Author's response to reviews

Title: Interaction and uptake of exosomes by ovarian cancer cells

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Version: 2 Date: 3 February 2011

Author's response to reviews: see over
Dear Editor,

I am submitting a revised version of the manuscript entitled “Interaction and uptake of exosomes by ovarian cancer cells” following the comments of the reviewers.

The changes made are contained in the following pages.

Hoping that the manuscript can be considered for publication in BMC Cancer,

Yours sincerely,

Dr. Júlia Costa
Principal Investigator
Head of Laboratory of Glycobiology
Reviewer’s report

Title: Interaction and uptake of exosomes by ovarian cancer cells

Version: 1 Date: 23 December 2010

Reviewer: Adrian Morelli

Reviewer’s report:

In the manuscript entitled “Interaction and uptake of exosomes by ovarian cancer cells” by C. Escrevente and colleagues, the authors showed the internalization of CFSE-labeled exosomes by ovarian tumor cells in vitro and explored the mechanisms of internalization of the nano-vesicles. They also studied the glycol-phenotype of the exosome surface and its potential role during their internalization by the ovarian cell line. Although, internalization of exosomes by different types of cells is not novel and some of the mechanisms have been already elucidated, the most important part of the study is in the second half of the ms, where the authors analyzed the pattern of glycolproteins expressed by the exosomes and their potential role during the uptake of the vesicles by the ovarian cells. The ms. is modest, but technologically well done. I do not have major comments save the fact that the text of most sections should be shortened, since the ms. is not a review on exosomes.

The text has been shortened by 17% based on word count.

Reviewer’s report

Title: Interaction and uptake of exosomes by ovarian cancer cells

Version: 1 Date: 19 December 2010

Reviewer: senfang Sui

Reviewer’s report:

Major Compulsory Revisions

General comments:

This manuscript shows that exosomes derived from the ovarian cancer SKOV3 cell line are internalized by the same cells. The authors generate exosomes labeled with CFSE, and show by light and flow cytometry that they are internalized via various endocytic pathways, and the internalization is energy-dependent and requires proteins from the cells and the exosomes. The
Exosomes were found to be enriched in specific mannose- and sialic acid-containing glycoproteins that could constitute exosome markers, but sialic acid removal seemed not to play a critical role in exosomes uptake. Broad cellular source and different protein and lipid composition of exosomes make the studying of exosomes uptake very complicated. Although this topic has been extensively studied elsewhere, the molecular detail concerning exosomes internalization in their recipient cells is still not very clear. In general, this paper provide some useful information about SKOV3 cell exosomes uptake, and it has some novelty in showing glycoproteins of exosomes derived from this kind of cell, though the current study is not in-depth. Thus, the manuscript may provide some clue for characterizing specific glycoproteins markers localized in exosomes from other cellular origin.

Specific comments:

1. In figure 1A, there are some colocalizations between “DAPI” and “CFSE”, why?

It is possible that there is fluorescence crosstalk between the two channels. This picture should be replaced.

Higher intensity green zones corresponding to CFSE-Exos detected with the “I3” filter from Leica (excitation range blue, BP 450 – 490) also faintly stained in blue using the “D” filter from Leica (excitation range UV+violet, BP355-425). Fluorescence crosstalk between the two channels could be admitted. Since we have not been able to clarify this matter, we propose to delete Figure 1A since Figures 1B and C provide enough evidence for the point that is made (uptake of exosomes by SKOV3 cells).

2. The authors stated that exosomes are taken up via various endocytic pathways, however, in figure 2B, why is the fluorescent signal of internalized exosomes so weak in most cells compared with the single cell in the “inset” in the same picture? The uptake efficiency is so low that one may think exosomes are not efficiently internalized in these cells.

Figure 2B has been corrected: i) the figure for colocalization with EEA1 has been replaced by another where exosomes are more visible; ii) visualization has been ameliorated. For that brightness and contrast were adjusted in order to better visualize the Exos-CFSE and the markers EEA1, LAMP1 and caveolin1. ImageJ software was used and the same adjustments
were made in all pictures (red – 8/245; green – 21/57). Then, the 2 RGB colors were merged and brightness and contrast were again adjusted similarly in all pictures (18/132). The inset has been corrected using Photoshop. The zone of interest was selected and the inset was prepared as follows: i) the area was selected and the following option was chosen: edit, preferences, general, interpolation, nearest neighbor image; ii) the selected area was copied to a new layer and magnified 100% to 400%. Using this procedure without other adjustments the image in the inset has a comparable intensity to the figure not magnified.

3. Also in figure2B, the scale bar is not correct. Obviously, the magnification is not the same between the “EEA1” and the other two pictures. This has been corrected.

4. In “results” section, first part “Uptake of SKOV3 exosomes by SKOV3 cells”, the fifth paragraph, since exosomes also contained lipid rafts domains, methyl-beta-cyclodextrin incubation may also disrupt the integrity of exosomes, thus affecting the exosomes uptake. I would suggest methyl-beta-cyclodextrin may first be incubated with the cells then washed out before adding exosomes for chase. The authors should make the protocol clearer in materials and methods. The protocol has been made clearer in “Materials and methods” (page 8, second paragraph). A sentence considering the possibility that methyl-beta-cyclodextrin could also affect exosome integrity has been added in “Results” (page 11, first paragraph).