Reviewer’s report

Title: Twist and Snai1 expression in pharyngeal squamous cell carcinoma stroma is related to cancer progression

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Reviewer: Patricia Reis

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Reviewer’s comments

The manuscript by Jouppila-Mättö et al. entitled “TWIST and SNAI1 expression in pharyngeal squamous cell carcinoma is related to cancer progression” assessed the expression of TWIST and SNAI1 proteins, by immunohistochemistry, in 109 pharyngeal carcinomas. The authors correlated the expression of these proteins in tumor epithelial cells and tumor stroma with clinical data of patients and tumor histopathological characteristics. They found that strong staining of TWIST protein in tumor stroma was associated with tumor relapse. In addition, TWIST and SNAI1 positivity in the stroma was detected mainly in tumors stage II or more advanced stages. Tumors that did not stain for TWIST and SNAI1 proteins in the stromal cells were mostly T1-T2 category and stages I and II of disease. The authors also reported that the absence of TWIST and SNAI1 staining in the stroma was significantly associated with a better 5-year disease-specific survival and better overall survival. The authors conclude that TWIST and SNAI1 expression in stromal cells is associated with tumor progression, and that negative expression of these proteins in the tumor stroma predicts better patient outcome.

The study methods are straightforward, appropriate and well defined; however the study hypothesis is not clearly defined in the manuscript. The authors’ conclusions need revision, since the authors did not show direct evidence for the role of TWIST and SNAI1 in pharyngeal tumor progression; they rather showed an association between strong intensity of staining of these proteins in the tumor stroma and clinical and histopathological characteristics (such as larger tumor size, advanced tumor stage) that indicate progressive disease.

Minor essential revisions

1. The authors need to correct the citation of genes and proteins throughout the manuscript: they should use capital letters for human proteins (e.g., TWIST, SNAI1) and capital italicized letters for human genes (e.g., TWIST, SNAI1).
2. The authors should replace “strong protein expression” by strong staining intensity”, as this is more appropriate for studies using immunohistochemistry.
3. The study hypothesis needs to be clearly defined in the manuscript.
4. Please add details about the patient sample selection - it appears that the patients were randomly selected, or were the patients chosen based on tumor
characteristics, disease stage or outcome?

5. In Figure 1, Panel D, the authors show positive SNAI1 staining in stromal cells; however there are few cells that are staining for SNAI1 protein. Which score was given for this particular case? Also, please indicate the tumor grade and stage for the samples shown in the figure.

6. The graphs showing disease-specific survival and overall survival analysis require better resolution and larger size, for improved visualization of the survival analysis results.

7. In Figure 2, the authors state that survival was poorer according to the number of transcription factors expressed in the stroma. This statement, which is also found in other parts of the manuscript, gives the reader the impression that several transcription factors were analyzed, while there were only two proteins analyzed in the study. The authors should simply state that patient survival was poorer when both transcription factors were positive in the tumor stroma.

8. On Page 8 (Discussion), there are grammatical errors and a typo that needs to be fixed. For example, "this data" should be replaced by "these data". The typo is in the word "condected", which should be "conducted".

Major compulsory revisions

1. In the Discussion section, the authors state that smoking can modulate the expression of EMT markers. This is an interesting finding or hypothesis that should be expanded and explained in more detail.

2. In the Discussion, the authors state that “there is variation between classifying strategies between different publications”, but they do not explain it further. This needs to be expanded and clarified for the reader.

3. The major point in the Discussion is the lack of a detailed discussion about how TWIST and SNAI1 may drive disease progression and metastasis in cancer and in pharyngeal cancer. In particular, the authors could expand their discussion about the findings of TWIST expression in epithelial cell nuclei. I suggest to include findings from a recently published manuscript entitled “Bmi1 is essential in TWIST1-induced EMT”, Nat Cell Biol. 2010.

4. The authors should add data from the work by Schwock et al. BMC Clin Pathol. 2010, which examined SNAI1 protein expression, by IHC, in oral squamous cell carcinomas.

5. Considering that loss of E-Cadherin protein expression has been correlated with positive SNAI1 and TWIST expression, do the authors have any supporting data regarding E-Cadherin expression in the pharyngeal tumors that show strong staining intensity of TWIST and SNAI1 in the tumor stroma or tumor epithelia?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.