Reviewer's report

Title: The type II transmembrane serine proteases hepsin and TMPRSS3 are associated with breast cancer survival

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Reviewer: Tiziana Triulzi

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The manuscript by Pelkonen et al. evaluated the expression at mRNA and protein levels of two transmembrane serine proteases (TTSP: TMPRSS1 and TMPRSS3) in breast cancer samples. Obtained results indicated that these TTSPs are expressed at higher levels in malignant tumors than benign lesions and that low TTSPs expression were independent prognostic factors for poor survival of patients with malignant breast carcinomas. Even though few studies regarding the expression of these TTSPs, especially TMPRSS3, in breast carcinomas are present in the literature, the study suffers from certain deficiencies (elaborated below) which affect the interest level in the manuscript or its relevance to publication in this version in this Journal.

1- The paper included several redundant analyses that make the paper hard to follow and not focused. The authors should choose to focus on TMPRSSs mRNA or protein expression levels and give corresponding results in the main text and put the others in the supplementary.

2- The authors concluded that both TTSPs are potential novel therapeutic targets. It is not clear how two molecules that are associated with bad prognosis if low-expressed could be considered as therapeutic targets. Instead, these molecules could be used as prognostic biomarkers.

3- It is not clear how the authors analyzed the immunoreactivity of TTSPs in tumor samples. What does the ‘median value of immunohistochemical scores’ means? Why did the author choose the median value? Usually, the immunoreactivity mean value of three areas of the tumor slides is used.

4- It is not clear how the authors analyzed the qPCR data. How did the authors use standard curves for sample quantification? The authors need to better explain these analyses.

5- In Table 1, the authors need to give data regarding patients’ treatment and insert analysis of TTSPs association with breast cancer survival according to treatment group if possible.

6- In Table 2, results regarding all covariates used in the multivariate analyses need to be included. Moreover the cut-off used to classify low tumors as low expressing need to be better specified.

7- Data presented in Figure 2 are redundant. Instead, Supplementary Table 1, in which association with all covariates is reported, could be moved as main table.
8- In Figure 3, it is not clear why the authors reported significances calculated by several tests. It looks like they do not know which one is the best for their data, since they are similar but not identical. Moreover, it is not clear why they reported association of Hepsin with survival in 10 years follow up. The authors need to show data, they have, in a 20 years follow-up and then discussed the results.

9- Data presented in Figure 4 are redundant and do not add any informative data compared to what presented in Table 2. This figure should be removed.

10- The discussion is too long and unfocused. The authors demonstrated an association between TTSPs expression and breast cancer prognosis, without any data regarding the biological role of these TTSPs in human breast cancer, thus the discussion (from line 419) regarding their pro-metastatic activity is confusing and useless. The part regarding matrilpase-2 is useless too. The authors need to focus the discussion on results presented in this study and discuss instead why the low levels of these two TTSPs are associated with poor prognosis even if their biological role suggested their pro-metastatic activity.

11- Limitations of the work need to be included in the discussion session.

12- Based on data the authors previously published in PLoS One, and on the evidence that mRNA expression levels of these two molecules resulted more prognostic than protein levels, it would be interesting to add in this paper information regarding the association between TTSPs expression and SNPs in TMPRSS1 and TMPRSS3 and association with breast cancer prognosis.

13- Validation of TMPRSS1 and TMPRSS3 mRNA expression levels as prognostic marker in public gene expression datasets should be included.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.