Reviewer's report

Title: WWOX protein expression varies among ovarian carcinoma histotypes and correlates with less favorable outcome.

Version: 2 Date: 3 May 2005

Reviewer: Hani Gabra

Reviewer's report:

General
This is a paper of major importance and is deserving of publication. This paper analyses WWOX expression in ovarian normal tissue and cancer by Western analysis and by immunohistochemistry. The paper analyses WWOX expression in ovarian cancer in relation to clinicopathological variables, and finds frequent loss of WWOX expression in ovarian tumors, particularly mucinous and clear cell carcinomas of the ovary. WWOX loss associates with shorter survival time for patients, and with stage 4 disease. WWOX loss is also associated with the PR negative state in ovarian cancer, but interestingly not ER loss (which is the case for breast cancer)

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
None

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.) I am unclear as to how the WWOX cutoff levels were decided on. It would be useful if the authors would address this issue. Why was a negative cutoff of 63 decided on?
2) Why was ER/PR status defined by <5%, 5-40% and >40%. Was this on the authors previous breast ca work? Previous publications have shown histoscores of >120 for ER and >70 for PR to be functionally important for endocrine therapy response in ovarian cancer. It is unclear what the basis for the cutoffs the authors used have been, and this should be explained.
3)Final sentence in the results is wrong. It should read either:
   “In the PR negative cases 29% (70/240) were WWOX negative, while only 14% (12/83) of the PR positive cases were WWOX negative” or “In the WWOX negative cases 85% (70/82) were PR negative, while only 15% (12/83) were PR positive”
4)In the discussion (p15) they state that the association of WWOX with PR but not ER “raise the question of whether the observed positive association between PR loss and WWOX loss is more a consequence of the predominance of WWOX loss in the two aforementioned [clear cell and mucinous] histotypes, rather than a direct mechanistic association between WWOX and PR”. Since there are only 10 mucinous and 19 clear cell cases (compared to 40 endo and 375 serous cases) this seems unlikely. But it can be easily checked by running the statistics of WWOX and PR association in the serous only (or serous plus endometrioid only).
5)Western blotting: It is not stated how the tissue used for WB was selected and provisionally processed, was it fresh, or snap frozen and used later? The types of tumours studied by WB are not stated, so one cannot assess whether the findings (regarding high and low expression) are concordant with the immuno- results.

6)Immunostaining: the expression of WWOX was assessed over the whole surface area of each core. It is not clear whether all cores had a comparable amount of the epithelial component of the tumour, which would make a difference. On the same note, the authors mention that for hormone receptor expression, they selected only cores that had at least 10% tumour (page 8) and assessed
only 323 cases out of the 444. This gives one the impression that the tumour content across cores was not fairly uniform. Could the authors please clarify these discrepancies?

7) Page 4, third line from the bottom; histotypes and not hystotypes.
    page 10, line 9, corpora and not corpi
    page 15, line 2, the authors call WWOX an enzyme. is this proven?
8) Are the authors sure that the mucinous carcinomas are not of colorectal origin

9) It is curious that the proportion of serous and stage 3 to other histotypes and stages is so overwhelming. Could the authors comment on selection bias of the TMA and the inherent problems associated with this when trying to correlate with general clinicopathological variables in ovarian cancer?

10) There are no positive and negative controls on the shown western blot. It would be helpful to show that the antibody is specific.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests, although WWOX is an area of my own research.