Reviewer's report

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

Version: 1 Date: 2 July 2010

Reviewer: Karina Dalsgaard Sorensen

Reviewer's report:

Major Compulsory Revisions

1

Background, last paragraph:
The description of the present knowledge about AGR2 and prostate cancer is not up to date and should include additional references, most importantly
A) the recent work by Zhang et al (Cancer res, Jan 1, 2010, 70(1):240-248) showing that AGR2 is a metastasis promoting gene in prostate cancer.
B) The previously published finding of AGR2 as a prognostic marker for PC survival, based on another TMA study (Zhang et al 2007; Prostate Cancer and prostatic diseases, 10, 293-300)

2

The scoring system for AGR2 used for TMA analysis is rather complicated. What is the interobserver variation on this? This is important when assessing the validity of a TMA study

3

The sample size in fig 1 is very small and highly overlapping with previously published work from the same authors (ref 5). The text in the results section should more clearly reflect the limitation in sample size as well as state which parts of the data set is new. The authors could perhaps include data from publicly available microarray expression data for AGR2 in prostate cancer

4

A loading control should be included for western blots in Fig 1B and 1C

5

The sample size in figures 3 and 4 are much higher that the number of patients represented on the TMA (stated as n=187 in Methods). This inconsistency needs to be explained.

6.

In fig 4 the authors use Gleason grade, and find a significant association with the AGR2 expression. In contrast, in Table 1, the authors find no association between AGR2 expression and Gleason score. The authors should comment on
this seeming inconsistency.

7. Tables 2 and 4: T stage is a well known predictor of PSA recurrence after prostatatectomy. Therefore, this variable should be included in the univariate and multivariate cox regression analyses performed for “all patients”

8. Table 2 includes Stage III and IV patients, but according to Table 1 only Stage III samples are represented on the TMA. This needs to be clarified. Have the authors investigated the possible difference in AGR2 expression and outcome for T3a versus T3b. It is possible that the proposed predictive role of AGR2 in high stage prostate cancer is a surrogate marker for T stage.

9. Fig 5. Why did the authors choose to use the 75th percentile of AGR2 expression for dichotomization in survival analysis and not the 50th percentile? There could be a risk of data fitting.

10. The discussion is somewhat unbalanced. The authors should emphasize the expression pattern of AGR2 in prostate cancer, the association with clinical parameters and outcome, and the possible biomarker potential of AGR2. The more speculative part on the possible function of AGR2 could be shortened and should be updated to include the most recent publications about AGR2 and prostate cancer (see Major revision, point 1)

11. In the conclusion, the authors should more clearly state the new findings in their study compared to already published work about AGR2. Their first finding seems to be a confirmation of already published findings (increased AGR2 expression in PC and HGPIN), while the second finding is new (possible association with biochemical recurrence after prostatectomy)

Minor Essential Revisions

1. Background, 1st paragraph:
   Are the 190,000 new cases of PC and 27,000 deaths in the US alone – please specify
   “false negative” should read “false positive”

2. The Methods section could be written more precisely and in somewhat more
Western blot, last paragraph: serum-supplemented (which serum, how much); 40µL analyzed on Western blot – but taken from which total volume of media? This is a general problem throughout the Methods section, which the authors may want to address to help the reader.

Fig 2, show image for negative control staining.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests