Reviewer's report

Title: AKAP3 correlates with triple negative status and disease free survival in breast cancer

Version: 2  Date: 14 December 2014

Reviewer: Clara NAHMIAS

Reviewer's report:

Major compulsory revisions

1. Percentage of cancer cells in tumor samples (cellularity) should be determined to be sure that loss of AKAP3 mRNA expression is not simply due to low content of cancer cells in each sample.

2. Internal controls of real-time PCR are lacking. Data obtained from AKAP3 qPCR need to be normalized relative to at least two endogenous genes (for instance RPLP0, EEF1G or PP1A), in order to account for extraction, reverse-transcription and possible degradation of RNAs. In absence of these controls, qPCR cannot be quantified and results are not conclusive.

3. An additional Table should be provided indicating AKAP3 expression levels (delta-CT and normalization relative to endogenous genes) in each sample analyzed (tumor and adjacent) and mentioning corresponding clinical data (age, size of the tumor ER, PR, HER2, treatment, time of survival etc)

4. A Figure should be provided (scattered dot plot) comparing AKAP3 expression (normalized to endogenous genes) in each tumor and adjacent tissue (classified according to grade, ER positivity, molecular subtype, treatment, etc) and compared with values for 15 normal breast tissues.

5. A Table should be provided showing median values (with standard error) for AKAP3 expression in tumor samples, adjacent tissues and normal samples.

6. Figure 1 is of very poor quality. What does neg-censored and pos-censored mean? Furthermore there is a problem with p values in left and right panels, as these seem to have been inverted. This may lead to false interpretation of the results.

7. Another figure should be provided showing overall survival (median time) and 5-year survival for patients from different groups expressing low or high levels of AKAP3 (cut-off defined as >2 fold or < 2 fold that of median value for normal breast tissue).

8. On line 140, authors write: “There was no association between AKAP3 expression in normal adjacent tissues and triple negativity, but there was a significant association between lack of AKAP3 in normal adjacent tissue and poor prognosis (p=.003)”. These two statements are contradictory and should be discussed in more details, as triple-negative breast cancer is a subtype of poor prognosis.
9. On line 212, authors write: “AKAP3 may act as inhibitor of proliferation since it was not expressed in higher stage and tumor size”. This conclusion is over-interpreted, as down-regulated genes are not all involved in inhibition of proliferation.

Minor essential revisions
1. Line 298: Table 1 (and not tabel 1)
2. Please mention full name of ACTB gene (actin beta)

Level of interest: An article of importance in its field

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