OPEN PEER REVIEW REPORT 2

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00394
Title: Crry Silencing Alleviates Tau Pathology by Regulating the Expression of Neuroinflammatory Cytokines and the Complement System
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COMMENTS TO AUTHORS

This is an important manuscript which describes the impact of silencing Cr1-related protein Y (Crry, the ortholog of CR1 in humans) in a mouse model of tauopathy. Authors showed Crry was located in microglia and was elevated in P301S compared with WT mice and increased with age. Crry knock-down reduced tau phosphorylation and activity of tau kinases GSK3B and CDK5; improved cognitive deficits; was neuroprotective; and unexpectedly reduced neuroinflammation as measured by the expression of IL-1β, TNF-α and IL-6. The levels of complement component C3 were reduced but C3b was increased following Crry-silencing in P301S mice.

Overall the study design and the experiments are good and the results are very interesting, though some revisions of the manuscript are required.

In particular, the discussion of how Crry silencing is protective needs significant attention. It is known that Crry has a) decay acceleration activity (i.e. increases dissociation/inactivation of C3 convertases) and b) Factor I cofactor activity (breakdown of C3b to iC3b). Therefore given a ~40% reduction of Crry it ought to follow that C3-convertases persist for longer and therefore more C3b is produced and that C3b cannot be efficiently degraded (indeed this is supported by C3 and C3b western blotting). These ought to be proinflammatory events. In Crry-/- mice, where the gene is deleted systemically from birth, there appears to be no C3 as a result of consumption - any the C3 which is produced is immediately converted to C3b. However, accumulation of C3b causes microglial priming, via interaction of C3b with CR3, which exacerbates neurodegenerative disease, in the context of EAE (Ramaglia et al., 2012). The fact that the data presented here indicate Crry silencing is beneficial in this context is remarkable and warrants proper discussion.

In addition, it is not sufficient to say that CR1 might be a therapeutic target in AD. The complement pathway offers several druggable targets but CR1 is not one of them. (Recommend reading review from Carpanini et al., 2019).

Specific edits

It is incorrect to refer to this model as a model of AD. It is a model of tauopathy or neurodegeneration.

Introduction

Page 5 line 39: Kunkle 2019 is the most up to date ref. or Bellenguez 2021 though this is on BioRXiv

Page 5 line 44: correction - through its effect

Page 5 line 49: "CR1 expression was recently confirmed to be related to the abundance of phosphorylated tau (Killick et al., 2013)" is not precise. Crry (not CR1) deletion was associated with reduced tau hyperphosphorylation (not expression). In addition I would suggest reordering this section - It is confusing to have this mention of the Killick study then Yoshiyama study then Killick study at the top of the next page. It reads as though crry was deleted from P301S tau Yoshiyama mice rather than WT mice. Suggest moving the sentence about yoshiyama to more relevant section.

Page 6 line 9: "However, these observations only illustrate that CR1 affects the expression of tau proteins, without explaining the exact mechanism of CR1's action on AD-related tauopathy." Again crry affects tau hyperphosphorylation not CR1 affecting expression.

Page 6 line 49: "The complement system has been shown to be involved in AD via neuroinflammation." This is very vague I'm not sure what is meant here.
Page 7 line 12: Hampton et al., 2010 use a different P301S tau model where the transgene is driven by the Thy1.2 promoter. For a list of references using the PS19 model see Alzforum database.
Materials and methods
Page 7 line 29 and line 51: it is unclear whether the location of the university and hospital have been removed for anonymous reviewing or are mistakes
Page 9 line 53: "100 μL/50 mL protein lysate RIPA" is this unit correct? Ul/mg perhaps?
Page 10 line 1: "After treatment with 5% Skim milk/BSA" in what? Presumably PBS? Tween?
Page 10 line 5: what concentration secondary?
Page 10 line 46: "rehydrated with ethanol" presumably serial dilutions of ethanol in water?
Page 10 line 48: "antigen retrieval in ice-cold acetone for 10 min" Acetone used as a fixative not for antigen retrieval
Page 10 line 51: "The samples were treated with BSA/Triton X-100 (0.1%)" What conc BSA? Diluted in what?
Page 11 line 7: within a given region? Or randomly across the whole tissue section?
Results
Page 12 line 43: "Crry upregulation was not as a result of the increased number of microglia in AD" It would be nice to see some comparative staining to support this claim if possible?
Page 13 line 17: "These results suggest that Crry expression was successfully inhibited by shRNA infusion." Is it possible to support this claim with staining showing loss of Crry in microglia?
Page 13 line 43: "DECREASE IN HYPERPHOSPHORYLATED TAU LEVELS FOLLOWING AFTER CRRY SILENCING" Following or after but not both.
Page 14 line 48: "While ventricle volume was increased, while Crry silencing decreased this change in ventricle volume in P301S mice (Figure 6G)." repeated use of while
Discussion
Page 16 line 44: "Microglia, the resident immune cells of the brain, mediated neuroinflammation is a hallmark of a variety of neurodegenerative diseases, including AD (Tang and Le, 2016)." Suggest delete "the resident immune cells of the brain" because by this stage in the paper readers should know!
Page 17 line 22: "CDK5 is a cyclin-dependent kinase, and also is tau kinases under both physiological and pathological conditions (Cortés et al., 2019)." The latter part needs rephrasing
Page 17 line 46: "In addition, we found that Crry silencing could decreased the neurons death" Correction to decrease
Page 17 53: "This confirmed that Crry silencing might improve AD." I'm not sure if this is suggesting silencing CR1 in human AD, which would be problematic, or repeating that Crry silencing is beneficial in this model of tauopathy which might need rephrasing?
Page 18 line 22: "It had reported that C3 was activated in human AD brain and was necessary for neurodegeneration in mice models of tauopathy," correction to has been
Page 18 line 39: "In conclusion, Crry might play a vital role in the AD murine model by significantly reducing tau phosphorylation and ameliorating cognitive dysfunction." Crry silencing, not Crry, reduces tau phosphorylation etc.