Author's response to reviews

Title: Human alpha-defensin (DEFA) gene expression helps to characterise benign and malignant salivary gland tumours.

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Author's response to reviews: see over
To whom it may concern,

herewith I am sending you the revised manuscript MS: 1341628070727951, entitled: “Human α-defensin (DEFA) gene expression helps to characterise benign and malignant salivary gland tumours.”.

Corrections in the manuscript text are highlighted in yellow, responses to the reviewers remarks are in red text colour in this letter.

Bonn, 4. September 2012
Reviewer 1

Reviewer’s report:

The authors compared their data with hBD from literature. Why authors have not performed the evaluation of these defensins in this work?

The first investigations concerning α- and β-defensins in head and neck cancers were carried out by our group:
Antimicrobial peptides were very well known from inflammatory diseases of the oral cavity as chronic periodontitis and the gene DEFB1 encoding for hBD-1 has been identified as a major periodontitis-associated gene. As we learned that it might have a function as tumorsuppressor in epithelial tumours of the urogenitary tract, we started trying to find out if it has a similar function in epithelial head and neck cancers. In several in vivo and in vitro studies we were able to trace alterations in the gene expression of β-defensins in head and neck cancers. These studies are already published elsewhere so we cited them in the manuscript [references 4, 5, 6, 11, 19, 21, 22, 24, 34 in the manuscript]. The present study on α-defensins is based on these works.

In introduction:
Which candidates have been detected?
I added some examples of candidate genes in the introduction. The citations 3, 4, 5 and 6 might be a good survey of the knowledge we have about the molecular pathology of these tumours.

Defensines should be corrected to Defensins.
Corrected as suggested.

Objective in the end of introduction is not clear.
You are right - the sentence was difficult to understand – I changed it and added two more sentences to make it more understandable.
In Material and methods:
Parotic should be corrected to Parotid

Corrected as suggested.

Was RNA extracted from all tissue or those selected areas?

Using microdissection, only from the areas containing tumour tissue— I added a few words to make this clear in the material and methods section.

Results presentation should be improved.

Corrected as suggested – I added a figure (fig. 1) to visualize our results.
Reviewer 2

1) Abstract. Abstract is badly written and both conclusions are not well substantiated.

This is the first study which investigates α-defensins in different salivary gland tumour entities our knowledge at this point of time is limited. In the past our group was one of the first which investigated β-defensins in different head and neck cancers. As defensins were mostly seen as important components of the innate immune system, we contributed to shed light on their functions in tumour formation and progression in head and neck cancers. Because of the described structural and functional similarities between α- and β-defensins we conducted this study. It’s the first of its kind.

2) Introduction. Paragraph one (P1). Listing of salivary tumor types in latin seems inappropriate.

Not the tumour types, but their main histopathological features are listed: …“a) myoepithelial components, b) basaloid components, c) epithelial components, d) lymphatic components and e) pleomorphic adenomas...”
Maybe you mean the specification of the six major salivary glands (Glandula parotidea, Glandula submandibularis, Glandula sublingualis)? In all our publications (for instance Wenghoefer et al. Nuclear hBD-1 accumulation in malignant salivary gland tumors. BMC Cancer. 2008, 8, 290. we had them in Latin). If Latin is inappropriate in this context I am open for your suggestions concerning the appropriate language.

Defensines section (correct word – defensins):

Corrected as suggested.

P1: there are mature, prepro, and proforms with different MWs.

Everyone knows, that of every peptide mature, prepro, and proforms do exist – so we refrained from stating this extra. The molecular weight of the defensins ranges between 3.5 to 6.5 kDa – which is stated in the text.
P2: theta defensins should be mentioned.

Theta defensins do only occur in monkeys but not in humans (Enteric alpha defensins in norm and pathology. Lisitsyn NA, Bukurova YA, Nikitina IG, Krasnov GS, Sykulev Y, Beresten SF. Ann Clin Microbiol Antimicrob. 2012 Jan 11;11:1. Review.) – for this reason they are irrelevant for this study.

P3: “involvement of defensins…for beta-defensins” should be better expressed. First sentence should be combined with the next one.

Corrected as suggested.

3) Methods. Sentence three in Quantitative realtime-PCR section is unclear and should be omitted.

The sentence was corrected to make it more understandable.

4) Results. Section is poorly written: no explanations, just a list of tables (repeating their content) and figures. Better to combine results and discussion sections and to place Tables 1 and 2 in the supplement.

I added a figure (fig. 1) to visualize our results and make them more understandable. Apart from that we think that the results and discussion section should be separated clearly. We see the results section as a summary of our main findings which are interpreted in the discussion. And the discussion section should not be a repetition the results. Both stand for themselves and should not be mixed up.

5) Discussion.
P1: “the transcripts of both genes were visualized by immunostaining”. This statement is obviously wrong.

The statement was corrected.
P3: Better to move historical facts in the introduction.

We generally agree – but as there is such a wide variety of salivary gland tumours I could not give a detailed overview about all of them. So I wrote a brief overview in the introduction and gave more detailed information about those entities we had interesting findings in the discussion.

P4: Better to move in the introduction.

Same as comment to P3.

P5 and 6: Too long (P6, the last paragraph, is one and a half pages long) and mostly useless – there is no discussion of molecular and cellular biology.

For a maxillofacial plastic surgeon treating head and neck cancers it is an every day experience that even tumours of the same or a similar entity have a different biological behaviour. Especially pleomorphic adenomas and cystadenolymphomas display interesting features concerning malignant progression and recurrence. For the surgeon this knowledge is particularly empiric. In these studies we accumulate molecular information about tumours we have treated more or less successfully. Retrospectively we know very well what happened to our patients, but in most of the salivary gland tumours we do not know why certain patients with a certain tumour have a certain outcome – so for the clinician every information about what distinguishes (differences in the defensin gene expression for instance) these tumours he treats is everything but useless – and could be relevant for a better treatment in the future.

There is no description of numerous figures, which are useless.

There are descriptions of the figures in the results as well as next to the figures themselves. I added the optical enlargement, which was missing in the description.

Reference one seems to be useless for most of the readers.
This is one of the most important reviews concerning diagnosis and therapy of salivary gland disorders for German-speaking maxillofacial plastic surgeons.

Sorry for the delay of the revision – if anything is still missing, please let me know.

Yours sincerely

Dr. Dr. M. Wenghoefer