Reviewer's report

Title: TGF-beta receptor 2 downregulation in tumor associated stroma worsens prognosis and poorly differentiated tumors show more tumor associated macrophages and lower TGF-beta1 expression in colon carcinoma: a retrospective study

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Reviewer: Diego Arango

Reviewer's report:

The authors have successfully fixed some or the issues raised in the initial review. However, some still need to be improved, and this reviewer would like to raise some new issues in an attempt to improve the study.

1.- Page 7 third paragraph: continues to say ‘Most tumors in the recent study showed no detectable TGF-ß receptor expression’. If this means these are results from an earlier study, please provide reference. If this refers to the results of the current study, please modify accordingly.

2.- At least twice in the results section (below), the authors make reference to situations where the mean (or the percentage) of a comparison is different but the p values are far from significant. These results should be presented more carefully since a p value of 0.459, for instance, means that if the experiment was repeated, it is very improbable that the same result would be obtained. Therefore, I would suggest reporting these results more along the lines of ‘no differences were observed in...’.

TMAs: ‘This difference was also reflected by increased 5-year survival (87% vs. 80%) and lower rate of distant metastases (19.4% vs. 30.0%) of the former group, although these differences were not significant (p = 0.261 and p = 0.241 respectively)’.

Components of TGF-ß pathway in tumor tissue: ‘Tumors with strong expression of TGF-ß1 (score 3) showed an excellent 5 year cancer related survival (100%) compared to tumors without expression (83.3%), but this difference was not statistically significant (p = 0.459)’.

3.- The authors should present micrographs of the IHC for the SMADs. This reviewer is particularly concerned with the results obtained for SMAD4 tumor levels. The authors have used a commercial antibody (Santa Cruz) that has been used before on colorectal tumors and the loss of SMAD4 expression has been reported to be frequent in these tumors (see for instance PMID: 12077092, 16144935 and 15814640). In earlier studies, this antibody was used at a 1/1000-1/2000 dilution and in the study by Dr. Bacman et al it was used at 1/50. I suspect this 20- to 40-fold difference could be responsible for this finding. The authors should comment on this potentially serious technical problem before the MS is published.

4.- The first 8 lines of the methods section in the abstract are part of one single sentence. It would be useful to break this point in several phrases.