Reviewer's report

Title: A Protein-Based Set of Reference Markers for Liver Tissues and Hepatocellular Carcinoma

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Reviewer: Jeff Leek

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Brief Statistical Review of: A Protein-Based Set of Reference Markers for Liver Tissues and Hepatocellular Carcinoma

Authors: Stella Sun, Xin Yi, Ronnie T.P. Poon, Chun Yeung, Phillip J.R. Day, and John M. Luk

Summary:

This paper describes a 2-DE proteomic experiment designed to identify housekeeping proteins in human liver tissue. The approach is to identify proteins whose measured intensity is similar across 105 cancerous, 103 non-tumorous cirrhotic and 116 normal tissues. The authors use a P-value threshold to identify potential candidates and study their distribution. They identify 3 stable protein markers and validate two in subsequent experiments.

Overall Comments:

The major critique suggested by the reviewer is that a large P-value threshold can not be used to identify proteins that do not change in an ANOVA. The referee is correct that statistical interpretation of large P-values as an indication of stability is inappropriate. However, it appears that the authors do not attempt to interpret large P-values as a measure of statistical significance; rather, they simply use the P-value as a ranking tool to identify potentially stable proteins for future study. Furthermore, they report the mean, standard deviation, and 95% confidence interval for the intensity of these proteins across the three groups. The authors should emphasize these points to reduce potential concerns about statistical interpretation.

Minor Essential Revisions:

(1) It is important to emphasize that choosing large P-values to pick candidates for reference markers does not imply any measure of statistical significance. Stating clearly that it is just a ranking tool used to identify proteins with potentially low variability across groups would reduce confusion. The multiple comparisons approach described on page 5 is also confusing. Since the P-value does not constitute a measure of significance in this case, there is no point in performing a correction for multiple tests.
(2) Some discussion of the reported means, standard deviations and confidence intervals with respect to the potential for reference markers would be very helpful since the selected markers can not be reported with a corresponding measure of statistical significance.

(3) It would also be useful to see some overall measure of the variability of each potential marker across all three groups (cancerous, non-tumorous cirrhotic, and normal).

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

**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.