 and CD163 antibodies to stain murine
macrophages should be amended.

6. to improve clarity and ease the reader, the data from the in vivo experiments
shown in fig5 should be moved at the beginning and presented as fig1.

7. the diagram shown in fig6 should be better schematized to improve clarity
(avoid repetitions and present a linear scheme).

Minor points:

1. In Material and Methods section, information is missing on 1. antibodies used
for western blot analysis (pg 9, line 2); 2. reverse primers used to amplify miR-16
and U6 (pg 9, line 2) 3. database/software tools used to predict miR-16 targets.
The two paragraphs about qPCR should be merged in a single one.

2. 4T1 murine breast tumor cell line growing upon sc injection in syngeneic
BALB/c mice cannot be defined a ‘xenograft’ model.

3. Authors should correct M1 with M2 and vice versa in the Abstract (line 11) and
in the Results section (pg 13, line 18-19).

4. Legends to the figures 2, 3, 4 (pg 26) and the figures should consistently
indicate if TAM used as control were treated with exosomes isolated from
untreated 4T1 cells, and if 4T1 cells were stimulated or not with EGCG.

5. Figures 3 and 4 are inverted. Statistical analysis is missing.

6. In figures 3, 4 and 5 specify that results are expressed as 2-##Ct.

7. Figure 5, what is DW should be specified. The legend in Figure 5G explaining
black and white bars is missing.

8. In Figure 6, IL10 is indicated instead of IL6.

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