Reviewer's report

Title: Circulating miR-200c and miR-141 and outcomes in patients with breast cancer

Version: 3
Date: 18 August 2014

Reviewer: Michael Georg Schrauder

Reviewer's report:

1. The question posed by the authors is defined and scientific as well as clinically relevant.
2. The methods are well described.
3. The data are not entirely sound and do not perfectly fit into the recent data by other groups. Different studies were not able to find an association of miR-200c or miR-141 with breast cancer. Moreover, the association of miR-200c and miR-141 expression with survival was not confirmed by different GEO data sets.
4. The manuscript adheres to the relevant standards for reporting but I found no reference that the presented data was made public on e.g. GEO?
5. The discussion and conclusions are well balanced but not completely supported by the data.
6. Most of the limitations of the study are clearly stated, but one major problem of RT-PCR-analyses is not discussed: sample normalization. The authors used U6 snRNA and 5S rRNA to control input variability and sample normalization. This approach is criticized by many experts. Citation: ‘5S or U6 were the two least stable RNA species in a panel of 12 RNA targets evaluated across 13 discrete normal human tissues.’ Taken from:

Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues.

Peltier HJ, Latham GJ.

Other examples from the literature:

U6 is unsuitable for normalization of serum miRNA levels in patients with sepsis or liver fibrosis.


snoU6 and 5S RNAs are not reliable miRNA reference genes in neuronal
differentiation.

7. The authors clearly acknowledge any work upon which they are building.
8. The title and abstract accurately convey what has been found.
9. The writing is acceptable.

- Major Compulsory Revisions
The first shortcoming of the work is the small number of patients (and especially controls) analyzed.
The second major point is the one step approach: no "screening step" (e.g. array or NGS) was conducted to identify candidate miRNAs, which could then be confirmed by RT-PCR. The candidate miRNAs were taken from previous studies in patients with gastric cancer.

3. The blood collection of 49% of the patients was done "after R0 surgery" of the tumor. This is in sharp contrast to the published work by Heneghan et al. (cit.: ´levels of miR-195 and let-7a decreased in cancer patients postoperatively, to levels comparable with control subjects´) Taken from:

4. The authors used a completely heterogeneous cohort of patients including patients with metastasis, with positive and negative hormonal receptors, HER2 positive and negative,.
Taken into account the major differences of distinct breast cancer subtypes and the small number of patients in this study this heterogeneity makes it very hard to interpret the data.

- Minor Essential Revisions
Space character is missing on page 7 line 17 ´141expression levels`
Declaration of competing interests:

'I declare that I have no competing interests'