Reviewer’s report

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

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Reviewer: Guy Adami

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Salivary extracellular vesicle-associated miR-512-3p and miR-412-3p as potential biomarkers in oral squamous cell carcinoma by Gai et al. is about an important subject area which has been somewhat confusing. It is quite hard to get accurate measurements of saliva miRNAs. The authors may be on to something by using extracellular vesicles but there needs to be more evidence that what they see is reproducible.

For analysis of miRNA levels by microarray there needs to be a false discovery rate, not a p value that is not corrected for multiple testing. Given the small number of samples tested this will only provide guidance on which miRNAs to focus on for the Rt-PCR quantitation. It is unclear if the subjects for the microarray assay and the Rt-PCR assay overlap. This is of most importance for the healthy controls as there may have only been 6 total. It is really for the RT-PCR data where the statistics are most important. For that reason it would be nice if there were 5 more OSCC subjects and 5 more healthy controls to verify the results shown. I would strongly recommend that. The ROC curves are nice but they are not quite enough to validate the value of these miRNAs shown in figure 2 as OSCC salivary vesicle markers.

It would be good to describe how miRNA were quantitated for the microarray assay. In regard to RNA controls, were all RNAs used?

Protein assay gave an interesting result. Was that done at least twice?

One sided Mann Whitney test comparing RT-PCR derived expression levels may not be ideal, why not used two -sided test?

"pathway analysis was performed by DIANA-mirPath v.3.0 [21] online software. miRNA targets were searched on microT-CDS [22]."
Pathway analysis based on miRNA target sequences in mRNAs that are linked to function has its problems. It is probably good that the authors chose to use nonexperimentally validated miRNA targets - microT-CDS. The authors may want to stress even more the speculative natures of its prediction of pathways.

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

Yes

**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

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I am able to assess the statistics

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