Reviewer's report

Title: Immunodetection of nmt55/p54nrb Isoforms in Human Breast Cancer.

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Reviewer: Dr Michael Fasco

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until I see revised version

This is a potentially interesting series of experiments to determine the mechanism whereby nmt55/p54nrb is apparently under-expressed in a subset estrogen receptor positive (ER+) breast tumors. nmt55/p54nrb participates in pre-mRNA splicing and may serve as a potential tumor marker for hormone/antiestrogen responsiveness since it is absent in estrogen receptor negative (ER-) tumors as well as the subset of ER+ tumors. An aberrant form of nmt55/p54nrb is identified using separate antibodies that have epitopes in the N- and C-terminal and center regions of nmt55/p54nrb. The approach of using antibodies with different epitopes arose from prior experiments that showed no obvious correlation between nmt55/p54nrb mRNA and protein levels, suggesting that a post-transcriptional change had occurred in at least some ER+ tumors.

Unfortunately, portions of this work and manuscript were apparently prepared in haste.

Major Criticisms:

1. The cornerstone of this work is the differential antibody reactivity shown in Figure 6, but it poorly presently and very inadequately explained. Do the authors really believe that all the proteins in this Figure have the same molecular weight? On page 11 they state that a possible cause for the differential antibody recognition could be proteolytic degradation, but immediately dismiss the notion because "the estimated molecular weight of 55 kD is unaltered in these variants". From Figure 6, it would certainly appear that the antibody positive protein in lane 9 is of lower molecular weight than the others, and those in lanes 6 through 8 may be lower than the nmt55/p54nrb in MCF-7 cells. Moreover, the tumor nmt55/p54nrb detected by antibodies NMT-4 and NMT-5 is much different in shape than in the MCF-7 cells, which could be due to the presence of multiple forms. Some mixing experiments would resolve these questions. Also some of the questions raised as to why antibody NMT-4 does not react with nmt55/p54nrb in tumors 8 and 9 (such as a point mutation) could be readily answered by PCR and sequencing of the region of interest. A splice variant could also be the reason, and its presence is readily detected by PCR.
2. The inability of NMT-4 to detect nmt55/p54nrb in tumors 8 and 9 is not entirely convincing. Antibodies do not have the same detection limits on Western blots so it is possible that the absence of reactive protein could be apparent and due to a sensitivity difference. nmt55/p54nrb in MCF-7 cells could be loaded at various concentrations, surrounding the estimated nmt55/p54nrb concentration in the tumor protein, and then detected with each antibody to eliminate sensitivity difference as a cause.

Minor Criticisms:

1. Abstract. Beginning assumes too much prior knowledge on the part of the reader. For example, in background says nmt55/p54nrb expression decreased in a subset of tumors. Later find out that subset is from ER+ tumors, but also decreased in ER- tumors.

2. Page 2 Result - 1st sentence. Why only ER-? Conclusion not justified from 1st part of sentence.

3. Page 4. Last Sentence. in large (ER+ ?) and/ or ER-. Again conclusion not warranted from sentence as written.

4. Figure 3B. Mislabeled or backwards. NH2 and C-terminals of translated protein are 5' and 3' respectively.

Competing interests:

None declared.