Dear Dr. Sarah R. Vaiselbuh,

Thank you for submitting your manuscript to Cancer Research Frontiers.

We have carefully considered your manuscript, and feel that **we might be able to accept it if you could respond adequately to the points that have been raised during the review process. Therefore, the decision is "Revision".**

We invite you to submit a revised version of the manuscript. You will need to provide:

1. A cover letter that includes a point-by-point response to all of the issues raised in the reviews. (See reviewers’ comments attached below.)
2. The manuscript (use the attached version) marked with your changes in order to facilitate review of your revisions. You may use Word with Track Changes, highlighting or colored text to indicate changes.

Please email back your revision within 30 days of the date of this decision.

If you choose not to submit a revision, please notify us.

Yours sincerely,

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**Reviewer 1: Minor revision**

In this paper, Dr. Vaiselbuh reviewed the roles of exosomes in cancer niche development, cancer diagnosis and prognosis as well as exo-therapy. It is a timely, helpful and detailed review of exosomes in cancer review; however, some questions need to be discussed:

1. Exosomes in the body fluid are accessible biomarkers for cancer diagnosis. However, what about their specificity, do they have markers indicating their origins and targets?
2. The methods how to isolate exosomes as well as how to analysis their contents are important for investigating exosomes in cancer research. They might be included in this review if possible.

**Reviewer 2: Minor revision**

This mini-review is on the whole a well-formulated contribution of current interest. There are some aspects that should be considered by the author, they are:
1. Historical background of exosomes. Historical aspects are often a ticklish task to deal with. There is no international consensus about the definition (and nomenclature) of extracellular vesicles (EVs) among which exosomes are prominent. Also, a majority of reports, so far, on exosomes are carried out on exosomes delivered by various cells grown in vitro while only a minority describes exosomes isolated from physiological media (body fluids). Hence, as given in a review by Marian Aalberts et al, Reproduction, 147 (2014)1-14, EVs (exosomes) in seminal plasma and prostatic fluid were first reported in the 1970s. Subsequently these vesicles were found to be exocytosed by the epithelial cells of the prostate gland and therefore formally could be denoted exosomes (at that time the generality of vesicle release from cells was not known) and the vesicles were denoted “Prostasomes” as given in the first review of 1985 on prostasomes/exosomes (Biochim Biophys Acta 822 (1985) 203-218). Another recognizing characteristic of exosomes is their unprecedentedly high membrane cholesterol/phospholipid ratio yielding high molecular ordering (Biochim Biophys Acta 984 (1989) 167-173).

2. The author discusses meritoriously the role of various forms of RNA in exosomes but should mention Argonaute proteins, which are known to bind miRNAs and transport them to recipient cells, e.g. vesicles in blood plasma, associated with Argonaute2, the key effector protein of a miRNA-mediated gene silencing mechanism (Proc Natl Acad Sci U S A 108 (2011)5003-5007).

3. The author should also briefly discuss the role of exosomes in multidrug (chemotherapy) resistance (MDR). One of the mechanisms leading to the MDR phenotype is the activation of efflux proteins belonging to the ATP-binding cassette (ABC) transporter superfamily. P-glycoprotein and the multidrug resistant protein 1(MRP 1) are members of this family with a putative role in cancer chemoresistance. There is an interesting recent report on MRP 1 localization in lipid raft domains and prostasomes in prostate cancer cell lines (OncoTargets and Therapy 7 (2014) 2215-2225).

Minor points:

Page 4, exosomes biogenesis in cancer. The second sentence (lines 3-5) is unclear to me. The first formation results in a so called “early endosome”(by invagination of the plasma membrane) and the second inward budding (on multiple loci of the endosomal membrane) gives rise to the intraluminal vesicles (forming the multivesicular body, MVB) that, after fusion between the membrane surrounding the MVB and the plasma membrane, are released as exosomes extracellularly.

The distinction between the findings of the Nobel laureates and the exosomes is not clearly expressed. These laureates studied the mechanism of release of solitary (free) molecules (extracellularly, either in an endocrinological system or a neurotransmitter system) confined within intracellular membrane-surrounded secretory granules (cf.: Douglas’ work in the early 1970s) and how the granules reached the plasma membrane and the fusion event between the two membranes resulting in the secretion of the granules’ molecular content (hormones or neurotransmitters). In case of exosomes, membrane surrounded vesicles were released extracellularly to reach (with their cargo) the target cells for delivery of a complex assembly of bioactive molecules.

Page 5, first line. The word “shedding” is not properly used in this context. Here it is understood “from the plasma membrane” meaning the formation of so called “ectosomes” that should be distinguished from exosomes.

Page 9, next last line. What is onco-exosomes? Does the author mean “oncosomes”?

Reviewer 3: Minor revision

The review by Dr. Vaiselbuh is dedicated to the role and diagnostic potential of exosomes in cancer. Exosomes are secreted, nano-sized vesicles that contain RNA species and proteins protected by a lipid bilayer containing specific transmembrane proteins. The biogenesis and role of exosomes in cancer is
discussed. The largest part of the review is dedicated to the clinical utility of exosomes in cancer: its diagnostic and therapeutic potential. The figures and tables are very informative.

Minor Remarks

P5: please update the online exocarta database with two new recent initiatives such as vesiclepedia and EVpedia


P6: Schonnenschein should be Sonnenschein

Exosomes are difficult to discriminate from protein aggregates and lipoprotein complexes; especially if using complex biological matrices. A recent effort comparing different exosome isolation methods revealed a disparity in the yield and content of “exosomal” RNA. The results of this study clearly reveal that minimal experimental requirements are necessary to define exosomes.


figure 1
why become the cells larger from top to bottom??
Nucleus and endosome should have similar size in all cells. Is there evidence that MVBs in cancer cells are larger than MVBs in normal cells.

(end)