Reviewer's report

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

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Reviewer: Petra Richter

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The study performed by Breliere and co-workers entitled „Tenascin-W is a better cancer biomarker than tenascin-C for most human solid tumors“ investigates the expression levels of Tenascin-W and Tenascin-C in a variety of human solid tumors (pancreas, kidney, lung carcinomas and malignant melanoma) compared to a panel of “normal” tissue. The main finding of the study is that there is a cancer associated protein expression of both Tenascin-C and Tenascin-W not or only to a significantly lesser extent occurring in “normal” tissue. Focussing on the comparison of the protein expression of Tenascin-C and Tenascin-W authors demonstrated that Tenascin-W is more “cancer specific” because there is nearly no expression in “normal” tissue samples except liver and spleen whereas Tenascin-C is expressed also in some other organs. Against that background, authors suggest Tenascin-W to be a very potent tumor biomarker for most human solid tumors.

The idea of the paper is of high clinical interest since the identification of new valid cancer biomarkers would crucially contribute to a faster diagnosis and a better surveillance after therapy. Methodologically, the study is performed very well. Protein expression is analysed by immunohistochemistry and western blot analysis as well. The number of cases investigated is adequate and the study results therefore representative. Results are presented clearly and in a good quality.

Nevertheless, there are some points of criticism:

1.) The authors do not consider the importance of the differential carcinoma associated re-expression of distinguishable isoforms of Tenascin-C generated by alternative splicing! In the view of the reviewer this is indispensable to evaluate the value of Tenascin-W as a tumor biomarker compared to Tenascin-C. At least the differentiation between large and small isoforms of Tenascin-C (Tn-CL and Tn-Cs) in carcinomas must be considered. The finding of the current study that Tn-CS is detectable in “normal” tissue is known for many years since these small variants are important components of the extracellular matrix muscles, bones and cartilage. The antibody used in this study detects all variants of Tenascin-C and not only the large isoforms. These facts are of great importance and must be taken into account by the authors at least in the discussion. To improve the significance of the study, Tenascin-W protein expression should be analysed in relation to the expression of different Tn-C splicing variants (detectable by
splicing domain specific antibodies which are available) reported to be re-expressed in a carcinoma entity specific manner.

2.) For immunohistochemistry, a biotinylated anti-mouse secondary antibody is used. Did authors perform Biotin blocking before? If so, it should be described in the material and methods section.

3.) In the present study, an expression of Tenascin-W in spatial association to blood vessel could be demonstrated. As one can see in Figure 5, there is a perivascular deposition. This deposition pattern outside the endothelial basement membrane has been already described for large Tenascin-C splicing variants in newly formed blood vessels of different tumor entities (Berndt et al. 2010, Histochem Cell Biol, Galler et al. 2011, Histochem Cell Biol). The reviewer encourages the authors to discuss these findings in the context of their own results. Furthermore, authors suggest Tenascin-W as a target “for selective delivery of anticancer medicine”. Here it should be discussed that due to the perivascular deposition pattern the accessibility from the blood stream is limited because therapeutic agents have to pass the vessel wall (endothelium and endothelial basement membrane) before the target structure can be reached. This should be discussed.

4.) Authors could show a cancer associated expression of Tenascin-W not occurring in most “normal” tissues. Is this really a cancer specific phenomenon or does this expression belongs to the complex reorganization of the extracellular matrix during processes of benign and malign tissue remodelling in general? More precisely: What about inflammation? This question should be taken into account at least in the discussion.

Taken together this is a well performed and presented study that merits publication in BMC Clinical Pathology after minor revision.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

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