Reviewer's report

Title: PKC alpha regulates netrin-1/UNC5B-mediated survival pathway in bladder cancer.

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Reviewer: Tatjana Simic

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The paper of Liu et al describes expression PKC alpha, netrin-1 and UNC5B in bladder cancer samples obtained from 120 patients and 40 specimens of adjacent non-tumor tissue as well as in bladder cancer cell lines. It also addresses the question of whether any of these parameters may have prognostic role in bladder cancer patients. Although the material presented is robust in terms of quantity of data and methodology used, there are some major concerns regarding the introduction to the problem and interpretation of the data. Besides, the paper requires extensive language editing.

- Major Compulsory Revisions

1. The research question posed by the authors can hardly be understood from the Abstract. It should be clearly pointed out why the Netrin-1 UNC5B signaling should be evaluated in bladder cancer. The sequence of presentation of the results does not correspond to that presented in the article. The conclusion refers only to the data obtained in in vitro conditions. There are abbreviations used without an explanation.

2. In Introduction the authors provided brief general information about Netrin-1 and UNC5 family of proteins. However, the potential significance of these proteins in bladder cancerogenesis is given in only one sentence with a bit confusing statement that „it is important to explore it’s potential mechanism on the regulation of bladder cancer apoptosis, which may unveil the functional diversity of this pathway.“ Besides, the signaling pathway that relates PKC alpha with netrin1/UNC5B has not been introduced at all. PKC alpha has just been mentioned in the last sentence of the Introduction. Therefore, the hypothesis which underlies this large study is not clear at all. The Introduction should be extended and focused to the potential role of PKC alpha, Netrin-1 signaling in the regulation of bladder cancer cells survival. Overall design of the study should be provided.

3. Methods section has been extensively written. Although this style is according to the journal guidelines, the description of methods still contains too many details such as frequency of rinsing and volumes of solutions added. Such information is usually the part of laboratory protocols, but rarely published except for novel procedures. It is not clear why only BIU and SV cell lines were treated with inhibitors and is RNA. Besides drugs should be omitted in the subheading. There are typing errors, such volume of „20ll“ in Real time PCR, „50 lg“ in Western Blotting... It should be pointed out that among so many data the overall
concept of the study can hardly be followed.

4. Results section is divided in too many subheadings and should be organized in a way to integrate information that focuses at one topic. The text of Results should be clear without the Figures - that is not the case in this paper. For example lines 186-190: „The western blotting of 6 samples were shown in Fig. 1B & D, and the optical density of the tumor (T) & normal (N) tissues was measured and expressed graphically (Fig.1C & E). Differences in T stage (T1, T2, T3 & T4) (Fig.2A & D) and histological grade (G1, G2 &189 G3) (Fig.2C & F) by RT-PCR were significant (P < 0.05). The western blot results also showed difference in tumor stage (Fig.2B & E).“ How much the mean expression of Netrin-1 and UNC5B change in tumors in comparison to non-tumor tissue? Could the expression be normalized according to standard purified proteins? Regarding the correlation between netrin-1 and UNC5B the authors report the „positive“correlation of both parameters with grade, and metastasis despite the fact that there was a „negative“ correlation between netrin and UNC5B. This might be the consequence of the unusual presentation of data, where, the expression of Netrin was presented as percentage of cases with low expression, while that of UNC5B as percentage of cases with low expression. This is a bit confusing. The key question is what did the authors mean by „high“ and „low“ expression of the parameters tested?

5. Discussion is too short and addresses the significance of Netrin-1/UNCB5 in apoptotic signaling with several conclusions regarding the role of this pathway in bladder carcinogenesis. However, none of the apoptotic parameters was determined in this study. On the other hand, the prognostic significance of the results obtained has not been addressed in the light of findings in other tumors.

- Minor Essential Revisions

1. It is not clear why only BIU and SV cell lines were treated with inhibitors and is RNA. Besides drugs should be omitted in the subheading. There are typing errors, such volume of „20ll“ in Real time PCR, „50 lg“ in Western Blotting...

2. Table 1 and 2 it should be clarified what is meant by high or low expression

3. Table 3 presents crude statistical data

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

Quality of written English: Not suitable for publication unless extensively edited

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' below