Author's response to reviews

Title: Tenascin-W is a better cancer biomarker than tenascin-C for most human solid tumors

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Author's response to reviews: see over
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Dear Editorial Team,

We thank you for giving us the opportunity to submit a revised version of our manuscript. We highly appreciated the thoughtful comments by both reviewers and have addressed all of their excellent suggestions in our revised version of the manuscript and have revised the figures accordingly. Our detailed answers to each point raised by the reviewers is given below.

We hope that our revised manuscript is now acceptable for publication.

Yours sincerely,

Ruth Chiquet-Ehrismann

Answers to Reviewer 1

Major compulsory revisions

1) Following the reviewer’s suggestion, we modified the manuscript by replacing overexpressed with detectable, not to overstate our findings, since we did not quantify the bands and only estimated the level of tenascin-W expression from the band intensity seen on the Western blots.
2) The text was modified as suggested (see answer 1)
3) Marker positions are now indicated in the Fig. 1A-C.
4) We have added the numbers in the pie chart of Fig. 2A as requested.
5) Fig. 2C has been changed as suggested, numbers have been deleted and the staining categories “absent, low, moderate” are indicated in the respective panels.
6) We included the staining categories in Fig. 3 as suggested.

Minor essential revisions

1-23) We agree with all of the suggested text changes/corrections and have incorporated all of them in the revised manuscript.

Discretionary revisions

1) We added in Materials and Methods: “After a one hour blocking step in 5% milk powder…”
2) All healthy kidney samples were scored as low expression or absent. This is now specified in the text: "immunohistochemical analysis revealed slight, but clearly visible expression of TNW in most of the control kidney samples while the remaining samples were unstained"
3) The quality of the sections of the indicated tumors was not high enough to be published, but the result was clearly visible. Thus we prefer not to present the pictures in a figure.
4) The suggested citation has been inserted.
5) We do not think a figure of the structure of tenascin-C and tenascin-W is useful for this paper, but instead we refer to a recent review where models of these proteins are presented: “(see [5] for information on the structure of tenascins).”
6) We have introduced abbreviations for tenascin-W (TNW) and tenascin-C (TNC) in the revised manuscript as suggested.

Answers to Reviewer 2:

1) We agree with the comments of this reviewer and have now discussed the fact that large tenascin-C variants are more cancer-specific than the total tenascin-C analyzed in the present study. We have discussed these aspects now in the revised Discussion section and have added the corresponding references:

“However, in the case of TNC certain splice variants with additional fibronectin type 3 domains have been shown to be more tumor-specific than the shortest isoform (23,24). Therefore, isoforms-specific antibodies against TNC can also be used as tumor-specific markers (24,25). This is also the case for another ubiquitous extracellular matrix protein, namely fibronectin, which was shown to have cancer-specific splice variants which can be used as targets for antibody-mediated therapy in human patients [26,27].”

2. We apologize for this omission. The slides were blocked before staining and we have added this in the Methods sections: “For chromogenic stainings, slides were first blocked twice for 12 min with the AB Block reagent (Ventana).”

3. We appreciate these comments and have added additional points of discussion as suggested including the relevant references:

“Interestingly, a similar deposition outside the endothelial basement membrane has already been described for large TNC splice variants in newly formed blood vessels [23,29]. Use of a renal carcinoma xenograft model has revealed that the source of these perivascular large TNC molecules were the carcinoma cells themselves [29]. It would be of high interest to establish whether carcinoma cells could also be the source of blood vessels associated TNW.”

We also have added in the conclusion section the suggested mention that successful treatment requires that the therapeutic agent has to be able to pass the vessel wall: “The perivascular deposition pattern of TNW however will limit this strategy to antibody-drug conjugates able to cross the blood vessel walls.”

4. We have added a further clarification concerning TNW expression in benign tissue remodelling and inflammation answering this important question by the reviewer: “We can not exclude so far a possible association between TNW and other types of tissue remodelling conditions such as inflammation or wound healing. However, we were unable to detect TNW either in biopsies from patients with inflammatory bowel diseases (even in areas where TNC is highly expressed – our unpublished data), nor in wound healing in the mouse (28).”