Author's response to reviews

Title: HMGA1 and HMGA2 expression and comparative analyses of HMGA2, Lin28 and let-7 miRNAs in oral squamous cell carcinoma

Authors:

Katharina A. Sterenczak (Katharina.Sterenczak@tiho-hannover.de)
Andre Eckardt (Andre.Eckardt@klinikum-bremerhaven.de)
Andreas Kampmann (Kampmann.Andreas@mh-hannover.de)
Saskia Willenbrock (Saskia.Willenbrock@tiho-hannover.de)
Nina Eberle (Nina.Eberle@t-online.de)
Florian Länger (Laenger.Florian@mh-hannover.de)
Sven Kleinschmidt (Sven.Kleinschmidt@laves.niedersachsen.de)
Marion Hewicker-Trautwein (Marion.Hewicker.Trautwein@tiho-hannover.de)
Hans Kreipe (Kreipe.Hans@mh-hannover.de)
Ingo Nolte (ingo.nolte@tiho-hannover.de)
Hugo Murua Escobar (hugo.murua.escobar@med.uni-rostock.de)
Nils C. Gellrich (Gellrich.Nils-Claudius@mh-hannover.de)

Version: 6
Date: 25 August 2014

Dear Editor, dear Editorial Team,

Thank you very much for the principal acceptance of our manuscript MS# 1457729371953663 by Sterenczak et al.. Please find enclosed the newly revised version of our BMC Cancer manuscript addressing the remaining issues:

We carefully addressed the additional comments by the reviewers and thank them again for the constructive review of the revised manuscript.

Thank you very much in advance for your consideration.

With my best wishes from Hannover,

H Murua Escobar

List of Changes
BMC Cancer Manuscript: 1457729371953663; Sterenczak et al. revision 3

Referee 3, Max Heiland:

Comment 1: “How the authors guarantee, that tumor cells obtained for culturing are really squamous carcinoma cells and not e.g. fibroblasts. Did they characterized these cells? Please extend the methods section and discuss.”
Answer to comment 1:
The samples of both origins were taken during routine surgery. The human as well as the respective canine tumour samples were divided in equal representative parts for pathologic analyses and cultivation.
To clarify the sample processing in our manuscript we edited the respective part as follows:

In the former paragraph (Section: Methods, Page: 9):
“Due to the possibility to access fresh neoplastic material of both species we decided to aim at an establishment of OSCC cell lines as tools for further experimental approaches. The successful establishment of new cell lines allowed us to compare the gene expression patterns of the neoplastic primary tissues and the cell lines of both species.
Two human cell lines were generated from freshly isolated squamous cell carcinoma biopsies derived from patient 4 and patient 12 (tumour staging see above) ....”

we added the following bold sentences (Section: Methods, Page: 9):
“Due to the possibility to access fresh neoplastic material of both species we decided to aim at an establishment of OSCC cell lines as tools for further experimental approaches. The successful establishment of new cell lines allowed us to compare the gene expression patterns of the neoplastic primary tissues and the cell lines of both species. The respective human and canine tumour samples were verified to be squamous cell carcinomas by routine histopathologic characterisation. The samples were analysed by either a human or veterinary pathologist respectively. Two human cell lines were generated from freshly isolated squamous cell carcinoma biopsies derived from patient 4 and patient 12 (tumour staging see above) ....”

Comment 2: “How many investigators evaluated the immunohistochemical signals? In case of different investigators, how were different results discussed and decided?”

Answer to comment 2:
The respective human and canine samples were analysed by either a human or veterinary pathologist depending on the sample origin. In the respective species all samples were analysed by the same pathologist.
In order to clarify this point we edited the respective parts in our manuscript as follows:

In the former paragraph (Section: Methods, Page: 9):
“Due to the possibility to access fresh neoplastic material of both species we decided to aim at an establishment of OSCC cell lines as tools for further experimental approaches. The successful establishment of new cell lines allowed us to compare the gene expression patterns of the neoplastic primary tissues
and the cell lines of both species.

Two human cell lines were generated from freshly isolated squamous cell carcinoma biopsies derived from patient 4 and patient 12 (tumour staging see above) ….

we added the following bold sentence (Section: Methods, Page: 9):

“Due to the possibility to access fresh neoplastic material of both species we decided to aim at an establishment of OSCC cell lines as tools for further experimental approaches. The successful establishment of new cell lines allowed us to compare the gene expression patterns of the neoplastic primary tissues and the cell lines of both species. The respective human and canine tumour samples were verified to be squamous cell carcinomas by routine histopathologic characterisation. The samples were analysed by either a human or veterinary pathologist respectively. Two human cell lines were generated from freshly isolated squamous cell carcinoma biopsies derived from patient 4 and patient 12 (tumour staging see above) ….”

Comment 3: “What was the idea of using Ki-67 antibodies, just to indicate proliferation activity? Is there a relation to the investigated targets?”

Answer to comment 3:

The initial idea to perform a Ki-67 staining in the IHC was to show the proliferative character of the analysed tissue sections. Additionally as stated in the introduction of the manuscript the re-expression of HMGA2 has been widely described to correlate with aggressiveness and malignancy of several tumour entities. Further HMGA2 is strongly expressed in embryonic development and has been shown to stimulate proliferation of different cell types. Thus, the staining of both targets appeared interesting for our study.

(Review on HMGA2 characteristics see e.g. “The HMGA proteins: A myriad of functions” by Isabelle Cleynen and Wim J.M Vand De Ven, 2008, International Journal of Oncology.)

Comment 4 : “- Discussion part, paragraph 3: The authors discussed the fact, that multivariate risk factor analysis demonstrated that HMGA2 expression serves as an independent prognostic marker for disease-specific overall survival. Do they mean disease-specific or overall or both, which is totally different..”

Answer to comment 4:

Our paragraph discusses the findings published by Miyazawa et al. 2004. Within this study the identification of prognostic factors associated with oral carcinoma specific death was performed using a multivariate risk factor analysis. As stated by the authors the variables included age, sex, T stage, N status, clinical stage, histological differentiation and HMGA2 staining. T stage and HMGA2 staining were found to be significant independent predictors of death from carcinoma. For overall survival, positive HMGA2 staining was found to be an independent predictor of reduced survival. (All other variables analysed were not associated
with survival according to the multivariate analysis).
We do realize that within our paragraph this fact is stated unclear and thus we have edited this part accordingly.

The former paragraph (Section: Discussion, Page: 18):
“HMGA2 was found to be expressed at the invasive front of oral carcinomas leading to the conclusion that –in contrast to HMGA1- HMGA2 immunostaining could be a potential prognostic determinant in stratifying patients into risk groups [11]. Further, multivariate risk factor analysis demonstrated that HMGA2 expression serves as an independent prognostic marker for disease-specific overall survival [11]. Contrary to this HMGA1 expression was also reported to be increased in head and neck carcinomas analysed via semi-quantitative RT-PCR and immunohistochemistry when compared to healthy mucosa samples [12] “

Was changed into the following new paragraph (Section: Discussion, Page: 18):
“HMGA2 was found to be expressed at the invasive front of oral carcinomas leading to the conclusion that –in contrast to HMGA1- HMGA2 immunostaining could be a potential prognostic determinant in stratifying patients into risk groups [11]. Further, multivariate risk factor analysis demonstrated that HMGA2 expression was found to be a significant independent predictor of death of carcinoma and an independent prognostic marker for disease-specific overall survival [11]. Contrary to this HMGA1 expression was also reported to be increased in head and neck carcinomas analysed via semi-quantitative RT-PCR and immunohistochemistry when compared to healthy mucosa samples [12].”

Comment 5: “Did the authors examined the correlation between HMGA1, HMGA2, let-7, mir-98, Lin28 expression and survival on their own data? Was there a difference between the metastasized and non metastasized cases? Please discuss”

Answer to comment 5:
In our study we did not analysed the correlation between the analysed targets and the survival time. The reason for these missing analyses is due to:
A) Especially in veterinary medicine, the patients presented to facilities of Veterinary Universities are in a way pre-selected. This means, that usually only cases in which common veterinaries are incapable to provide accurate treatment options the cases are referred to academic institutions. This implies that the presented patients are often in late stages of the diseases. Following surgical treatment veterinary patients are released with the respective owners. In many cases these patients are not presented at the University again. Further, some patients get euthanized in the following periods by local veterinarians. In contrast to humans, veterinary cancer cases are not reported to a central register and thus accurate data mining in larger sample set is not possible in dogs.
B) As stated before, the limited number of analysed cases herein would not allow
generating powerful analyses for the issues. Thus, we opted to keep our analyses descriptive.

However, to address this point in our manuscript we added the following paragraph in the discussion of our manuscript (Section: Discussion, Page: 21):

“A correlation between the analysed targets and the survival time was not focussed in this study due to the limited number of analysed cases. Additionally, regarding canine patients, an accurate follow up is often hindered by the fact that veterinary patients are frequently not represented at the academic institution as owners follow treatment at local veterinarians. Furthermore, central cancer register for canine patients, as present for human cancer patients, does not exist. However, due to the similarities in canine and human cancer presentation, as reported herein, basic research and the development of clinical regimens in either of the species provide valuable solid data for the respective counterpart.”

Comment 6: “- in what sense do the authors consider their targets as prognostic tools? To differentiate between metastasized and non metastasized cases, between limited and advanced tumor size etc? Would their tools be usefull during follow up after treatment? Please discuss..”

Answer to comment 6:
Within our study we referred to the term “prognostic tool” addressing two major meanings.

On one hand we were interested if the targets could be used as early stage markers to predict the malignant potential of a tumour and thus be of use for the optimisation of therapeutic options. On the other hand we were interested to see if the tumour invasive potential could be characterised by one of the targets. Our results and the results of the groups working in the field indicate that of the analysed targets only HMGA2 showed potential for risk assessment. To clarify this we stated in our discussion that our results affirm the findings by Miyazawa for HMGA2 and added the sentence (Section: Discussion, Page: 18):

“HMGA2 was found to be expressed at the invasive front of oral carcinomas leading to the conclusion that –in contrast to HMGA1- HMGA2 immunostaining could be a potential prognostic determinant in stratifying patients into risk groups [11]. Further, multivariate risk factor analysis demonstrated that HMGA2 expression was found to be a significant independent predictor of death of carcinoma and an independent prognostic marker for disease-specific overall survival [11]. Contrary to this HMGA1 expression was also reported to be increased in head and neck carcinomas analysed via semi-quantitative RT-PCR and immunohistochemistry when compared to healthy mucosa samples [12].”