Reviewer's report

Title: AIB1 gene amplification and the instability of polyQ encoding sequence in breast cancer and cell lines

Version: 1 Date: 2 February 2006

Reviewer: Beatrice Orsetti

Reviewer's report:

General
This manuscript by Lee-Jun C. Wong and colleagues reports copy number changes and sequence instability of AIB1 polyQ encoding sequence in breast cancer cell lines and tumors. The issue addressed concerns the relationship between amplification and sequence variability of polyQ encoding sequence of AIB1 gene in breast cancer.

Real time Q-PCR and PCR/Cloning/Sequencing have been used and are well appropriated methods.

--------------------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1- AIB1 polyQ domain sequence variability could be of interest in breast cancer because of its possible implication in aggressiveness or resistance to therapeutical treatment. However the question asked is not clear, and the descriptive data obtained on cell lines in this study do not give enough clues to understand the biological impact of sequence variations in cancer cells.

2- Only few information is available for derived cell lines. Authors should add a reference or give detailed explanations on how these cell lines were obtained.

3- Concerning real time Q-PCR analysis for copy number evaluation in highly rearranged cell lines one gene as reference is not sufficient and especially B2 microglobulin gene as explained in ref n°28 included in the manuscript. The method used for copy number calculation should be better described.

4- The interpretation of decrease of amplification in various MCF-7 variants should be discussed. In my opinion, clonal heterogeneity is important in this cell line (Nugoli et al. BMC Cancer. 2003 Apr 24;3:13) and could explain genomic variability upon selection conditions or increased number of passages (as for p19 and p72). It should be interesting to do FISH using a specific probe of AIB1 in order to (1)-compare copy number obtained by both techniques (2)- evaluate heterogeneity of each cell line in term of level of amplification and possibly type of amplification (HSR, Dmin).

5- The set of primary breast tumors is too small and should be enriched particularly in ER+ tumors.

6- The number of variant patterns observed in cell lines could be biased by copy number level of the gene due to the PCR/cloning sequencing strategy. Indeed, in amplified cell line, the predominant pattern is the « parental » one, suggesting that the parental allele is amplified and overrepresented in PCR/cloned fragments. It is remarkable that the number of sequence variants observed for non amplified samples is often larger, possibly because of reduced bias. The authors should try to increase the number of sequenced clones in amplified cells.
7- Consequence of these somatic mutation on transcripts size and relative abundance as well as protein levels should be evaluated.

8- In order to evaluate the consequence of such sequence variability on breast cell lines, (shorter pattern, longer pattern) authors should select cell clones bearing mutated patterns for further studies using cell cloning strategies.

9- No sequence data on primary breast tumors are shown. No technical reasons are given.

-------------------------------------------------------------------------------

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1- The title « AIB1 gene amplification and the instability of polyQ encoding sequence in breast cancer and cell lines » is not in concordance with the work as no breast tumors were explored for sequence instability.

2- In material and methods, authors should indicate clearly the number of cell lines and primary tumors used in the study.

3- No clinical information is available for tumors except the estrogen receptor status. Hystological types, and tumor grades should be added at least.

4- In material and methods authors write that they used 7 blood genomic DNA as a control and in the result section mention « 48 age matched control individuals ». The relationship between the two sets of samples is not clear.

5- Authors write that « at least 8 clones for each sample were picked for sequencing » but in table 3, the total number of sequenced clones is 7 (MCF-7 for example) or even 5 for LCC2.

-------------------------------------------------------------------------------

Discretionary Revisions (which the author can choose to ignore)

What next?: Reject because too small an advance to publish

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

- I declare that I have no competing interests