OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-23-00714
Title: Disentangling brain PrPC proteoforms and their roles in physiology and disease
Reviewer's Name: Mariana Juliani Do Amaral
Reviewer's country: Brazil

COMMENTS TO AUTHORS

This perspective article is exciting and very interesting work about the influence of PrPC proteolytic fragments in prion diseases development. The authors mostly comment about their recent work in JBC (PMID: 36565989) in which they developed a specific WB method to probe the endoproteolytic processing of PrPC. This manuscript is well-written and before I recommend it for publication, I would ask the authors to address the comments pointed out below.

Major

Line 12- '(…) and Parkinson's diseases.' A very important work that highlights the involvement of PrPC in other NDDs is Corbett et al., 2020 (PMID: 31853635). This work deserves to be cited here as well.

Line 23-I suggest to replace 'less prominent in physiological conditions' to 'mostly found in pathological conditions' or something related, since most studies associate beta-cleavage to oxidative stress.

Similar to the sites for alfa-cleavage (H109 and K110) please state what amino acid residues could serve as sites for beta-cleavage and gamma-cleavage, respectively.

From line 19 to 34- Missing references after explaining each proteolytic processing. Ideally, cite the original articles that firstly described cleavages and not just the review from Glatzel's group (Linsenmeier, 2017).

Line 45- I suggest to tone down this statement. Although the existence of proteolytic fragments of PrPC is unbiased, the pathophysiological significance of these fragments remains under discussion.

From line 14 to 20- I suggest this paragraph to be greatly shortened, and instead make a Figure illustrating the different animal models used in this study. The Fig. would be a lot clearer for the reader. Perspective articles can have up to 2 Figs, then this would make Fig. 2 (panel A).

It's quite interesting that the C1 fragment is higher in expression as compared to FL-PrPC. Could you include in Fig. 1 the percentage of abundance of each fragment in the healthy brain? Besides each fragment would fit well. In addition, can you include besides each fragment what process generates them (alfa-, beta-, gamma-, etc…).

Line 36- It has been shown before that the C-terminal domain of PrPC forms the structural core of amyloid aggregates (reviewed in PMID: 29514050). This is also supported by the 3D structure of three PrP amyloid-like fibrils solved by electron microscopy (PDB Id.: 6uur, 6lni, and 7lna) that comprise the C-terminal. However, authors state that C1 (comprising residues 109-230) is not aggregation-prone. What is the basis for this argument?

Line 28- 'have not' should be 'do not have'.

Line 38- please include a reference that supports neuroprotective roles of PrPC fragments.

Line 51- less affected by 'these phenomena' (in plural) since many factors can affect the stability of PrPC fragments.

Line 61- mounting evidence suggest the existence of cytosolic PrP that harbor the ER signal peptide (SP) (works from Lindquist's lab, Hegde's lab and PMID: 16908519). Is this pool of cytosolic PrP included in your FL-PrPC quantification? If not, I recommend the investigation of that in a future study as including an antibody for the SP would be relatively simple.

What protease(s) are involved in alfa-cleavage? Similarly, what triggers beta-cleavage? Please comment on that within the text.
Page 4- Line 10. Please review grammar of this paragraph, starts with 'Despite with...' which is non-standard English.
Page 4- Line 28. What kind of 'assemblies associated with other NDDs' do you argue about? Could you cite examples within this statement?
Page 4- Line 29. 'spontaneous misfolding of PrPC'. This is imprecise. Do you mean through a shed seed of PrPSc? Please rephrase to explain better.
Line 32- Absolutely. This is a great point raised by the authors. I hope the authors can further address that in a future study, this would make a great contribution to the field.
Line 35- Because of the intrinsically disordered nature of the PrPC N-terminal, I believe it is mostly degraded by cellular proteases hampering the accurate quantification of N-terminal fragments by WB. You previously commented on that, but now assumes tout court that N-t fragments are minority in the brain. Could you rephrase that to account for the limitations on detection of N-terminal fragments?
The section 'The role of N-t proteoforms in prion diseases' is very interesting. I would recommend that authors summarize the correlations of cleavage products and pathogenic mutations in a Figure. For example, Fig. 2 (panel B).
Kostylev et al., 2018 (PMID: 30401430; Supplementary Fig. 1D) have estimated the concentration of PrPC to be about 400 nM (human brain homogenate) and about 4 μM extracellular PrPC. Also, PrPC is found at approximately 10 nM in the cerebrospinal fluid (Dorey et al., 2015 PMID: 25559883) and at 0.06 to 1 μM in the plasma of healthy individuals (Llorens et al., 2020 PMID: 31216593; Yao et al., 2021 PMID: 34537219). Can you discuss estimated physiological concentrations of PrPC in the light of your fragment's quantification by WB?
The section 'Conclusion and future directions' would be enriched with comments from Glatzel's group study (PMID: 34818048).

Minor
Please verify unnecessary use of acronyms and correct typos.
Line 19- Hys109, should be His109.
Line 39- FL-PrPC- please include 'full-length' inside brackets since it's the first time this acronym (FL) appears in the text.