Reviewer’s report

Title: Analysis of angiogenic markers in head and neck squamous cell carcinoma - gene and protein expression

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Reviewer: Bernhard Frerich

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In their study “Analysis of angiogenic markers in head and neck squamous cell carcinoma – gene and protein expression”, the authors examined alterations in angiogenic genes in 83 OSCCs and found specific overexpressions in tumors compared to normal mucosa samples. Additionally, OSCCs with lymphatic spread showed a higher expression of VEGF and ANGPT-genes.

Abstract: Decent. The total number of healthy mucosa samples is not given as well as the total number and names of angiogenic genes under examination (VEGF ANGPT and EFNB). Immunohistochemistry is abbreviated as “IHC” in the text without explanation (f.e. “immunohistochemistry (IHC)”). In the title, head and neck squamous cell carcinoma is named. Though, in the abstract, oral squamous cell carcinoma is addressed. These two entities should be differentiated and not mixed as seen within the text. The study seems to focus on oral entities only.

Introduction: Angiogenesis of OSCC also influences prognosis as seen in current publications. The study of Kämmerer et al. 2014, should be added and discussed (“Angiogenesis-related prognosis in patients with OSCC – role of the VEGF +936 C/T polymorphism”). The aim of the study is clearly stated and the chosen genes (VEGF ANGPT and EFNB) are appropriate candidates for this approach.

Materials and Methods: Did the study have a prospective or a retrospective approach? I am not deeply enough into the topic to review the microarray assay procedures even though they seem to be the same when compared to similar publications. Immunohistochemistry was assessed on deep frozen samples? Did the authors see all vital tissue in all samples? From which region was the sample taken? Was there a histopathological evaluation before stating that it was OSCC in the respective samples? Most likely, necrosis should be seen as well. Why did the authors chose CD31? CD34 staining might even achieve better results when compared to CD31. Please discuss. Please indicate the localisations as well as the TMN (or even UICC) stadia of the tumors in a separate table. Again, the number of control samples is not given. Why did the authors examine only 14/83 samples with immunohistochemistry? F.e., there is only 1 case of a T3 OSCC. For a good correlation with microarray results, all samples should be stained accordingly. The results obtained by this analysis cannot be described as significant in any way. By the way, what did the authors mean with “CD31 is not detected in the analysed samples”? Was immunohistochemistry conducted on sheer epithelial samples? No vessels seen at all? Why should these samples be
of interest when talking about angiogenic genes? The sentence that “poor prognosis was associated with increasing T stage” is not part of the results obtained by immunohistochemistry. Additionally, data of prognosis are not given within the manuscript at all. Please compare with similar publications and adapt.

Results: When talking about prognosis, were all OSCC-samples resected with safety margins? Which ones? Obviously, lymphatic spread is classified into involvement of lymphatic nodes. T1-T4 are not stages, but categories of the TNM-system. Did the authors also try to calculate with different nodal involvements (N1, N2) or invasion of lymphatic vessels (L1)? Onset of metastatic disease would be an important parameter, accordingly correlation to tumor stage I-IV would be of interest. Why are patients’ habits reported as the authors did not correlate them with their findings? Immunohistochemistry see above.

Discussion: Lacks adressing the clinical relevance.

Tables: Giving percentages is not recommended in sample sizes <100. Did the authors try to stated descriptive p-values after Bonferroni correction as well? These values should also base on t-tests correlating the single stages with the gene expression profile. By the way, p-values are sufficiently stated as p<0,05 or even p<0,001.

Figures: the figures for revision are way too small and not of a good quality.

Recommendation: Major revision.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

no competing interests