Reviewer’s report

Title: HIF-1 transcription activity: HIF1A driven response in normoxia and in hypoxia.

Version: 0 Date: 24 Dec 2018

Reviewer: Chitra Kannabiran

Reviewer's report:

The study is a characterization of the HIF1A -dependent gene expression profiles and methylation status of the genome in neuroblastoma cells, by knockdown of the HIF1A gene using shRNAs under normoxic and hypoxic conditions. In addition, the authors have examined methylation profiles under hypoxia using methylation chips. Thus, they identify sets of genes regulated exclusively in hypoxia, exclusively in normoxia and those regulated by HIF1A. To address the concern of the effect of NMYC amplification in the NB cells primarily analysed, the gene expression profiles were confirmed in a different cell line without NMYC amplification. Though the methods and analyses of the gene expression and methylation overall are appropriate, one concern with such studies is the use of established cell lines such as neuroblastoma, regardless of their N-MYC status. These cells tend to show a deregulated gene expression that may not respond to changes in the environment, and are not comparable with normal cells.

It is not clear how the authors evaluated for hypoxic status of the cells after their exposure to low oxygen conditions. There should be an independent test (not expression of HIF) to check that hypoxia is established in these cells. This aspect is missing from the study. Is it conclusive that just 2 hrs of incubation in low oxygen made the cells hypoxic?

Another concern I have is that as seen from Figures 1A and S3, in both cell lines used, the knockdown of HIF1A does not appear to be complete in the presence of hypoxia (lanes 4-6 in both figures). If there is "leaky" expression of HIF1A despite knockdown, the gene expression data obtained may not be specific for HIF1A-inducible genes.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

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.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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