Reviewer's report

Title: Increased expression of urokinase plasminogen activator and its cognate receptor in human seminomas

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Reviewer: Helge Taubert

Reviewer's report:

The manuscript from Ulisse et al. investigates the mRNA expression of members of the urokinase plasminogen activator system (uPA, uPAR, PAI-1 and PAI-2) in 14 human seminomas and 6 normal tissues and correlates the expression levels in the tumor tissue with clinical data. The study provides interesting results as an increased expression of uPA (6.25-fold) and uPAR (3.8-fold) in seminomas compared to normal tissues and a significant association between uPA expression level and patient’s age.

However, there are several questions to be answered:

Major Compulsory Revisions

1. General: There is only a small number of patients with seminoma (n=14) investigated in this study what makes statistical evaluation not very much reliable.

2. Were the seminomas investigated by Western hybridization and by immunohistochemistry a part of those studied for mRNA expression? There are 8 patients with seminoma from the Regina Elena Cancer Institute and 10 patients’ samples were investigated by IHC but from the 6 patients’ samples from BioChain Institute only cDNA was available (?)


4. Material and Methods: Which antibodies were applied for detection of uPA and uPAR?

5. Material and Methods: Why did the authors use western hybridization instead of available ELISA kits?

6. Material and Methods: Does the ## Cp method mean the ## Ct method or what does it mean?

7. Was a reference gene measured in the mRNA expression analysis (actin is mentioned as positive control)? The correlation of expression levels in normal and tumor tissues could differ when the tissues are from different patients/probands because of different freezing and storage conditions. All data should be normalized to actin. It is not possible to calculate the ratio of the average of
mRNA expression in 14 tumor samples and 6 normal tissues (tumor tissue and normal tissue was only matched for three patients!?).

8. Pearson correlation is based on a normal distribution of measured values, did the authors test this? If there is not a normal distribution authors should use Spearman correlation.

9. Results: Can an uPA immunoreactivity positive in 3 out 10 samples be significant?

10. Discussion: As for the introduction authors should cite relevant review articles f.e. after the sentence on page 12: “The aberrant expression of uPAS components in malignant tissues…”

11. Figure 1: The mean values are shown in the figure are different from the values in the text on page 10 (uPA: 3.8-fold and uPAR: 6.25-fold).

12. Figure 2C: The molecular weight standard should be given in 2C as it was done in 2A. Did the uPAR protein stain in the range between 55-60 kDa? Why did the authors no western hybridization for PAI-1?

13. Figure 3: Did the tumor sample characterized by IHC originate from a patient listed in table 1. If yes which sample was it?

14. Fig. 4: As in comment 7, please, use actin mRNA expression as reference gene for normalisation. Calculate the measured gene expression (uPA, uPAR, respectively) per actin only in the tumor tissue and then correlate it to the clinical factor. It is difficult to rely on statistics for tumor stage when tumor stage is only known for 8 cases. As in comment 8, please, test if your measured values allow a Pearson test if not calculate them according to Spearman test and change Fig.4. Why is no correlation for PAI-1 and PAI-2 mRNA with clinical factors expression made?

Minor comments:

Material and Methods: Extraction and analysis of mRNA: 0,5µM#0.5µM, 1,25ng#1.25ng
Substrate gel electrophoresis: 0,03% # 0.03%

Level of interest: An article of importance in its field

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