Reviewer's report

Title: Expression of pS2 in prostate cancer correlates with grade and Chromogranin A expression but not with stage

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Reviewer: Jens Hansson

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General
The stated aims of the current study were to investigate the expression of pS2/TFF1 in malignant prostatic tissue and to correlate this with neuroendocrine differentiation, clinical stage and grade. Earlier research has however already shown that pS2 protein is expressed in a subpopulation of prostatic neuroendocrine cells, and in both secretory cells of relapsed tumors, and in malignant cells of untreated tumors – but in the latter case only in cells immediately adjacent to neuroendocrine cells (Bonkhoff et al, 1995), implicating a functional relationship. Colombel et al reported as early as 1999 that there is no correlation with prostatic pS2 mRNA expression and tumor stage or grade, and Yang and co-workers measured pS2 protein concentrations in hyperplastic and neoplastic prostatic tissues, but found no significant difference between the two, nor any correlation to PSA levels or androgenic status. PS2/TFF1 has been reported to be involved in the development or progression of gastric cancer if lost through promoter methylation (and hence silencing of its expression) (Carvalho et al 2002), suggesting that pS2 is a tumor-suppressor. TFF2 upregulation is on the other hand a negative prognostic factor in gastric cancer (Dhar et al 2003), implicating TFF2 as a tumor-promotor. The role of trefoil factors in tumorigenesis still remains controversial however, and there is a lack of data concerning their role in prostate cancer. However, the question posed by the authors is not new, and it is a shame that they did not include more novel aspects in their study, e.g. study the function of pS2 in the prostate gland, or - since pS2 is a secreted protein - measured the pS2 levels in blood (ELISA) and correlate this to neuroendocrine differentiation etc. It would also have been of interest to included expression of TFF2 or TFF3, to include the whole trefoil family, or if focus only is laid upon TFF1 – then the authors could have included in situ hybridization of the mRNA expression (Dhar et al, 2003, reported a discrepancy between mRNA and protein expression of TFF2 in gastric cancer patients). There are, however, more severe concerns, relating to the data gathered and conclusions drawn by the authors in the present manuscript, as well as other aspects, all of which should be addressed by the authors before possible publication:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1) Language: The English grammar should be revised by one of the authors.
2) Title and Abstract: The title is fine as such, but it is stated that “…pS2..correlates…not with stage”, while the abstract says that “…pS2 was correlated with…clinical stage…” in the method section, - and then in the result section it is stated that “…there was no significant correlation between pS2 expression and the stage of disease”. First of all, no results should be stated in the method section, this should only be stated in the results section, and secondly – the statement should be coherent.
3) Introduction: The authors start off by elaborating about prognostic markers, and the need for new markers in prostate cancer, and this ends in a statement that neuroendocrine differentiation and pS2 may have a potential role as prognostic markers, and then also with the aims of the present study (see above). In the discussion, the authors end by concluding that the present study has shown that neuroendocrine differentiation and pS2 expression have independent prognostic value. Since the authors did not state that they intended to study the prognostic implications of pS2 expression (or neuroendocrine differentiation) in their aims, they ought to do this in the introduction – if, and only if
they include data to support this in the result section (currently, there is no such data in the result section), if not, then the authors should rewrite the introduction (and discussion). The introduction should highlight relevant facts for the study in question – neuroendocrine regulation, cancer and signaling pathways – (they may for example include information about the trefoil factor family, and their function in gastrointestinal cells (these molecules are known to be upregulated in response to injury and to be involved in defense and repair), their association with neuroendocrine cells or neuroendocrine products (somatostatin and VIP is known to regulate their synthesis/ expression in the gastrointestinal tract), and their intracellular signaling pathways (pS2/TFF1 is known to inhibit cell proliferation and to protect cells from p53 dependent and p53 independent apoptosis, while TFF2 is known to stimulate proliferation and is a motogen)).

4) Methods and Materials: It is unclear and confusing when the authors write that “95 malignant CONSECUTIVE specimens were obtained from 84 patients” in the first line of the material and methods section. What does this mean? Did they not skip any patient/specimen? It is even more confusing since they wrote that “RANDOMLY selected samples of 95 prostatic specimens from 84 patients were evaluated” in the abstract. What should it be? Random or consecutive - and if consecutive – what does this mean? The authors should explain/change this, and give the same information in both sections! Furthermore, in the results section the authors present information about the number of patients and their clinical classification. This information should be moved to the Material and Methods section – and the authors have to add information about the number of metastatic cases (M1), as this is not given in the current manuscript (but only if metastatic samples were investigated, see below).

5) Results: Here the authors state that “pS2 was CONFINED to malignant cells” (line 5), but they also state that “pS2 reactivity was detected in normal and hyperplastic acini” (line 13-14). Clearly, then, pS2 was NOT confined to malignant cells. The authors must change this, and give the readers information about the number of specimens and patients positive for pS2 expression, including pathological data, preferably in the format of a table. The table should also contain information about CgA staining, and if pS2 and CgA is co-localized. This would best be done by changing Table 1 to contain all this relevant information. Stage: again we have the problem with pS2 expression and possible correlation with stage of disease: the authors state that “expression of pS2 was correlated with the stage of disease in Figure 1.” As noted above, the authors tend to give different statements regarding correlation between pS2 expression and stage. There is neither any information given in the text or in tables to support either of the conclusions, and no statistical analysis is presented in the text, tables or figures. And as noted above, the authors did not include any information about the number of patients with distant metastases. So, we cannot draw any conclusions – nor can the authors, since they did not investigate the expression of pS2 in metastatic lesions – at least not if we believe what they stated in the material and methods sections, i.e. that only "resections and prostatectomy samples were investigated". Accordingly, the authors must omit such information, and instead focus on the data actually sampled. The authors must also present appropriate statistical analysis. Grade: the authors state in the result section of the abstract that “pS2 expression in prostate cancer significantly (p<0.001) correlates with histological grade”, but in the Result section proper, the authors do not have this statement, the information given instead is “the expression of pS2 in various prognostically and therapeutically distinct groups based upon the grade of cancer is described in figure 2”. This last sentence is confusing, and does not give the reader any information whether or not pS2 correlates with tumor grade (and no prognostic or therapeutic information is given in the figure or text). This information should be presented in the text, and the numbers behind the statement should be clearly presented in Figure 2. However, if we look at Figure 2, we see information differentially presented as described in the Material and Method section. In the figure, patients are divided into 4 different Gleason sum groups (G2-4, G5-6, G7, and G8-10), but in the Material and Method section, the authors state that the patients should have been divided into three groups (G 2-4, G 5-7, and G 8-10). In Figure 2 there is a histogram, where “% expression of pS2” is presented (ranging from 6 to 21%). However, it is not stated what the % expression refers to. Is it the percentage of positive acini present in whole mounted specimens, as stated in the Methods section? (If so, then only 15 patients was analyzed). Or is it number of specimens/patients positive for pS2? If one looks at Table 1, it is stated that 9 specimens (or is it patients? This information is not given, but
should be given by the authors) are positive for pS2. However, there is no information about the number of patients in each group, or the number of patients positive for pS2, making it impossible for the reader to draw any conclusions, and no statistical analysis is presented in the text of the Results section. But as noted above, the abstract states that “p<0.001”, however, in Figure 2 we are presented with a p value greater than 0.001 (p>0.001). This would indicate that there at least is no statistical significant correlation between grade of disease and pS2 expression. However, little to no information is provided so the reader can check for him/her self. The authors must present all relevant information, and they also must use appropriate statistical analysis methods. The authors have applied Students t test to correlate pS2 expression with tumor grade, however, when comparing proportions, especially whenever expected number of frequencies in any group is low, one should use Fischer’s exact test. Then the authors state that 2/3rd of pS2 positive cells also stains for CgA, but they have not presented any information to support such a statement. In order to make such a statement, the authors must present either pictures where pS2 and CgA are double labeled, or present pictures of consecutive sections of alternating pS2 and CgA staining. However, the authors do not seem to have used either method, since no such methodological information is given in the Materials and Method section.

6) Discussion: The first paragraph (the authors discuss why they did not use tissue extracts) is unnecessary and should be omitted. In the third paragraph the authors discuss neuroendocrine differentiation and points out that their present work shows that “NE differentiation not only correlates with tumor grade but also has independent prognostic value”. However, the authors have not presented any information in the Results section to support such a statement, and thus should be omitted. In the third paragraph the authors also refer to a work by Colombel et al , where he and co-worker found no correlation between pS2 and tumor stage or grade. And that is it – the authors do not discuss this in context of their own results, which should be done! In the fourth and last paragraph the authors conclude that pS2 correlates with tumor grade and that pS2 expression has independent prognostic value. Once again, the authors did not present any information in the Results section to make such a conclusion, nor did the authors mention that they collected information to be able to do this in the Materials and Method section. Since the specimens used in the study were collected between 1991 and 1998, the authors should present the 5-year overall survival rates of patients with pS2-negative and pS2-positive status, and 5-year disease-free survival rates for pS2-negative and pS2-positive cases. Survival curves should be presented, plotted by the Kaplan-Meier method, and statistical significance between groups should be determined by the log rank test. A multivariate analysis (Cox stepwise regression analysis) should be done to detect the independent prognostic factors. If this is not done, then the authors should omit any conclusions regarding prognostic values!

7) References: The first sentence in the introduction is “The biological potential of prostate cancer is extremely variable (1)”, (1) referring to an article by Ather himself. To start with, the sentence is confusing – what does “potential” mean? Progression would be a better word. Secondly, the article by Ather does not investigate the “biological potential” of prostate cancer, but is a review article about neuroendocrine differentiation. A more appropriate article to reference to would be e.g. Scher, Cancer, 2003:97(3 Suppl):758-71. The authors then discuss NE differentiation and its role in ascertaining the biological aggressiveness of the tumor (3) in the introduction (first paragraph). However, this reference number does not deal with NE differentiation at all – instead the authors should refer to an article by a work that does exactly that (e.g. Di Sant’Agnese or Abrahamsson etc). Next the authors state that pS2 is implicated in the pathogenesis and progression of neuroendocrine tumors, referring to Wang et al (5). However, it would be more appropriate to refer to Bonkhoff et al (1995), since he and co-workers described pS2 expression in the prostate and in prostatic NE cells etc. The authors also states that pS2 is linked with NE differentiation and poorer outcome, referring to (7), i.e. Lugmani – who investigated pS2 immunoreactivity in the prostate, but reported in that article that “no reactivity for pS2 was seen in…prostate”. Well, the list goes on and on and on. The authors must rewrite the reference list – it should be up to date and cover all appropriate references necessary!
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes

Declaration of competing interests: None.