Reviewer's report

Title: HMGA1 and HMGA2 expression and comparative analyses of HMGA2, Lin28 and let-7 miRNAs in oral squamous cell carcinoma

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Reviewer: xiaomei lu

Reviewer's report:

Sterenczak KA and colleagues report the trend that HMGA2 was consensus higher in OSCC from both human and dog, whereas HMGA1 showed no significant expression variation and that miR-98 was down-regulated in human but let-7a and miR-98 was up-regulated in canine OSCC tissue samples, based on rather limited number of cases. The trends reported in the study is of interest and of potential clinical importance, albeit not as novel as suggested by the authors. At least, some of the main conclusions have already been described for other types of cancer cell lines and tumors. It could have been better had the authors analyzed HMGA1/2 or let-7 family members’ expression with clinico-pathological information of OSCC from human or dog. The article is well-written; methods used are well-described and appropriate, however, the data and conclusion drawn were not experimentally solid on the rather limited number of cases, which is also my most concern.

Major comments:

(1) In introduction section, the introduction would benefit from shortening, which was wordy and too long to read and contains some redundant information.

(2) In material and methods section, the number of tissues from human and canine respectively was too insufficient to provide statistical analysis. Moreover, the staging and grading of the tissue samples from canine should be presented as did the tissue samples from human.

(3) In material and methods section, all the real-time RT-PCR primers as well as accession numbers in Gene bank involved in the present study should be provided either in manuscript or in supplementary, to make reproducible and allow for readers’ following.

(4) The relevant results (qRT-PCR and IHC) regarding HMGA1 should be juxtaposed with HMGA2 in results not mentioned and placed in supplementary.

(5) In statistical analysis, the specific statistical methodology employed in the study needs to be provided. Such as student’s test, one-way ANOVA or rank sum test. It was too broad to say p value <0.05 was considered statistically significant.

(6) In discussion part, the conclusion of the study is very likely strongly biased by the choice of so limited cases from both human and dog as it stands. Considering the great variability between individuals, all the trends described
here is at best suggestive rather than conclusive, and the correlation between HMGA1/2 and let-7 family is indirect and inferable rather than direct and experimentally solid in light of lack of Luciferase reporter assay on cell line level.

(7) There were some overstatements of significance of results in the discussion and over-interpretation of existing literatures. The discussion section would also benefit from shortening and succinctness.

Such as

In discussion section, page 17, line 14 “our findings in humans and dogs strongly support……in both species”, I disagree with these statement. Because, there is no data at all provided in this paper that relates to “prognosis”. So, it would be suggested that the authors should delete the statement.

In discussion section, page 18, line 20-23, the statement that “our immunohistochemical findings in canine ……at the invasive front in canine oral cancer”, is also overstated. In the study, there is no data presented at all that supports the role of HMGA2 in the cellular behaviors in OSCC. Suggest deleting these statements without experimental evidence.

In discussion section, page 19, line 2-4, the statement that “furthermore, miR-98 was ……and miR-98 in OSCC cell line samples” was also lack of evidence results. The correlation between miR-98 and HMGA2 is at best inferable without the biochemical analyses (classical luciferase reporter assay), and may be biased due to rather limited cases in the case of individual variability. So, it would be better to rephrase or delete.

(8) In the reference part, both the volume and page number were missing in the reference [33] and [34], please check it.

Specific comments:

Figure 1. Figure 1A and 1B was alphabetically mislabeled with C and D, please check it.

Figure 1A and 1B were factually the same results showing the same information, which plotted with different internal control. Suggest choosing one from the two kinds of similar results presented. In the legend to figure 1, the statistical method used should be detailed rather than presented with p value. The same holds true for Figure 2A and 2B.

Figure 3. Without comparison with normal control tissues, whatever human or dog, it is not clear how to interpret the immunohistochemical data. What's more, regarding Figure 3 and figure 4, the magnification labeling should be uniform, either labeling with scale bars or magnification times. Additionally, The IHC results shown should be quantified, at lest semi-quantified. As stated, the author employed weak, moderate and strong to semi-quantitatively evaluate HMGA2/1 expression, however, there is no mention that the quantitation of HMGA2/1 in figure legend as well as results section.

Figure 5. In the legend to figure 5, as stated above, the statistical method used
should be detailed and provided; moreover the calculated method of HMGA2 and miRNAs should also be given both in legends and manuscript where appropriate. Take, for example, regarding miRNAs 2-##t method was properly used whereas normal mRNA of gene of interest, standard curve method using proper standard to construct calibration curve for real-time RT-PCR may be first of choice. The same holds true for the legend to figure 6.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

Declaration of no competing interests