Reviewer’s report

Title: Overexpression of NIMA-related kinase 2 is associated with progression and poor prognosis of prostate cancer

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Reviewer: Ronald Simon

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Zeng et al. present functional and IHC data on the prognostic role of NEK2 in prostate cancer. The group performed NEC2 knockdown in one prostate cancer cell line and studied the effects of cell growth in vitro and in vivo. In addition, IHC was performed in 99 prostate cancers. RNA expression data of the Taylor study are used to estimate the prognostic impact of NEK2. Although interesting, the study is somewhat limited by the facts that there were no own follow-up data to address the important question of the prognostic relevance of NEK2, that the number of samples is very limited (<100), and that the functional analyses were limited to one cell line.

Major compulsory revisions

- NEK2 may have a specific nuclear function connected to regulation of survival and apoptosis (Naro et al., Nucl. Acids Res 2013) in addition to its role for centromere maintenance. This should be added to the introduction and also discussed in the light of the functional studies, given that there might be mostly cytoplasmic staining in normal prostate but nuclear staining in high-grade cancers.

- It seems that only one cell line (LNCAP) was analyzed. Please discuss possible limitations of the study results and conclusions if they are based on one experiment only.

- Same with the xenograft: please specify how many independent experiments were done.

- Associations between NEK2 up-regulation and Gleason/stage were only marginal in the Taylor dataset (RNA) but more evident in the IHC data. Please discuss.

- The conclusion, that NEK2 is of prognostic value for predicting outcome of PCA recurrence (discussion and abstract) is reaching too far based on the facts that 1) prognosis data were from a different group (Taylor data), 2) from a very limited number of samples (n=160), and 3) that no multivariate analyses were included. I would suggest concluding that these are promising preliminary findings, which prompt for larger validation studies. I would also discuss limitations of RNA screening studies and emphasize the advantages of in situ analyses like IHC. Unfortunately, follow-up data were lacking in this study, but there may be PCA TMAs available with FO data for subsequent studies.
Minor essential revision

- NEK2 has three splice variants (A-C) with possibly different roles. Please clarify in the introduction.
- Please define PSA recurrence or refer to Taylor et al.
- Please give details on the cell lines in the Materials/Methods section.
- Were normal prostate epithelial cells microdissected before RNA analysis? Was there NEK2 staining present in non-cancereous prostate cells, which might have an impact on RNA analysis?
- An IRS was used to quantitate NEK2 immunostaining, and the average IRS was only about 5 even in high-grade cancers. Why was it so low? It would be helpful to give the fraction of entirely negative (intensity 0) cancers and also of strongly positive cancers (i.e., 3+ in #50%).

Discretionary revisions
- none

Level of interest: An article of limited interest

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

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