Reviewer’s report

Title: Differential expression of THOC1 and ALY mRNP biogenesis/export factors in human cancers

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Reviewer: Melissa J. Moore

Reviewer’s report:

This manuscript describes a comparative analysis of the expression patterns of the mRNA export factors THOC1 and ALY in tumor samples, with the hope of establishing a link between the levels of these factors and tumorogenesis. Unfortunately, as described in detail below, much of the analysis is incomplete and/or repetitive of other previously existing data. Therefore, this manuscript adds little to our knowledge regarding any potential roles of mRNA export factors in cancer.

Major Essential Revisions:

(1) On page 4, the authors note that THOC1 has been implicated in several cancers and claim that: “However, neither the pattern of expression of THOC1 and other THO components in different tumors and the possible mechanism underlying this process are known”. This claim is the primary motivation for this study. Unfortunately, the authors have failed to take notice of numerous cancer biology studies that utilized microarray analyses, expressed sequence tags, or other genome-wide expression profiling methods such as SAGE. Even though the focus of such studies was not necessarily changes in THOC1 and ALY’s expression in cancer, they have nonetheless collected expression from a large number of cancerous cell lines, tissues, samples. These datasets are publicly available through many databases including ArrayExpress (http://www.ebi.ac.uk/microarray-as/ae/), Human Gene Expression Map (Luk M, et al. A Global Map of Human Gene Expression. Nature Biotechnology. 2010), Novartis Gene Atlas (http://biogps.gnf.org) and several others. In fact, a quick search using Human Expression Map reveals many studies measuring THOC1 expression using microarrays and suggests that there is a large variation in THOC1 expression across breast cancer cells.

Similarly, immuno-histochemistry for THOC1, and ALY was previously carried out in a large number of normal, cancer tissues/cell lines. It is encouraging that the authors’ data is in good agreement with these previous results. This previous dataset is available through the Human Protein Atlas (http://www.proteinatlas.org/) and recent description of the project is available in Berglund L et al. Mol Cell Proteomics 2008 (10):2019-27.

If the authors wish to publish their results, they must consider these previous results in their analyses. The authors should cite these works, and
interpret/discuss their work in comparison to these existing datasets. As now written, the current manuscript provides minimal little new information about the proteins of interest.

(2) On page 2, the authors claim that “These results suggest a differential connection between tumorogenesis and the expression levels of human THO and ALY. This study opens the possibility of studying mRNP biogenesis factors as putative players in cell proliferation that can have a key impact on tumor development”. However, I don’t think the data presented are sufficient to support such a claim. This study is entirely observational in nature, and in cancer tissues/cell lines hundreds of genes are known to be differentially expressed both at the level of mRNA and protein. Furthermore, cancerous cell populations are highly heterogeneous. In any particular instance, the population of cancer cells will vary in their overall expression profiles and specifically their THOC1 and ALY expression. To suggest any physiological relevance for the observed differences in expression profiles additional evidence is needed. Unless the authors provide additional evidence, they should appropriately constrain their interpretation of the data.

(3) On page 6, the authors note that the level of HPRT mRNA was used for normalization in their qrt-PCR analysis. However, existing evidence indicates that this is not an appropriate choice. HPRT1 gene levels have been observed to vary substantially across various cancer cells (search for HPRT1 using 15.meta.groups as a factor at the Human Expression Map: http://www.dev.ebi.ac.uk/microarray/hge/HGE.jsp). Therefore, a better normalization procedure (e.g., the one described in Vandesompele, J et al. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology) would have to be employed before one can make any conclusions from the qrt-PCR data.

(4) Throughout the paper there is a general lack of appropriate statistical analysis. For example, the data presented in Figure 1 are discussed in terms of arbitrary thresholds of 0.5/1.5 fold change. However, we have no sense whether these represent statistically significant deviations. Further, reporting only fold-change can be misleading. A 1.5 fold change from 100 units of expression to 150 units of expression will generally be more significant than a change from 0.0001 expression units to 0.00015 units due to measurement errors at lower expression levels causing higher variance in observed values. These effects should be considered and the significance of the observed changes reported.

Minor Essential Revisions:

(1) On page 2, hybridization is misspelled as “hibridization”. Same error occurs at
multiple places in the paper.

(2) On page 6, the authors wrote that they used “a ji quadrade test”. This result was not mentioned in the Results and in the figure legends the authors refer to the standard chi-squared test. I believe this “ji quadrade test” is a typo as I have never encountered this test and was unable to find any previous reference describing it.

(3) On page 2, it is unclear what is referred to in last sentence of “Background”: “Given the importance of the maintenance of genome integrity and its(?) link with cancer”.

(4) In general, the paper will benefit from some language correction. The writing is somewhat redundant, especially in the discussion.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I declare that I have no competing interests