Author's response to reviews

Title: TGF-beta receptor 2 downregulation in tumour-associated stroma worsens prognosis and high-grade tumours show more tumour-associated macrophages and lower TGF-beta1 expression in colon carcinoma: a retrospective study

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Author's response to reviews:

Dear editor, dear reviewers,

in the second revised version of our article we made some more changes as follows:

reviewer 1 (Diego Arango; Referee 2: http://www.biomedcentral.com/imedia/1265052256141823_comment.pdf):

Dear reviewer,

Thank you for your comments. In the following we tried to improve the text according to your suggestions:

1. We changed the term "recent" to "present".

2. We avoided to note non-significant differences with the exception of TGF-beta-Rs expression in stroma and its' relation to distant metastases, where p-values were below 0.1 and this non-significant trend is in line with the significant differences in nodal status and survival, which we think should be at least be mentioned.

3. We can't understand, why you didn't mention this aspect mentioned in the first revision. Nevertheless we try to explain our view concerning Smad4 expression in colon cancer, but want to point out that our emphasis is on the stroma component and not on tumor tissue in the present study.

In the present study we optimized the immunohistochemical methods including dilutions of antibodies including Smad4 and in this case using dilution of 1:50 the best results could be achieved. For the Smad4 antibody (sc-7966) Santa Cruz also recommends dilutions between 1:50-500 and others have also used higher concentration than you did (e.g. PMID: 11891193). To show you some staining results we included histological pictures (see additional file 1: Smad3+Smad4 jpg). We noticed that you used these lower antibody concentrations of 1:1000 and 1:2000 in your work. We can only speculate that the difference might be due to different antigen retrieval procedures, although the solution we used (target retrieval solution pH6 = TRS6 by DAKO) is also based on citrate buffer as you described in methods sections of your paper, or other variations in methodological aspects.
In fact we found no Smad4-negative tumor cases in our series, but we don't think this is due to overstaining. We also didn't use a scoring system to evaluate the intensity of Smad4 expression, as you did in the mentioned papers and so several of our Smad4 positive cases would fall into the group of "low Smad4 expression". This might explain, why in our study we weren't able to confirm your finding of Smad4 being a prognostic marker in colon cancer. Nevertheless if you compare the rate of cases negative for nuclear Smad4 expression in our series (14% or 44 of 305 tumors), it is not too far from your results and for nuclear Smad4 expression we have shown an association to lymph node metastasis. But there was no correlation to prognosis in our study. We think, this could be explained if you take a look at the survival rates in our study compared to yours. The 5 year cancer related survival was almost 80% in stage III tumor patients of our study compared to less than 40% in your study. In our opinion this high survival rate points to a better local tumor control and fewer local recurrences. This might have been achieved through more radical resection with higher numbers of resected lymph nodes and better local control in our series (see also PMID: 17333036). In view of this we think in our study the influence of low Smad4 expression on survival is eliminated through improved surgical quality. We think chemotherapy (in our study about 30% of stage III patients received adjuvant therapy with 5-FU) had no further measurable effect in this group in our study. In summary we think in view of high survival rates and good local tumor control in our study the importance of low TGF-SZ-R2 expression in tumor associated stroma for prognosis is even more emphasized.

We added these thoughts to the discussion section and hope you accept this view.

4. The sentence is split into several smaller ones.

reviewer 2 (Yoshifumi Nakayama; Referee 1: http://www.biomedcentral.com/imedia/1562069031411793_comment.pdf):

Dear reviewer,

Thank you again for your comments. We took most of your suggestions and changed the text as follows:

1. We splitted the relevant sentences into smaller ones.

2. We now only use the terms "low-grade" (i.e. grade 1 and grade 2) and "high-grade" (i.e. grade 3 and grade 4) in title, text and tables and explain them twice in the abstract and in table 1. We think it's now easier to follow the comparisons concerning histological grade of differentiation.

3. In table 1 we use internationally accepted terms for localisation in the lower intestinal tract as suggested in the "International Classification of Diseases". We do not share the opinion of the reviewer and think it's better to use the exact terms, which could easily be summarized to get larger groups, such as for instance right colon (i.e. caecum + colon ascendens).

4. In table 3 multivariate analysis we combined the lines for lymph node status and stage, as in the present study these groups (and the corresponding scores) are identical.

We hope the changes now meet your expectations and look forward to your decision.

Yours sincerely