Author's response to reviews

Title: SOX2 Expression Correlates with Lymph-Node Metastases and Distant Spread in Right-Sided Colon Cancer

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Author's response to reviews: see over
Dear Professor Patel,

Thank you for reviewing our manuscript and giving us the possibility to submit a revised version. We have appreciated the reviewers' helpful comments and have adapted the manuscript accordingly.

Please find below a detailed list addressing the reviewers' comments and suggestions point by point. Changes made within the manuscript are indicated in yellow colour. We are confident that we have answered the open questions satisfactorily and hope that the manuscript is now acceptable for publication in *BMC Cancer*.

Yours sincerely,

Andreas Jung Ph.D.      Jens Neumann, M.D.

To Referee 1 (Ida RK Bukholm):

“Regression analyses for distant metastases where all known histopathological risk factors are included for colonic and rectal carcinomas separately”

Referee 1 recommends an evaluation of histopathological parameters by use of multivariate statistical analysis. This is not possible since the study design, utilizing a matched case control-study, is not suitable for such an analysis. The matched case-control design of our study was controlled for the potential confounder’s pT-stage, grade and localisation. Age and gender are also similar in both groups. We hope you agree that we can do without a multivariate analysis under these conditions.

Furthermore, the study was limited to the right-sided colon. Therefore no patients with rectal cancer were included into the analysis. However, we agree that this fact is not evident in the previous submitted version of the manuscript and apologize for this misunderstanding by our side. We modified the Manuscript in order to clarify this point.

“Do immunohistochemistry for SOX2 and beta catenin in matched normal mucosa for all patients and use cut off value according to expression level in the normal mucosa?”

SOX2 is only expressed in a minority of normal colonic tissues (in our collection of colon cancers 4%). This finding has also been reported by other groups (Ong et al.,
Modern Pathology (2010) 23, 450-457). Due to the small number of positive cases (N=4) no statistical correlation could be obtained. Nevertheless, we added detailed information on the SOX2 expression within the normal colonic tissue into the result section and discussed the findings accordingly. Furthermore an additional figure (figure 2), showing the expression pattern of SOX2 within normal colonic tissue, has been added.

“Exclude statistics analyses on 8 patients with co expression of SOX2 and beta-catenin”

To clarify the value of the different marker combinations we performed a subgroup analysis for SOX2 and β-catenin (high SOX2 and β-catenin vs. high SOX2 and low β-catenin vs. low SOX2 and high β-catenin β-catenin vs. low sox2 and β-catenin). Combined high scores of SOX2 and β-catenin revealed the highest risk for locoregional- and distant spared. In contrast absence of both markers was associated with reduced rates of metastases. The results were presented in a new table (new Table 2) and the text of the results and the discussion were adapted accordingly.

To Referee 2 (Vittorio Colantuoni):

(1) “Association of SOX2 negative cases with tumour grade, nodal status and metastases should be discussed”

We included information on the correlation of SOX2 negativity with clinico-pathological parameters to the results and discussed the topic accordingly.

(2) “SOX2 expression in the cytoplasm and normal mucosa”

We added detailed information on the expression pattern of SOX2 in normal colonic tissue to the manuscript. Furthermore we included a new figure showing the SOX2 expression within normal tissue (see also referee 1, section 2). In addition we included information on the cytoplasmic expression of SOX2 in the result section (see also referee 3, section 3).

(3) “More information are required to correlate the SOX2 expression levels”

Detailed information on the correlation of SOX2 and β-catenin with clinico-pathological parameters including sex, age, T-category, nodal status, presence or absence of distant metastases and histopathological tumour grade are available in Table 1.

(4) “Does the nuclear positivity correlate with a higher rate of liver metastases and tumour progression?”

Unfortunately there was a mistake within table 1 and subsequently in the text of the manuscript. The correct frequency of high nuclear β-catenin is 38 of 114 cases
(33.3%). Within this group 27 patients (71.1%) developed liver metastases during the follow up period. Thus, high nuclear expression of β-catenin is associated with a higher frequency of distant metastases (p=0.001). Table 1 and the text of the manuscript have been corrected accordingly. We do apologize for this inaccuracy.

(5) “Interaction of SOX2 and the β-catenin transcriptional activity”

Referee 2 recommends discussing the functional relationship of SOX2 and β-catenin. We included a whole new paragraph to the discussion of the manuscript dealing with this point.

(6) “SOX2 protein expression in addition to β-catenin”

It was not our intention to state that a combination of SOX2 and β-catenin may be a possible prognostic marker-combination in the management of colon cancer patients. We apologize for this misunderstanding. To clarify this issue we modified the text of the discussion accordingly.

(7) “Correlation of SOX2 with other stem-cell-markers”

In previous studies we analyzed the expression of CD133 within our set of right-sided colon cancers. Since the CD133 expression failed to show a significant correlation with SOX2 and the data on CD133 has already been published (Horst et al., Journal of Pathology 2009) we decided not to include these data into the results of the present manuscript but raised this issue in the discussion. In the revised version we discussed the relationship of stem-cell markers such as CD133 and the Wnt-Pathway in colon cancer.

(8) “Correlation of the SOX2 expression with the localization”

The study was limited to the right-sided colon. The pairs of tumours were matched according to their localisation. See also referee 1, section 1.

To Referee 3 (Jahn M Nesland):

“The authors should separate Discussion and Results”

We made modification on the text of the results and discussion to clarify this issue.

“A material with follow up information should be presented. What is the outcome for the various groups (beta-cat and Sox2 pos, beta cat pos and Sox2 neg etc)? What about the double positive group?”

Our study utilized a case-control design. As end-point we defined the presence or absence of distant metastases (liver) within a follow up period of five years. Therefore, due to the design of the study no data on the overall and disease free
survival of the patients could be derived from our collection of colon cancer patients. Furthermore, correlation of overall and disease free survival with SOX2 expression in colon cancer has been published by other groups. In our study we intended to clarify, if the poorer outcome of colon cancers with high SOX2 expression reported in the literature is associated with an increased occurrence of distant metastases. However, we agree that in the previous submitted version of the manuscript this fact hasn’t become apparent. We modified the Manuscript in order to clarify this point and apologize for this misunderstanding by our side.

As recommended an additional subgroup analysis was calculated (high SOX2 and β-catenin vs. high SOX2 and low β-catenin vs. low SOX2 and high β-catenin vs. low sox2 and β-catenin). These results were presented in a new table (new Table 2) and the text of the results was adapted accordingly. See also Referee 1, section 3.

“Cytoplasmic staining for Sox2 was not evaluated!”

Referee 3 stated that cytoplasmic staining for SOX2 was not evaluated. In fact SOX2 showed absent or only weak and unspecific cytoplasmic positivity. Therefore cytoplasmic SOX2 expression was excluded from further analyses. However, we added this information to the manuscript. See also referee 2, section 2.

To all referees:

We made modifications to the text of the discussion in order to appropriately reflect the changes listed above. The manuscript was proofread by a native speaker leading to minor changes in the text. All modifications were indicated by yellow colour.