Reviewer’s report

Title: A calcium-binding protein S100P in normal and malignant human tissues

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Reviewer: Sven Diederichs

Reviewer’s report:

General

Parkkila et al. describe the expression levels of S100P mRNA by qRT-PCR and S100P protein by IHC in normal and malignant tissues. For their IHC studies, they have generated a new monoclonal antibody for S100P. They discuss the implications of their findings for the use of S100P as tumor marker or therapeutic target. The manuscript is carefully written and the data well presented. However, while their studies are of interest, two major issues need to be resolved before the revised manuscript can be accepted for publication in BMC Clinical Pathology.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) Incomplete characterization of the novel antibody

The authors need to characterize their new antibody in more detail - or present data already obtained in Supplementary Figures or at least "For Review Only". They mention Western blots for endogenous and ectopic S100P, which should be shown - also comparing the commercial antibody with their new. In addition, they mention that their antibody generates less background in IHC (p. 11) - the data to back this claim should also be presented. Also, in the Discussion section (p. 11), the authors state that the IHC staining is "similar in most cases" - however, as far as I understand, the two antibodies are only compared in a single figure (Fig. 2A vs. Fig. 2B). If other IHC experiments were also carried out with both antibodies, the authors should annotate or show them. Lastly, the authors could significantly strengthen their point - if the new antibody works well in Western blots as described - by comparing at least in a single experiment e.g. tumor and normal tissues to verify their results with a second method at the protein level.

2) Discussion of the data & Conclusions

In the Abstract, Discussion and Conclusion sections, the authors conclude that the use of S100P expression as tumor marker my be limited due to its wide expression in both normal and tumor tissue and that it may represent a promising therapeutic target.

In the light of the data presented in this manuscript, I would draw the opposite conclusions form their data: As the authors have listed in their well-versed Background section, S100P has been found by multiple studies to be overexpressed in cancer vs. normal tissues and implicated in the tumorigenic process. In fact, the only data that Parkkila and colleagues show directly comparing expression levels in normal and malignant tissue from the same organ (Fig. 5) precisely recapitulate previous findings: The S100P expression is much higher in tumor than in normal tissue. This finding would further corroborate the potential use of S100P as tumor marker rather than its limitations. In addition, the authors would need to discuss that despite significant expression in normal placenta, S100P might still be a reliable marker of malignancy in other tissues. The only limitation of the use of S100P as tumor marker derived from their data could be invoked from Fig. 6 showing large variation of S100P expression between different tumors derived from the same organ.

On the other hand, the use of S100P as therapeutic target is much more compromised by the expression in some normal tissues than its use as tumor marker. Therefore, their study would more likely indicate limitations than advantages of S100P as therapeutic targets.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Methods - qRT-PCR: The authors should state whether the S100P primer pair spans an exon-exon-junction or whether the RNA was treated with DNase before generation of cDNA to exclude DNA contamination - especially given the observed discrepancy between mRNA and protein expression
e.g. in stomach vs. esophagus.

2) Methods - IHC: The composition of the Wash Buffer should be given.

3) Figure Legends - Fig. 2: The explanation of the arrows in different panels does not match the arrows in Fig. 2 (Fig. 2C does not have arrows, but Fig. 2E & 2F).

Discretionary Revisions (which the author can choose to ignore)

1) Discussion: The authors discuss the expression data in leukocytes in great detail on p. 12 - though they only present a single figure panel on their mRNA expression which they show not always correlates to protein expression and is generally low in leukocytes.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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.