Author’s response to reviews

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

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Author’s response to reviews:

To the editor of BMC Cancer,

Enclosed please find our manuscript BCAN-D-17-00903 revised according to Reviewer suggestions and a point by point response to Reviewer comments. All changes to the manuscript are colored in purple.

As you suggested, we asked to a native English speaking colleague to copyedit the manuscript.

To Reviewer 1

We thank the Reviewer for the comments and suggestions.

1) As suggested by the reviewer we now specify that the multiple test correction was not done. The information has been added in Materials and methods, line 223, page 11.
2) The normalization of individual qRT-PCRs was performed using snoRNA RNU6B and miR-191, as described in Materials and methods, lines 184-186, page 9.

To Reviewer 3

We thank the Reviewer for the encouraging comments and we followed the suggestions to furtherly correct and improve the manuscript.

1) We agree that, if the change is not statistically significant, there is no change. Therefore, according with reviewer suggestion, table 4 has been removed and the data have been added to supplementary materials in Additional file 4: Table S2, reporting the entire results from array analysis. The sentence indicated by the reviewer has been removed (Results, line 270, page 13).

2) We totally agree with the reviewer that saliva and salivary EVs are not the same thing and the content in miRNA and other molecules may vary a lot. Nevertheless, endogenous controls are usually stably expressed in different specimens, but also in different sources, such as cells and extracellular vesicles. For instance, SnoRNA RNU6B is commonly used for both cells and EVs. Unfortunately, a few data are available on endogenous controls in salivary EVs, so we focused on miRNAs which were reported to be stably expressed in saliva. Then, based on array results, we selected the miRNA stably expressed in all the tested samples. qRT-PCR experiments on the larger cohort of patients confirmed that RNU6B and miR-191 were expressed in all samples with low variability among samples, and thus suitable as endogenous controls, as described in Materials and methods, lines 183-187, page 9.

3) Figure 2B has been modified according to reviewer’s suggestions.

4) We now provide a better explanation. We performed qRT-PCR on all eight miRNAs detected only in patients, as described in Materials and methods, line 180-182, page 9. Five miRNAs resulted to be increased in patients compared to controls, but not significantly. Two miRNAs were confirmed to be expressed only in patients, while miR-645 was comparable to controls. Results are shown in figure 2B and described in Results, line 277-289, page 13-14.

5) We now define RQ in foot note in table 2.

6) According with reviewer suggestions, the manuscript has been corrected, see Discussion, lines 384-395, page 19.
7) We agree with the reviewer that the sentence at line 321 can be misleading, thus it has been removed. As previously discussed, we completely agree that the content in miRNAs can vary from whole saliva and salivary EVs. For this reason, our results were not directly compared to results obtained from whole saliva specimens.

Since the aim of this work was the detection of candidate biomarkers, the discussion was focused on the analysis of other findings supporting the possible role of the candidate miRNAs in the pathology. On the other hand, some possible explanation for differences of our results compared to the literature are discussed at lines 395-398, page 19. On the basis of reviewer suggestion, a few lines on the possible differences of salivary EVs and saliva have been added, see Discussion, lines 398–402, page 19.