Author's response to reviews

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

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PDF covering letter
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Carole Mongin, Ph.D.
Editorial Assistant
BioMedCentral

Re: Pavao et al., “Immunodetection of nmt55/p54nrb Isoforms in Human Breast Cancer”
Revised Manuscript

Dear Dr. Mongin,

Attached please find the revised manuscript entitled “Immunodetection of nmt55/p54nrb Isoforms in Human Breast Cancer.” We would like to thank the reviewers for their constructive and helpful criticism, which enabled us to improve the manuscript tremendously. A point-by-point of the changes and explanations made in the manuscript is given below.

Thank you for all of your efforts regarding this matter

Sincerely,

Matthew Pavao, Ph.D.
1. Dr. Sutherland raised an important point in that it would be more informative if the characteristics of the tumors used in this study were provided. In our previous study (Traish et al., Diagn Mol Pathol, 6:209-21, 1997), we attempted to correlate nmt55/p54ⁿrb protein expression with various tumor clinicopathological characteristics in a series of human breast tumors. In this present study, however, the human breast tumors were procured under a separate Institutional Review Board (IRB) of the Boston University, which did not include obtaining tumor clinicopathological characteristics or any other clinical information on the patients. The breast tumor tissue obtained and utilized in this study was considered discarded tissues and was not used for clinical assessment. For these reasons, we made no attempt to obtain any clinical data. Also, for the same reasons, tumor pathological characteristics were not available to us as non-clinical researchers due to the constraints of the IRB.

We fully agree with the reviewer that the availability of these parameters would have provided more detail. However this is beyond the scope of this manuscript, since the focus of this study was to characterize the biochemical characteristics of the different nmt55/p54ⁿrb protein isoforms. One aim of this study was to specifically investigate the relationship of nmt55/p54ⁿrb protein expression with that of estrogen receptor (ER) at the biochemical level. We have included a statement within the discussion section of the manuscript (see e.g. pgs. 12-13) to address the issues discussed above.

2. The discussion has been reasonably shortened according to the reviewer’s suggestion.
Reviewer: Dr. Michael Fasco

Major Concerns:

1. Dr. Fasco clearly points to the brief written description/analysis accompanying Figure 6, which is a key figure in this manuscript. We agree with this concern and have made every effort to provide succinct information in the revised manuscript pertaining to Figure 6.

As to the inquiry regarding the evidence for nmt55/p54nb in Figure 6, we do believe that most of the detected nmt55/p54nb proteins have similar molecular weights. However, it is possible that potential degradation or post-translational modifications may have altered electrophoretic mobility in some tumor samples. For instance, the band representing nmt55/p54nb in lane 9 appears smaller and this may be attributed to proteolysis.

We agree with the reviewer that multiple nmt55/p54nb isoforms may be present in these tumor samples. In the course of our study, we have carried out mixing experiments, similar to those suggested by the reviewer, to resolve the issue of multiple nmt55/p54nb isoforms. For instance we had mixed nuclear extracts from MCF-7 cells with nuclear extracts from several tumors. In other experiments we have mixed nuclear extracts from tumors expressing nmt55/p54nb and from tumors not expressing nmt55/p54nb and carried out SDS/PAGE coupled with Western blot analyses. The data from these experiments proved inconclusive.

In view of the constructive criticism, we have now expanded the explanation of the data in Figure 6 and thoroughly discussed the potential discrepancies regarding protein molecular weights and the presence of multiple nmt55/p54nb isoforms (see e.g. pgs. 11-12).

We agree with the reviewer that point mutations or splice variants could be readily detected utilizing PCR coupled with sequencing techniques. However, we will not be able to implement these experiments with the same tumor samples used in this study because all of these samples were fully utilized and no tumor tissue remains to carry out such a study.

Because of the early detection of human breast tumors and the need to have receptor assay procedures carried out prior to obtaining tissue for research, most of the tumor tissue sample was small and was utilized in this study to characterize the biochemical nature of the protein. No sufficient tissue samples remain in our tissue bank that will allow us to obtain enough RNA to carry out the reviewer’s proposed study. Since the IRB for this particular study has expired as of June 2001, and in order to do this experiment, we would need to initiate a new study, which would require us to file and obtain approval for a new IRB to procure tumor tissues. Indeed, in our future research investigations we will screen tumor tissue and those found not to express nmt55/p54nb (based on NMT-4 antibodies) will be selected and screened by PCR and sequencing techniques to determine if the inability to detect nmt55/p54nb by NMT-4 polyclonal antibody is due to single point mutation or other mutations. We have now added this point in discussion in the manuscript (see e.g. pg. 12).

2. We agree with the reviewer that antibodies have varied detection and sensitivity limits when using Western blot analysis. However, we do not feel that the absence of the nmt55/p54nb
protein band, tested with polyclonal antibody (pAb) NMT-4, in the tumor samples shown in lanes 8 and 9 is due to its limits of sensitivity. We wish to point out that detection of the nmt55/p54\textsuperscript{nrh}, in the proteins extracted from the MCF-7 sample shown in lane 1 and the tumor sample shown in lane 6, were not limited by NMT-4 antibody sensitivity in Western Blots. The nmt55/p54\textsuperscript{nrh} protein expressed in these samples was detected at similar intensity with all three antibodies, including monoclonal antibody NMT-1 (which we expect to have the lowest sensitivity). Furthermore, the consistent, specific, tissue staining for nmt55/p54\textsuperscript{nrh} protein using Immunohistochemistry shown in Figures 7 and 8 with pAb NMT-4 and pAb NMT-5 correlates with the Western blot data in Figure 6 and preclude the notion that the absence of protein bands detected by Western blot is attributed to antibody sensitivity. Thus, the reason that pAb NMT-4 does not detect nmt55/p54\textsuperscript{nrh} protein in a number of tumor samples lies in potential changes in the protein domains.

Minor Criticisms:

1. We are limited by journal’s submission rules in being as detailed as possible regarding background information in the Abstract. We have attempted to be extensive in explaining our previous studies in the background section of the manuscript.

2. We agree with the reviewer that the discussion/conclusion regarding Figure 1 was not clear. We have rewritten this paragraph accordingly (see e.g. pg. 6).

3. As with the previous point, we agree with the reviewer that we were not clear in this portion of the manuscript regarding tumor size and ER status and have re-written this section (see e.g. pg. 4-5).

4. We thank the reviewer for pointing out this labeling discrepancy in Figure 3B. Figure 3B has been revised to indicate the correct 5’ and 3’ regions.

We have modified the manuscript according to the Reviewer’s comments. We thank you and hope that the revised manuscript is acceptable for publication.