Reviewer's report

Title: Characterization of Beta2-Microglobulin Expression in Different Types of Breast Cancer

Version: 1 Date: 14 August 2014

Reviewer: Kakoli Das

Reviewer's report:

- Major Compulsory Revisions
The authors Kesheng Li et.al. identify expression of a well-known housekeeping gene, #2-microglobulin in breast cancer molecular subtypes. They show differential protein expression but not gene expression of #2-M in the different subtypes. However, the expression has no correlation with patients' clinico-pathological parameters. The study has several limitations.

1. It is stated in the abstract that the purpose of the study was to investigate the mechanism of #2 –M action in breast cancer. However, the authors have not done any functional analysis to show the mechanism of #2 –M action which is warranted.

2. Conclusion in the abstract is usually drawn from the results of the study which is ambiguously stated.

3. Primer sequences in the methods section for real-time PCR have not been verified. Usually the sequences are verified using BLAST or BLAT after they are designed. Surprisingly, the #2-M primers sequences that the authors have designed do not belong to #2-M.

4. The authors have not mentioned the purpose of using p53 and ki-67 antibodies for their experiments.

5. In the Result section (silencing effect), it was mentioned that 3 siRNAs were used. However, the authors did not elaborate on the three siRNAs and the rationale behind using three. Why only siR-3 showed significant downstream effect?

6. What is the clinical significance of #2-M expression in this study? Was there any correlation with overall survival and treatment status of the patients?

7. The results have not been adequately discussed. The only reason suggested was that #2-M might be regulated by different signaling pathways. Which pathways and how are they regulated? There is repetition of sentences in the discussion. The study did not provide enough information about these. If the title states “characterization of #2-microglobulin….”, I think clinical significance and functional analysis would make it a complete study.

- Minor Essential Revisions
1. The quality of English could be better. The manuscript was not spell checked.
The authors confused between ‘straining’ and ‘staining’ a number of times. In the abstract methods section “lewkmia” should have been spell checked.

2. Figure 5 could be avoided as it does not add any value to the manuscript.

3. Statistical analyses were performed with an older version of SPSS 11.5 while IBM-SPSS version 22 is mostly used nowadays.

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

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

- Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?
  Response: No

- Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?
  Response: No

- Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?
  Response: No

- Do you have any other financial competing interests?
  Response: No

- Do you have any non-financial competing interests in relation to this paper?
  Response: No

If you can answer no to all of the above, write ‘I declare that I have no competing interests’ below. If your reply is yes to any, please give details below.

I declare that I have no competing interests