Reviewer’s report

Title: Differential expression of anterior gradient gene AGR2 in prostate cancer

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Reviewer: Yong-Jie Lu

Reviewer’s report:

The authors have performed a comparative analysis of the transcriptomes of cancer cells and normal cells. The observation that AGR2 was over-expressed by ~50 fold in a Gleason 3+3 tumour compared to the luminal cells led the authors to explore AGR2 as a potential biomarker. The observations from the transcriptome analysis were validated by immunohistochemistry on prostate tissue microarrays as well as Western blot analysis on cell-line and tissue lysates. The relationship of ARG2 expression to histopathology and cancer outcome was investigated. The authors show that with increasing Gleason score, there is a trend towards lower AGR expression and lower AGR was correlated with disease recurrence in patients who had originally presented with high-stage primary prostate cancer.

The result generally is in conflict with a previous publication (Zhang et al, PCAN 2007; 10:293). However, the potential reasons have been discussed and the inverse correlation with cancer progression has been supported from the observation in breast cancer. Therefore, the data is worthy to be published. However, there are many issues/points that should be addressed.

- Major Compulsory Revisions

1. As the sample sets for microarray and Western analysis is very limited, the message of this study is mainly based on IHC of TMA samples. The specificity of the antibody has to be validated in this study using Western blotting and/or IHC of known positive and negative samples.

2. The purity of cancer cells is a big issue in prostate cancer study. From the description in the method, samples were not microdissested for Western analysis. How can the purity of cancer cells be guaranteed and what is the purity?

3. Array results of this study should be excluded from the introduction. The introduction part should include a brief summary of the current knowledge of progression makers of prostate cancer.

4. In paragraph 2 of the Result section, the authors should clarify two sentences: Secreted AGR2 can be detected in cultured media from CL1 cells/ the commercial antibody IC3 cannot detect native AGR2 as secreted by CL1. Are the authors referring to the use of two different antibodies, or a difference between protein purification (native Vs denaturing conditions)?
Minor Essential Revisions

1. While the samples used in other analyses were indicated in the methods, the information for clinical samples, including the number of samples, analysed by Western was not given. This should be added. P6 line 4 from the bottom: What concentration of collagenase did the authors use? The authors attempted to confirm the gene expression array results seen for the G3 (G3+G3) and G4 (G4+G4) tumours by Western blot analysis of prostate cancer tissue compared to normal prostate epithelium. This is a good approach, however, the authors showed only 4 prostate cancer cases, three G3+G4 cases and one G4+4 case (Figure 1B). This is not a sufficient sample set, nor is it representative of the cases they had previously analysed. Ideally G3+G3 and G4+G4 should be shown and more than one cases of each should be presented.

2. Last second paragraph in the Discussion: The AGR2/CD10 sub-classification of prostate cancer is interesting and closely relevant to this study. This data should be presented in this manuscript.

3. Last paragraph in the Discussion: Details of the future plans shouldn’t be included in the manuscript.

4. As AGR2 expressed differentially between Gleason 5 and 3/4, splitting TMA samples into two Gleason score group 2-6 and >6 is not useful for this study. The expression in each grade group should be considered.

5. Tables 1 and 3, please include pT in footnote.

6. Figure 2. In figure legend, the authors should indicate the Gleason score of the adenocarcinoma samples displayed and which ones are weak, moderate or strong staining. Non-immune rabbit IgG image is not shown. For this reason this information should be removed from the figure legend and discussed in the Results section.

7. The following words/phrases should be more accurately used
   A. Outcome usually refers to survival data. The world ‘outcome’, when is related to AGR2 expression in this manuscript, should be replaced by ‘cancer recurrence’.
   B. ‘Affymetrics DNA array’ should by replaced by ‘Affymetrics expression array’.
   C. Methods section paragraph 1: ‘Bladder cancer epithelium’ is not a good term.
   D. Methods: Western blot analysis paragraph 1: ‘70o’ should be replaced with ‘70oC’ and ‘4o’ should be replaced with ‘4oC’.
   E. Line 4 paragraph 1 of Discussion: ‘message’ should be replaced with ‘mRNA’ or ‘transcript’.
   F. Line 1 paragraph 2 of Discussion. ‘trend’ cannot be used on microarray results from clinical samples as there were only two cases.

Discretionary Revisions

1. Why only transcriptomes from two prostate cancer samples (Gleason 3+3 and
Gleason 4+4) were used for the analysis? There are many datasets available in public database, therefore a large number of samples should be analysed to detect common changes, which can be used as tumour markers.

2. In the comparative analysis of the prostate cancer Vs normal transcriptomes, how were normal basal and luminal cells separated? Many people believe that basal cells are the origin of PCa, so in the microarray analysis, the expression changes compared to both luminal and basal cells should be considered during candidate gene selection.

3. Paragraph 3 in the Discussion section is largely not relevant to the results.

4. For Western Blot analysis (Figure 1B, 1C), the authors used PSA as a loading control. Is this a good loading control for prostate cancer? Why standard housekeeping genes, such as #-actin, was not used as a control. For Western Blot analysis, loading control results should be shown in (Figure 1B, 1C).

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.