Reviewer’s report

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

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Reviewer: Anne Hamburger

Reviewer’s report:

Maresh et al present data indicating that the expression of AGR2, a homolog of the Xenopus laevis gene XAG2, is increased in prostate cancer cells compared to normal prostatic epithelium. However, there was a tendency towards lower levels of AGR2 with increasing Gleason score. Relatively lower levels of AGR2 were predictive of disease recurrence in patients with high-stage, but not low stage, primary prostate cancer. It is postulated that AGR2 might serve as a biomarker for prostate recurrence.

Previous groups have demonstrated that AGR2 expression is increased in prostate cancer. Thus, much of the paper is confirmatory of previous findings. The observation that AGR2 expression goes down in more advanced stages of prostate cancer is new, but contradictory to findings of other groups. The fact low AGR2 expression predicts recurrence in advanced prostate cancer patients is novel.

Although this paper brings up several intriguing points, several issues weaken the manuscript.

Major Compulsory Revisions

Methods

1. Page 6, para 2. It is not clear how many cell transcriptomes were interrogated. The number of samples in each of the different categories were not presented (Fig. 1A). The variability and the p values for the intensity values were not presented. It was unclear if the data for the cell lines were generated by this group or from the database.

2. There is lengthy description of the preparation of the tissue lysate media (p. 6, para 3). However, it is unclear from Figure 1B if cell lysates were examined or lysate media. Quantitation of the lysate media is extremely difficult. Although the authors using anti-PSA as a loading control, there are difficulties with using PSA as a marker. In addition, loading controls are not presented, nor are the TIMP1 studies to demonstrate the number of prostate cancer cells in the preparation. It would be important to quantity both secreted AGR2 and AGR2 from whole cell lysates.

Results

3. The transcriptional analysis indicates that G4 tumors express about 10 fold less AGR2 message compared to G3. However, the TMA data indicate that expression is approximately equal. This discrepancy should be addressed.
4. The number of samples in Fig. 1B is extremely limited, making it difficult to draw conclusions regarding AGR2 expression. More samples are needed. There are no loading controls for Figures 1B or 1C (secreted AGR2). Data from other cell lines would strengthen the paper.

5. Figures 3 and 4 187 patients were evaluated by TMA. What do the n values under the bars indicate? Are these all the spots? What was the variability among spots for individual patients? Boxplots of the IHC staining would be more informative. Although there was a difference in AGR2 staining between Gleasons grade 4 and 5 by IHC (Fig. 4), it appears that comparing Gleason 2-6 and 7-10 show no differences (Table 1). How do the authors reconcile this?

6. Higher grade patients with high PSAs showed lower AGR2 expression (Table 3). This is a novel finding. Are there any data in the literature or from this group indicating a relationship between PSA and AGR2 expression?

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:

We have recently published a paper on AGR2 expression in prostate cancer (Zhang et al. Cancer Res., 2010). However, I do not feel that I have a competing interest in relation to this paper.

There is no financial competing interest.