Reviewer’s report

Title: Expression of endosialin is associated with the progression and with the more aggressive biological characters of rectal cancers with a clinical trial of preoperative radiotherapy

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Reviewer: Hellmut Augustin

Reviewer’s report:

The authors describe an interesting observation of Endosialin expression in colorectal tumors. Endosialin expression by epithelial tumors is in fact novel and therefore requires rigorous confirmation. In this regard, the manuscript clearly lacks necessary controls to emphatically validate the claims. Importantly, performance of Endosialin expression in the absence of co-staining for carcinoma cells, endothelial cells, pericytes and fibroblasts is not appropriate. Given the extent of the authors’ claims, it is necessary that the authors perform the relevant co-stainings.

Major compulsory revisions

Figure 1 is not at all mentioned in the Results section. The validity of the antibody has been previously demonstrated. The Western blot therefore appears to be irrelevant in this study.

The detection of ES expression in normal mucosa and in rectal carcinoma which is the prominent finding in this study as described in the figure (2A-D) is rather incomplete and inconclusive.

A major concern is the finding that Endosialin is expressed by tumor cells, which is not reassuring. The performance of just one staining is not sufficient to draw such a conclusion. Importantly, the co-staining of Endosialin along with relevant tumor cell markers, endothelial markers and stromal markers is required.

For the whole of Figure 2, it is important to stain for the expression of fibroblasts and pericytes. For the fibroblast staining, at least one of the common markers such as vimentin, desmin should be used. In the case of pericytes, NG2 co-staining should be performed. Since many of the above markers are not exclusive for pericytes and fibroblasts and are often expressed interchangeably, it is necessary to co-stain with e.g. endothelial markers.

Similarly, in the case of Figure 2B and 2C, it is important to co-stain for the expression of endothelial markers such as CD31 or CD34.

Finally, the authors show a diffuse Endosialin staining pattern in tumors in Figure 2D. Therefore, it is absolutely required that the authors perform co-stainings with a relevant tumor marker.
Despite a correlation with markers, which positively correlate with poor prognosis, the authors state that the Endosialin expression does not correlate with long term survival of patients in either of the studied group. This is rather surprising and should be part of the Discussion. Additionally, this conclusion should likely be incorporated into the title of the article.

In Table 3 and Table 4, the number of patients used by the authors is not clear. For example, the authors use in the non radiotherapy group a cohort of either 43 or 46 patients. In the radiotherapy, it fluctuates from 40 to 35. The derivation of percentages for variables needs to be clarified.

Minor Essential Revisions
It is recommended that the authors rearrange sentences in some paragraphs for better flow and readability. In general, the authors should describe each of the group (RT and the non RT group) separately and completely prior to describing the other group. For example on page 3, the fourth sentence can be placed at the end of the paragraph.

The Figures 2 A-D have different magnifications and the magnification needs to be shown. Additionally, the stage of tumor needs to be mentioned for the pictures whenever relevant. It is important to mention if the pictures in Figure 2 were obtained from the same patient or if those are representative images from either group.

A legend (footnote) for the tables should be include.

Discretionary Revisions
In table 3, the authors have used Dukes´ stage for determining the status of the tumor. It is advisable that the authors use the latest AJCC/TNM system.

For fibroblasts, it would be advisable to co-stain for the expression of alpha SMA which would reflect the myofibroblast population.

Additionally, the written style could be improved. The article contains a number of sentences which are either not clearly written or are incomplete or incoherent.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'