Reviewer’s report

Title: Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma.

Version: 1 Date: 23 Nov 2017

Reviewer: Guy Adami

Reviewer's report:

"Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma," has been greatly improved. There are still some things that need to be made clear and several that need to be altered but hey do not retract from the value of the paper that discusses the analysis of miRNA from salivary extracellular vesicle obtained by a new proprietary method.

Line 268 Finally, EVs from OSCC patients showed an up-regulation of 55 miRNAs, compared to controls (Table 4).

If the change is not statistically significant then it is not a change - otherwise it is confusing.

Line 184 SnoRNA RNU6B and miR-191 were used as endogenous controls. They were previously described as stably expressed in saliva samples [18-20] and qRT PCR array analysis showed a stable expression in tested samples.

Saliva and extracellular vesicle from salvia are not the same thing. I am not sure if all those references are necessary. What is more important is that the reference RNAs chosen are constantly present and show minimal between sample variability versus the common miRNAs.

Figure 2B If p values are given for two miRNAs that do not show a significant difference but trend toward significance then all 4 miRNA in 2B should be labeled with the p value, miR-337-5p and mir-520d-3p.

Line 280 The qRT-PCR analysis of miRNAs detected only in patients by qRT-PCR array showed that miR-27a-3p, miR-337-5p, miR-494-3p, and miR-520d-3p were overexpressed in patients but still present in controls (Fig. 2B).
How was the decision made to focus on these miRNAs present only in OSCC patients in the first assay, and not test the remaining 4 miRNAs from Table 3.

Table 2 please define RQ in footnote.

Line 384

In the literature as up-regulated in whole saliva or plasma of OSCC patients and have been proposed as biomarkers. For example, we observed an up regulation of both miR-31-5p and miR-31-3p in OSCC patients compared with controls (Table 4). Several studies have reported that miR-31 is overexpressed and/or involved in OSCC [58-63].

This must be change. Table 4 does not show a statically significant change. So you should not write that it changed.

Why is it written that saliva and saliva extracellular vesicle give the same miRNAs (Line 321) when there are very different results than what some of the other references looking at saliva miRNAs in HNSCC and normals? For example, the results are quite different from that of Momen-Heravi F., 2014, reference 20, yet there is no discussion of that and it is used a s a reference for choosing internal standards for RNA. That does not really make sense.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Unable to assess

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics
Quality of written English
Please indicate the quality of language in the manuscript:
Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.