OPEN PEER REVIEW REPORT 2

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Title: Silenced Vav1 attenuates the inflammatory response and neuronal apoptosis in cerebral I/R rats by repressing microglial and NLPR3 inflammasome activation
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COMMENTS TO AUTHORS

In this work entitled "Silenced Vav1 attenuates the inflammatory response and neuronal apoptosis in cerebral I/R rats by repressing microglial and NLPR3 inflammasome activation" the authors evaluate the role of Vav1 in the deleterious processes observed after reperfusion. This work provides new information in the field to understand the pathophysiological mechanisms underlying inflammation and neuronal apoptosis during I/R injury. However, some issues need to be addressed.

Major comments

1. Introduction section. In its present form, the relationship between the inflammasome NLRP3 and Vav1 protein is not well established. This relationship is based on evidence showing that the interaction between both factors is mediated by IP3K. The authors may want to review Inoue et al 2012. Sci Signal. 5(225):ra38 as another potential evidence of the interaction of NLRP3 and Vav1.
2. Material and methods section. The weight range and age of the animals used in the study should be included in this section. Besides, it is highly recommended to include the in material and methods section the brain area where the immunohistochemical analyses were performed. It could be assumed that the authors assessed the penumbra zone, but this should be stated in the manuscript.
3. Results section. It is unacceptable that the authors only show the western blot membrane of one replicate. In its present form, it is impossible to reach any conclusion from all the western blot analyses. Indeed, western blot analysis is a semi-quantitative experimental approach where the optical density of the bands can be quantified with many different software. I would question the relevance of any of this data without quantitation. The authors must provide a figure with the quantitative values for all the western blot analyses.
4. Results section. The conclusions obtained from IHC analysis are very subjective and based on qualitative rather than quantitative data. The authors need to provide quantitative data for these analyses. As an example, it is possible to quantify the colocalization percentage by using Mander's overlapping coefficient or the signal intensity with the total corrected fluorescence. Indeed, the authors may be aware of this since they mention in lines 255-256 that Vav1 knockdown decreased the ratio of TUNEL-positive cells, but it is unclear how was that ratio calculated.
5. Results section. It is astonishing that all the cells from the control group look the same in Figures 5A and 5D. Did the authors perform any additional normalization analysis? If so, please provide this information.
6. Discussion section. In general, the discussion of the results obtained needs to be improved. In its present form, it is essentially based on the citation of articles that only explain more or less the results obtained. The discussion should be modified to provide a more integrative view of the results and frame them in the context of the available literature and knowledge gaps. Besides, there is no discussion about the neurological impairments which are important to understand the role of Vav1 on the neurological function outcome after I/R.
7. Discussion section. The Vav1 siRNA was administered before MCAO. Therefore, the implications

of this fact need to be further discussed in terms of the role of Vav1 in the prevention of the immune response establishment and what could potentially happen if Vav1 siRNA is administered after the MCAO when the immune response is already active. This is important since most of the current therapy is used once the ischemia has occurred rather than to prevent this condition.

Minor comments
Material and methods. The information provided by different datasets (such as GEO) is valuable. Therefore, the use of GEO datasets should be included in the material and methods section and properly cited.