Author's response to reviews

Title: Extracellular vesicles: potential applications in cancer diagnosis, prognosis, and epidemiology

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Version: 2 Date: 22 February 2015

Author's response to reviews:

The manuscript has been revised based on comments from both reviewers. A point by point description is given below.

Reviewer 1: Vilma Martins

Reviewer's report:

In this paper, the authors have pointed the recent progress in understanding extracellular vesicles (EVs) biology and the role of them in cancer, discussing the utility of EVs for cancer diagnosis, prognosis, epidemiology and therapeutic opportunities. Although the subject is interesting, I found that the manuscript is a bit preliminary and skinny. The following issues will need to be adequately addressed prior to acceptance for publication.

Major Concerns:
(1-) In introduction part, the authors have cited 29 references; however 20 of them are literature review. It is suggested that the authors include high impact research articles. For example, in the second paragraph, the authors could cite different manuscripts describing the extracellular vesicles secretion by platelets, neutrophils, reticulocytes, macrophages, megakaryocytes, monocytes, B and T cells, mast cells, and endothelial cells.

Response:
Original references have been included and reviews have been removed.

(2-) At the beginning the second paragraph, the authors summarized the cancer development and progression; however, they did not cite any reference.

Response:
Reference has been added.

(3-) In the third paragraph of the introduction, for the sentence “These changes include the development of EVs and release of cargo that may serve as biomarkers for disease diagnosis, prognosis, and epidemiology”, the authors cited references related to circulating tumor cells not to EVs (references 12 - 16).
Response:
This point has been corrected.

(4-) About EV biogeneration, the authors have not described the endocytic and Exocytic pathways. It is important describe the proteins involved in the different steps of endocytic and exocytic pathways, such as Alix, Tsg101, Rab5, Rab7, Vps4, Vps36.
Response:
Both pathways with new references describing different proteins have been added in the revised manuscript.

(5-) In EV biogeneration part, the authors could describe in a separate paragraph the role of E6/E7 proteins in MVs composition and secretion. Moreover, they could present other proteins that are involved in the regulation of EVs releasing. Recently, Ostrowski et al. (2010) showed that Rab proteins (Rab27a and Rab27b) control the exosome secretion pathway.
Response:
The specific reference identified by the reviewer and related information have been added.

(6-) In EV biogeneration part, the authors mentioned that lung cancer cells release MVs into circulation and into lung pleural effusion. The authors could develop a separate paragraph about this issue, including more research articles.
Response:
A separate paragraph has been developed based on reviewer’s comments.

(7-) In using EVs to determine cancer aggressiveness part, the authors could describe the participation of exosomes in the formation of primary tumors and metastases (Peinado et al. 2012).
Response:
Based on the information in this specific reference, new information has been added.
(8-) In the page 4, line 3, the authors describe the content of EVs, and did not cited the Thakur, 2014 paper in Cell Research, where is described the presence of double-strand DNA inside EVs. Theory described by Lee in Biochemical and Biophysical research communication, 2014.

Response:
Both references have been added.

(9-) In page 9, lines 17 to 23 and page 10, lines 1 to 4, authors back talk about EVs as a biomarkers in a paragraph intended to describe the therapeutic purposes of the EVs. These sentences need to be relocated.

Response:
Sentences have been relocated.

(10-) Is there reference to support the propose to remove vesicles to prevent metastasis and tumorigenesis (in page 11, line 2 and 3)?

Response:
A new reference with description has been added.

(11-) In page 16, during figure description, in line 5 authors say that “exosomes also can be formed directly from the plasma membrane”, without reference. The current literature show that microvesicles can be formed directly from plasma membrane, not exosomes.

Response:
This point has been corrected.

(12-) The title of the Figure 1, does not describe the entire content of the figure. The figure does not explain the process of generating EVs, and comprise others aspects not related with the title.

Response:
Figure legend has been modified.

Minor Concerns

(1-) Page 4, line 17, maybe the authors would like to write about the involvement of EVs in intercellular communication not intracellular communication.

Response:
The involvement of EVs in both types of communications has been described in the revised version of the manuscript.
In figure 1B, C and D the proportions of the organelles (Golgi and Endoplasmic Reticulum), nucleus, MVB and EVs are not correct

Response:
Corrected figures with revised legend have been added.

Reviewer 2: Rui Henrique

Reviewer’s report:
This review is focused on the role of extracellular vesicles in tumorigenesis and on how this knowledge may provide relevant information and tools for cancer patients’ management.
Despite the wealth of existing information on this subject, the manuscript is rather short and it is not always apparent why certain data is mentioned and other is not.
Along the texto there is sometimes a lack of clarity as well as the repetition of ideas (e.g., the potential epidemiological impact of EV which is referred to several times).

Response:
The manuscript has been revised based on these comments.

Language could be refined to provide a more dynamic texto with a better connection between sentences and statements.

Response:
Special attention has been paid for better connection between sentences and statements.

Major compulsory revisions
- The section on EV biogeneration would benefit from a more detailed and updated description of the process, the mechanisms involved and the main players

Response:
This section has been added.

- In the EV functions, there is a lack of information concerning the genomic DNA content. Some recent studies using NGS found that double-stranded genomic DNA spanning all chromosomes may be detected in the serum exosomes of
patients with pancreatic cancer (Kahlert et al, 2014). This type of information must be included

Response:
New reference have been added per reviewer’s suggestions.

- The section on methodology for isolation and analysis of EV will benefit from the inclusion of a Table summarizing the most relevant of those issues. More updated references should be used.

Response:
Methodology section and a new table have been added.

- The section on the pathological functions of EVs seems to be misplaced as it would sound more rational to place it before the use of EVs in diagnosis.

Response:
The pathological section has been moved based on reviewer’s suggestion.

- The first paragraph in page 10 does not seem to belong to a section on therapeutic purposes and should be re-located to another section.

Response:
This specific paragraph has been moved.

- There are many references which are themselves reviews on the subject or related subjects. Although this contributes to the economy of the text, it fails to provide the most important and higher impact references in the field which are the original reports.

Response:
High impact references have been included.

- In Figure 1 legend, it is stated that exosomes may also be formed directly from the cell membrane. This is not stated in the text (namely on page 3, line 10). Moreover, no reference is provided for this statement.

Response:
These points have been corrected in the revised version of the manuscript.

Minor essential revisions

- Page 3, line 7, should read "endolysosomal"

Response:
This point has been corrected.

- Page 5, line 13: please replace "lung pleural effusion" by "pleural effusion due to lung cancer"

Response:
This point has been corrected.

- Page 9, line 9, should read "neoplastic cell-to-normal cell"

Response:
This point has been corrected.

- Page 12, line 2, should read "providing"

Response:
This point has been corrected.

- In Table 1, in several places, it should read "was conducted on"

Response:
This point has been corrected.