Author's response to reviews

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

Authors:

Susanne Jung (Susanne.Jung@ukmuenster.de)
Sonja Sielker (Sonja.Sielker@ukmuenster.de)
Nikolai Purcz (Purcz@mkg.uni-kiel.de)
Christopf Sroll (christoph.sroll@med.uni-duesseldorf.de)
Johannes Kleinheinz (Johannes.Kleinheinz@ukmuenster.de)

Version: 4 Date: 20 March 2015

Author's response to reviews: see over
Dear Editor and Reviewers,

We would like to thank you and the reviewers for the re-assessment of our manuscript entitled “Analysis of angiogenic markers in oral squamous cell carcinoma - gene and protein expression” (MS: 1003841322142338). Most of the helpful comments and suggestions for improving the manuscript have been incorporated into the revised and updated version. The manuscript was revised by language. The inserted tables were completely overworked and the abstract corrected, respectively. In this letter, we provide a point-by-point response to each addressed comment; the corresponding alterations in the text have been highlighted. Therefore, we hope that the manuscript is now acceptable for publication in Head & Face Medicine.

Sincerely yours,

Sonja Sielker, PhD and Susanne Jung, MD
Review 1:

Major concerns:

*The reviewer remarked that the lymph node involvement is not distinguished well enough. Especially the expression of EFNB2 in smaller tumours with a lymphatic spread should distinguished more.*

We overworked the old tables 2 and 4 and created a new one (now table 2). In this new table data for smaller and larger tumours are site by site with ones with and without lymphatic spread. Data should be now more clearly presented.

**Tab. 2: Expression factors of angiogenesis-related genes and IRS score related to T and N in OSCC samples.**

We consider the correlation between survival in OSCC and tumour biology and lymphatic spread more.

p. 9:
Further studies have indicated a strong correlation between lymphatic spreading and survival in OSCC [13, 14]. Jang et al. showed a significant correlation between tumour dimension and biology to lymph node metastases and survival in HNSCC [15].

We also distinguished the expression of EFNB2 more.

p.7/8:
... EFNB2 showed a contrary gene expression in small and larger tumours with lymphatic spread. The expression increase in smaller tumours and decrease in larger tumours in contrast to tumours without lymphatic spread (Tab. 2).

p.11:
Gene expression of EFNB2 correlated with tumour size and tumour differentiation. Also a stronger overexpression was observed in tumours with lymphatic spread against those without.

*The reviewer criticized the incorrect use of “T stage” instead of T as a category of the TNM-system.*

The incurred use of the term stage instead of category was corrected.

*He also says that “even the harvesting of tissue from a tumor specimen should have been made with agreement of a pathologist, and that should be mentioned...”*

Of course we harvest tissue only after confirmation by a pathologist. To avoid any misunderstanding we mention it on page 5 “patients with a histological diagnosed squamous cell carcinoma of the oral cavity” and on page 7 “histopathological confirmation of OSCC” in the previous manuscript.
The reviewer suggested merging data for T1 and T2 and for T3 and T4 in a smaller tumour and larger tumours group.

We picked up the suggestion and formed two groups’ small tumours with the data of T1 and T2 categories and larger tumours with the data of T3 and T4 categories. The data were recalculated and presented now in table 2.

The reviewer also remarked to present the data in the UICC stage form. Additionally he suggests merging the date of stage I and II and of stage III and IV.

We regrouped and recalculated the data. Data are now presented in table 3. We observed similar results comparing to tumour size

Table 3: Expression factors of angiogenesis-related genes and IRS score related to UICC in OSCC samples.

In subdivision of the UICC classification similar results could be observed...

We detected an unanticipated shift in gene expression in classification III. This shift is not allegeable only with tumour size. A merging of the groups will not abrogate the results.

The reviewer observed that “the assessment of the grading does not rely on clearly reproducible features and lacks inter-rater and intra-rater reliability”. And he criticised that “hence conventional grading makes not much sense as a standard for comparison with genetic characterization”.

We knew that grading is a histopathological analysis of the tissue. We knew also that the data are defined by human and that the characteristic of the collected data more randomly compared to other science based classification. Nevertheless data are collected by well-trained person. We were able to observe statistically relevant alteration in gene expression with regard to grading. In our opinion it is consequent to show the results.

Remarkable results identified by our data were the 2.7- and 2.9- fold gene expression level of VEGF and ANGPT2 in poorly differentiated tumours with lymph node metastasis versus those without.

Gene expression of EFNB2 correlated with tumour size and tumour differentiation.
Further and minor concerns:

The reviewer remarked that the assertions in the text and the data presented in the tables are not consistent, especially for PECAM1/CD31.

The data are regrouped and recalculated so most of the assertions in the text are adapted. For all data one way ANOVA was accomplished and differences in expression were analysed on a level of significance of $p < 0.05$.

The gene expression of PECAM1 shows in smaller tumours a higher expression as in larger tumours. Also is the gene expression in tumours with lymphatic spread higher as in those without lymphatic spread. This pattern was also observed in moderate and poorly differentiated tumours.

In our samples pool we have only two well differentiated tumours. It is quite obvious that statistical analysis with a group of two is not possible. We inserted the data for an improved comparability.

The reviewer also remarked that “the Ang1/Ang2 ratio is not depicted, values are not given, a suited presentation of the corresponding statements in the abstract is lacking”.

The ratio is now presented in tab. 5.

Tab. 5: Gene expression ratio of ANGPT1 to ANGPT2

Gene expression ratio of ANGPT1 against ANGPT2 in smaller and larger tumour is shown in tab. 5. The lacking information is inserted into the abstract. The ANGPT1/ANGPT2 transcription ratio was found decreased in larger tumours and especially in tumours without lymphatic spread.