Reviewer’s report

Title: Expression of BMI-1 and Mel-18 in breast tissue - a diagnostic marker in patients who have already been diagnosed with breast cancer

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Reviewer: Olaf J.C. Hellwinkel

Reviewer’s report:

Riis et al. presented an analysis on two Polycomb Group (PcG) genes - Bmi-1 and Mel-18 - and the potential value of their expression products as early breast cancer markers in normal tissue.

It is known, that these genes code for epigenetic silencers involved in maintaining cellular identities and that their deregulation can result in cancer. The study aims to compare the expression of both genes in normal breast epithelium of cancer patients and to relate it to the level of expression in breast epithelium of healthy women. As expression shifts of these genes in unsuspicious mamma-tissues could indicate occult (pre-)malign processes within the breast, such findings could improve the stratification of women at risk of developing malignancy.

The principal idea of the study is interesting and innovative. The analysed collectives (79 tumors, 23 adjacent cancer free tissues from tumor patients and 12 healthy controls) are compact but sufficient for a pilot study. The analysis of both, the mRNA expression level and the protein expression level (using immunohistochemistry) enhances the dimensionality and improves the informative data base of the study. In deed, the authors report Bmi-1 to be upregulated in tumor-adjacent normal breast tissues, while Mel-18 expression is repressed. These differences are quantitative at the RNA-level, while the protein-expression results are less informative but seem to support the RNA-data. These results principally support the suggestion of an applicability of Bmi-1 and Mel-18 expression data as early breast cancer markers.

However, there is quite a number of issues and/or problems in the design of the study and the paper composition, which need to be commented and/or corrected thoroughly.

Major points:

• First of all, information on tissue processing is insufficient. Were the tissue samples obtained for RNA-isolation analysed by histopathology? This is highly important to ensure/exclude the presence of desired/undesired tissue compartments. Were obtained defined tissue compartiments eventually by microdissection?

• As Bmi-1 and Mel-18 are involved in cell aging, possible age dependent
expression differences can be assumed. Therefore, information on the age
distributions of the individuals within the tested collectives is mandatory. What
about the median age (and quantiles) of the normal control individuals? Are
eventual differences between the tested collectives significant?

In this respect, a table of the collectives with all relevant clinical data would be
fine and could highly improve the comprehensibility of the paper!

• As stated by the authors, micro-arrays were made to assess mRNA-level
differences. Such experiments give valuable information about
whole-transcriptome variabilities between the tested tissues, however,
quantitative information given by this technique is less reliable then such
obtained by (real-time) RT-PCRs. If RT-PCRs were not performed, the authors
should declare why and discuss the consequences.

• Information about the applied statistic tests is missing!

• Results section - “Immunhistochemistry”: The text is vague and unspecific. A
table with the summarizing the specific results would be helpful.

• The composition of the discussion should be improved. Also, English spelling
and grammar is partially insufficient. A correct, concise and sound expression is
essential to enhance the understandability of the discussion.

Minor points:

• English spelling in the materials and methods- and the results section is
sometimes weak and should be corrected.

• Immunhistochemistry evaluation is described confusingly.

• Figures are explained insufficiently. The application of suitable dimensions of
the Y-axis could allow an intuitive understanding (“x-fold transcription compared
to …” or so).

• The application of the word “expression” in the results section (text and figures)
is partially not appropriate. Better use transcription for the data shown in figures 1
to 3!

• Control collectives are not denominated consistently in text and figures. This is
confusing.

• Results section - “Bmi-expression…” : The p-value of 0.046 for Bmi-1
upregulation is significant, but not highly significant! What about the effect size
(see criticism above about the figures)?

• Results section - “Significant inverse correlation”: Although inverse transcription
trends are unequivocally observable, it is problematic to utilize the term
“correlation” for a comparison of continuative variables (transcription means) in
nominatively “ascending” categories (describing cell dedifferentiation steps from
normal to tumor). What kind of statistics was applied here? What is the p-value?
Level of interest: An article of importance in its field

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'