Author's response to reviews

Title: Immunohistochemical molecular phenotypes of gastric cancer based on SOX2 and CDX2 predict patient outcome

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Author's response to reviews: see over
To the Editors of BMC Cancer,

We would like to thank you for the opportunity to respond to the reviewers and submit a revised version of the manuscript “Immunohistochemical molecular phenotypes of gastric cancer based on SOX2 and CDX2 predict patient outcome”. We were very happy to learn that the reviewers did not have methodological criticisms nor suggest additional experiments. The reviewers have asked for additional explanations on some of the experimental decisions, which are all addressed in the revised version of the manuscript (marked-up in red) and described point-by-point below.

We will be looking forward to hearing from you.
With my best regards.
Raquel Almeida

Answers to Referee 1

- **Why did authors use the combined expression of SOX2/CDX2 in survival analysis? Please explain more about this point. I think the association of SOX2 and CDX2 with more and less aggressive to be weak as a reason.**

**Reply:** We agree with the Reviewer that this option is insufficiently explained in the original version of the Ms. CDX2 is associated with differentiation whereas SOX2 has dual roles, both in gastric differentiation and stemness properties. This suggested that different combinations of these transcription factors could impact tumor behavior. We explored their association with patient survival individually and found that SOX2 and CDX2 expression were inversely associated with this parameter as well as with clinicopathological features. Thus, we hypothesized that the combined expression could lead to a better segregation of patients into prognostic groups putatively impacting on treatment or follow-up options, which is the key message of this study. We extended the description of these results in the revised version of the Ms.
• If you conduct retrospective analysis to identify prognostic value of survival, you should conduct multivariate analysis. What was the results when the multivariate analysis for overall survival carried out by using the baseline factors and SOX2 profile?

Reply: We fully agree with the Reviewer that multivariate analysis is important in this study. It was already included in the original version of the manuscript (Table 2) but we extended its description, in the revised version of the Ms, in the Results section as well as in the Table legend. Our option was to perform multivariate analysis (HR adjusted for age and sex using Cox regression) for the SOX2/CDX2 profile because it showed the highest impact on survival, using univariate analysis.

• Previous study reported that SOX2 has been suggested to have loss of expression, however, this report do not confirm it. Please discuss more about this point.

Reply: We agree with the Reviewer that this is an important issue to be clarified in further studies as results in the literature remain conflicting. As mentioned in the original version of the manuscript, the earliest papers on SOX2 and gastric cancer refer loss of SOX2 expression in this type of tumor. In our opinion, this is interpretative and based on the fact that SOX2 is always expressed in the gastric mucosa and in only about 50% of gastric carcinomas. In fact, we observe the same. However, since SOX2 is not expressed in all gastric cells and it is not known which is the cell that becomes transformed we don’t think this interpretation is appropriate. Furthermore, these studies suggest that SOX2 might be a tumor-suppressor gene for which there isn’t convincing evidences in the more recent publications as well as in our results. We have clarified this issue in the revised version of the Ms.

• “Sox2”should be corrected to“SOX2”

Reply: Thank you for pointing this out. It has been corrected in the revised version of the Ms.
Answers to Referee 2

- The authors are asked to explain why the FISH analysis was performed only on 21 samples.

Reply: In this study, we searched for copy number alterations in SOX2 locus using two methods: FISH analysis and copy-number PCR. The first was performed in 21 cases and the second in 62. We set up the FISH experiment in a subseries of 21 cases (~10% of the total number of cases), representative of the different SOX2 expression. The results obtained showed that SOX2 gene amplification was a less frequent event than copy number gain, which is highly prevalent and significantly associated with SOX2 expression. Therefore we decided to extend the genomic analysis using the CNV assay rather than FISH.