

Reviewer's report

Title: Over-expression of AhR (aryl hydrocarbon receptor) induces neural differentiation of Neuro2a cells: Neurotoxicology study

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Reviewer: Thomas Gasiewicz

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General

The manuscript by Akasoshi and coworkers examines the effects of aryl hydrocarbon receptor (AhR) over-expression in murine Neuro2a neuroblastoma cells. The data presented suggest that the differentiation of these cells is altered by AhR activation. The authors further suggest that this may be a good model for studying the relationships between AhR activation and developmental neurotoxicity. While the data might be of interest, the studies are incomplete and there is no compelling evidence presented to indicate that this is a valid model for studying AhR-regulated neuronal differentiation or the relationships between AhR activation and developmental neurotoxicity caused by exposure to exogenous AhR ligands. There are also several technical issues with the manuscript that make the data and their relevance difficult to interpret. Some additional data would be useful. This represents a good beginning to a manuscript that needs more data to be convincing.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

3. The rationale for using the Neuro2a cells is not defined. Why is this a valid model for studying neuronal differentiation or AhR-mediated neurotoxicity? What exactly do these cell represent in the brain? Additional consideration of this is needed here.
4. The authors draw conclusions regarding AhR activity following over-expression from a very limited data set, i.e. CYP mRNA expression). The studies should include XRE binding/transactivation analysis as well.
5. It would also be useful to know the relative expression of AhR protein in both the untransfected and transfected cells. The finding that AhR may have transcriptional activity in the absence of known AhR ligand suggests an abnormal phenotype that may not be relevant to a neurotoxicity model. A comparison of relative AhR expression levels and activity in this model to those present in brain cells exposed to exogenous AhR ligands would be useful.
6. The authors claim that proliferations rates are reduced but fail to provide a compelling data set to support this contention. There may be other reasons for the decreased DNA content, i.e. loss in cell viability or apoptosis. Although the text indicates that there was no cell death or apoptosis, no data was presented and it is not clear exactly how this was determined. There is a need to modify the experimental design so that a more rigorous measurement of proliferation (as well as viability and apoptosis) is used.
7. The TH mRNA data are interesting, but additional studies are needed to determine TH protein and functionality (i.e. TH protein, activity, dopamine measurements). The assessment of other markers of differentiation would also be useful.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The manuscript needs editing for grammar and syntax throughout.
2. Introduction: This is presented in a very superficial manner. There is a great deal of background and key references related to AhR expression, activity, and neurodevelopmental effects that are missing. While the Introduction need not be encyclopedic, additional information is needed here especially if the authors are suggesting that their system is a good model for developmental neurotoxicity.
8. P. 6, line 10 from bottom, "AhR was decreased by about 50%." Actually, the figure indicates only about 30% decrease.
9. Discussion: There seems to be much focus on HES in the discussion, especially since no data on HES

are presented. If AhR regulates HES through interaction with XREs, the expected result might be opposite to what the authors are reporting in this manuscript.

Discretionary Revisions (which the author can choose to ignore)

What next?: Reject because too insignificant for publication in any journal

Level of interest: Too insignificant to warrant publication in any journal

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

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.