Reviewer's report

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

**Version:** 4  **Date:** 11 June 2012

**Reviewer:** David S Guttery

Reviewer's report:

Brellier et al. hypothesize that tenascin-W (TNW), a member of the tenascin family of extracellular matrix glycoproteins, is a more specific and broader cancer biomarker than the far more extensively studied tenascin-C (TNC). They substantiate this hypothesis by showing (by Western blotting) that TNW is either present at very low levels or completely absent in a battery of normal adult tissues, but is present at high levels in pancreatic and renal carcinomas. Furthermore, they show that TNW is highly expressed in kidney tumours and malignant melanomas by TMA. This very interesting study is well thought out and a number of methods are used to substantiate their findings, with the experiments performed well and in a correct manner, with all necessary controls present. However, there are a few questions and typographical errors/grammar corrections which I feel need addressing before the manuscript should be accepted.

Major Compulsory Revisions:

1. Results (Tenascin-W is overexpressed in pancreas carcinomas): Title, the authors state that Tenascin-W is “over expressed”, how was this quantified and what is TNW overexpressed compare to? Normal tissue? I would suggest either quantifying the band intensities on the Western blot (using Image J for example, as the authors have done for the TMA experiments) and comparing that to normal tissue (or even VCL expression) or replacing “over expressed” with something like “increased band intensity was found on a Western blot”. Also, lines 4 and 5 state “very high levels of tenascin-W” or “moderate levels”, if making that statement I think you need quantification.

2. Results (Tenascin-W is overexpressed in kidney tumors): Line 3, again, “very high levels, moderate etc” stated. Please either quantify or change as suggested above.

3. Figure 1A, B and C: Please add markers for protein size.

4. Figure 2A: Please indicate numbers for each section of the pie chart as this will give a clear indication of the distribution.

5. Figure 2C: I really like this panel but please replace numbers #34, #33 etc with the respective staining category name (i.e. absent, low, moderate and high) as this will make it clearer, in my opinion.
6. Figure 3A: Again a beautiful panel but please add staining classification to the left of the panel as although it is explained in the legend I think it would be immediately clear if the figure was labelled this way.

Minor Essential Revisions:

1. Abstract (Background): Line 3, please replace “least described” with “least studied”

2. Abstract (Background): Line 6, please take out “general” from the sentence “tenascin-W could serve as a general cancer-specific extracellular…”

3. Abstract (Methods): Line 3, please write as “kidney and lung, as well as melanomas and compared them to healthy tissues”.

4. Abstract (Results): Line 2, please replace “human adult organs in normal, non-pathological conditions” with “human adult organs under normal, non-pathological conditions”.

5. Abstract (Conclusions): Line 1, please replace “tenascin-W to most solid” with “tenascin-W to a broad range of solid”.

6. Background: Line 8, should read “specifically expressed in the tumor stroma”.

7. Background: Line 14, please remove “the” and “organism” and change to read “in adult tissues”.

8. Background: Line 15, please change “wounds” to “wound-healing”

9. Background: Line 17, please change “been performed and” to “shown that”

10. Background: Line 17, please change “tenascin-C expression shows…” to “tenascin-C expression could have…”

11. Methods (Western blot analysis): Line 2, please state how much protein was loaded.

12. Results (Tenascin-W is not detectable in most adult organs): Line 1, please remove “normal”.

13. Results (Tenascin-W is not detectable in most adult organs): Line 3, please remove “Only” and also please change “liver very faint…” to “liver extracts very faint…”

14. Results (Tenascin-W is not detectable in most adult organs): Line 9, please replace “lower tenascin-C isoform” with “lower molecular weight tenascin-C isoform”.

15. Results (Tenascin-W is not detectable in most adult organs): Line 13, please change “also can..” to “can also..”

16. Results (Tenascin-W is overexpressed in pancreas carcinomas): Please
change all incidents of “pancreas carcinomas” to “pancreatic carcinomas”

17. Results (Tenascin-W is overexpressed in pancreas carcinomas): Line 11, please change “additional high isoforms..” to “additional higher molecular weight isoforms..”

18. Results (Tenascin-W is overexpressed in kidney tumors): Line 13/14, please change “expressed easily detectable expression of tenascin-W (ie moderate or high)” to “expressed moderate or high levels of tenascin-W”

19. Results (Tenascin-W is overexpressed in kidney tumors): Line 16/17, please remove “as no number appears in the upper right “triangle” of the table” and put brackets around “Fig. 2B”.

20. Results (Tenascin-W is overexpressed in kidney tumors): Line 23, please change “proof” to “prove”.

21. Discussion: Line 15, please change “appeared in” to “appeared on”.

22. Figure Legends (Figure 2): Line 2, please change “stained for of tenascin-W” to “stained for expression of tenascin-W”

23. Figure Legends (Figure 3): Line 1, please remove “malignant” as it is not an IHC analysis of just malignant melanoma samples, but primary tumours too.

Discretionary Revisions:

1. Methods (Western blot analysis): Line 5, it would be useful to know long the membranes were blocked for.

2. Results (Tenascin-W is overexpressed in kidney tumors): Line 12, was expression of TNW in the control kidney samples scored? If they all came out as low then no need for anything extra but if any came out as moderate or high then I think it would be good to add a chart to show this.

3. Results (Tenascin-W is expressed around blood vessels): Line 10, it would be good (although not essential) to show co-localisation in the tumours you described and further substantiate your findings instead of just saying “data not shown”.

4. Discussion: Line 46, after “called cancer stem cells (CSC) or cancer initiating cells” it would be good to have a reference, for example Beachy et al (2004) Nature; 432, 324-331.

5. Additional figure: I think it would be useful to add a figure illustrating the structures of TNW and TNC. Although these are readily available in a number of review articles, I think it would be nice to have a visual indication in this manuscript.

6. Is it necessary to type the full name of tenascin-W and tenascin-C throughout the manuscript? You could just abbreviate them after first use.
Level of interest: An article of importance in its field

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