Author's response to reviews

Title: Epigenetic regulation of matrix metalloproteinases expression in ameloblastoma

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Author's response to reviews: see over
Epigenetic regulation of matrix metalloproteinases expression in ameloblastoma

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Answers to the reviewers:

Reviewer 1:

Methods:

Biological feature of tumor is related to its histological type. Therefore, you should clarify the histological type of ameloblastoma were used in this study.

As suggested by the reviewer, we included the histological type in the Methods section. The samples comprised eleven solid-multicystic follicular ameloblastomas and one unicystic case.

Result and Discussion:

You should describe the histological findings of ameloblastoma were used for this study. If possible, please discuss relationship between the epigenetic regulation of MMPs and histological type of ameloblastoma.

As all but one ameloblastoma samples were follicular type we could not analyze the molecular data according to the histological type.

Reviewer 2:

Authors should specify which type of ameloblastoma belong (desmoplastic ameloblastoma, unicystic ameloblastoma, solid-multicystic ameloblastoma or peripheral ameloblastoma). Specify the variant of ameloblastoma of this study. Exist some differences in the epigenetic regulation of matrix metalloproteinase expression in the different variants of ameloblastoma?

The clinical and histological types of ameloblastoma were included in the methods section. One case was unicystic, while all the others were solid-multicystic ameloblastomas. So, we could not test any association between tumor type and the molecular results.

The reviewer consider important describe in the table 1, the variants of ameloblastomas studied, and a more precise location (eg., Anterior region, mandible body, and posterior region).

As most of the cases were solid follicular, we have included this information only in the methods section. As suggested, the specific location was included in the table. Thank you for this comment.

Discretionary Revisions
Matrix metalloproteinases are involved in the degradation of collagen. If within the sample there was a desmoplastic ameloblastoma would be interesting that the authors discuss what is role played by MMPs in the demoplasia.

Although this is an interesting suggestion, there wasn’t any case of desmoplastic ameloblastoma.

Reviewer 3

Major compulsory revisions:

The authors have evaluated the methylation status, protein expression and transcription of MMP-2 and 9 in ameloblastoma to assess its role in the expression of genes in ameloblastoma behavior. Though it is a novel study, the authors have concentrated more on the methylation status and have not related this to the transcription and protein expression and their relevance in detail. The discussion needs elaboration and the findings are not clearly presented. What were the histological types of ameloblastoma used, would the histological type show variation in these genes? Did the gingival samples exhibit inflammation which I assume is a distinct possibility, if so would that affect the expression? Why was protein expression not done on gingival samples? Overall the results have not been discussed clearly, make it simple concise but more clear interpretation of the results is warranted. Once answered, the article the article can be published.

Thank you for these comments. We incorporated in the discussion comments about the importance of methylation to gene and protein expressions. All the ameloblastomas showed increased transcription and MMP-9 protein expression and were mostly unmethylated for MMP-9, so it was not possible to assess if the transcription of the gene was correlated with its methylation status. However, our study suggests that the increased transcription of MMP-9 in ameloblastoma could be possibly influenced by unmethylation of the gene. As all but one ameloblastomas were follicular type, we could not analyze the molecular data according to the histological type. The clinical and histological types of ameloblastoma were included in the methods section.

It is well established that inflammation is a condition that influences methylation profile. To avoid this bias in the study, particularly in regard to control samples, we analyzed clinical gingival inflammation before collecting the gingiva samples by Gingival Bleeding Index. No bleeding on probing was identified in the cases collected. Therefore, the gingival samples did not show clinical evidence of inflammation. This information was included in the manuscript. We included in the results section that analysis of protein expression in healthy gingiva was not performed due to scarcity of tissue samples. We feel that by incorporating your suggestions, our manuscript has now improved and information are more clearly presented.