Reviewer's report

Title: S100A16 Promotes Differentiation and Contributes to a Less Aggressive Tumor Phenotype in Oral Squamous Cell Carcinoma

Version: 3 Date: 10 May 2015

Reviewer: Tamotsu Kiyoshima

Reviewer's report:

Comments to the Author

The authors, through their paper entitled "S100A16 Promotes Differentiation and Contributes to a Less Aggressive Tumor Phenotype in Oral Squamous Cell Carcinoma" provide some interesting information regarding the tumor suppressive function of S100A16 in OSCC. Although this manuscript evokes interest in me, I have some concerns that need to be addressed. Minor Essential Revisions are required before publication.

Some suggestions are listed below:

Although authors used a composite score that took into account not only the number of positive cells, but also the grading of the immunostaining intensity to more accurate the interpretation of immunohistochemical analysis, it should be specified followings;

1) how many microscopic fields were investing, the objective used and how many cells were counted (minimum 5000 cells),
2) how the authors decided the criteria for the grading of the immunostaining intensity,
3) how many pathologists performed immunohistochemical evaluation,

Authors collected 82 OSCCs (FFPE) in this study. Immunohistochemical data was based on only 65 OSCCs (FFPE). Please let me know the reason.

S100A16 showed a strong membranous expression in the committed/differentiating epithelial cell layers. Meanwhile negative or weak cytoplasmic staining of S100A16 was found in the basal cell layer (stem cell compartment). Please discuss the mechanism underlying different regulation of membranous and cytoplasmic S100A16 expression between supra-basal and basal layers.

The discussion related to the function of S100A16 in OSCC is too superficial. The putative mechanism underlying down-regulation of keratinocyte differentiation markers by reduced S100A16 expression and/or the relationship between keratinocyte differentiation markers and S100A16 should be discussed in the "Discussion" section. Additionally, if authors have ideas, add the description of the putative mechanism underlying down-regulation of S100A16
expression during OSCC carcinogenesis.

L38: This part is “Methods” part in the “Abstract”. Could authors please confirm which “was correlated” or “was examined the correlation” is suitable?

L122: Authors commented “NHOM, 31 formalin fixed-paraffin embedded (FFPE) and 44 frozen” below. Authors should confirm which “65” or “75” is correct?

L299: Please confirm which “score was down-regulated” or “score was decreased” is suitable?

Add the explanation of the dotted/slashed bars in the Figure legends of the Figure 5.

In Tables and supplemental Tables, the space should be inserted between a number and “(“.

In addition, several typos and grammatical errors are still remained in the manuscript. “functions” (L61), “perforemed” (L284), “S100A16- xenografts” (L446), "S00A16" (L809) and so on should be correct in the revised version. Please carefully and carefully check and correct point-by-point.

**Level of interest:** An article of outstanding merit and interest in its field

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

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests' below