Reviewer's report

Title: PEST-containing nuclear protein mediates the proliferation, migration, and invasion of human neuroblastoma cells through MAPK and PI3K/AKT/mTOR signaling pathways

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Reviewer: Hanna Rokita

Reviewer's report:

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The Authors present the results of their studies on a role of PEST-containing nuclear protein (PCNP) in neuroblastoma. They have used two approaches, gene knockdown and overexpression, to elucidate PCNP role in proliferation, migration and invasion of two human neuroblastoma cell lines, SH-SY5Y and SK-N-SH. They have also introduced xenograft tumor model in nude mice to evaluate an effect of PCNP in in vivo conditions. It is important that they show PCNP regulatory effect on the MAPK and the PI3K/Akt/mTOR pathways in the studied neuroblastoma cells.

The Authors should address following comments:

1. Key amino-acids residues of ERK1/2, JNK, p-38, PI3K, Akt and mTOR, that are phosphorylated, are indicated only in the subchapter of the "Methods" section titled "Western blotting" with the list of anti-phospho antibodies, while the information should be used in description of results with clear indication on a role of specific phosphorylation in activities of the proteins. The third page of "Discussion" on an effect of PCNP knockdown on decrease in phosphorylations of p38, JNK and ERK1/2 requires such explanation. Next, on the same page, a sentence on "PCNP over-expression significantly induced apoptosis by inhibiting the phosphorylations of PI3K, AKT and mTOR" should be better explained.
2. Both human neuroblastoma cell lines used in the study, SH-SY5Y and SK-N-SH, are not identical. They differ at least at the level of GD2 ganglioside expression (SK-N-SH is negative, while SH-SY5Y is positive). The Authors write only in the Discussion (lines 47-57), that the cell lines "possess several properties of neuronal cells". They should comment on the facts, especially, when they show some differences e.g. in Bax protein levels (Fig. 5b vs 5c) or "tumor inhibitory rate" (Fig. 8e).

3. The last sentence in the "Conclusions" should be modified as it is difficult to agree with the statement that PCNP constitutes a unique therapeutic target. It is rather "a potential therapeutic target".

4. Lines 39-42 second page of "Discussion" - PCNP could modulate the apoptotic level of human neuroblastoma cells" is unclear - it is rather: "PCNP has pro-apoptotic function in neuroblastoma".

5. Some western blot presented in Fig. 5a and Fig. 6a should be of better quality.

6. In description of western blotting (in "Methods" section) "protein kinase B" as synonym of Akt should be omitted as it is not used in the whole text.

7. The Authors use semi-quantitative RT-PCR to quantitate PCNP mRNA, moreover there is no information (or a reference) on sequences of primers for GAPDH and thermal profiles.

8. It should be "cleaved caspase-3" rather that "Cleaved Caspase-3".

9. In the list of references, ref. 14, 35, 40 and 41 have titles with words starting from capital letters.

10. Two panels of Fig. 3e and 3f, should be clearly explained - which one comes from migration test and invasion test.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

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