Peer Review File

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Reviewer Comments

The manuscript titled, "Complement C3 derived from astrocytes is activated in patients with TSC and mediates immune injury: an integrated bioinformatics analysis from Bi Zhang and colleagues and submitted to Translational Pediatrics attempts to provide mechanistic insight into the developmental disorder Tuberous Sclerosis Complex (TSC). To achieve this goal, the group performs a re-analysis of a previously published and described dataset from (https://onlinelibrary.wiley.com/doi/10.1111/j.1750-3639.2009.00341.x). The bioinformatic re-analysis identifies numbers of mRNA transcripts differentially expressed that considerably differ from what was previously published but do identify many of the same differentially expressed mRNAs, most notably, C3 that is part of the complement pathway. However, it is unclear whether this data, which technically was collected, processed, and analyzed by a different group could ethically be re-analyzed and published. I think the data, could potentially go in supplemental data and results could be described, but with greater emphasis on the fact that this work is from a different group. Some journals would not accept this practice, but I can only assume in some bioinformatics journals this may be allowed. Several other groups have also published data on C3 in TSC and the complement or inflammatory cascades. This fact alone demonstrates the authenticity and reproducibility of the manuscript which is notable. Further, the authors provide GO analyses, KEGG pathway analysis, and IPA analysis software interrogation of findings. The authors subsequently confirm C3 mRNA transcript levels in cortical tubers by analyzing a different published data set that also already emphasized the inflammatory changes in cortical tubers.

Perhaps the most notable findings is that deletion of Tsc2 in U87 cells increases C3 mRNA and protein levels. This is critically important since inflammatory changes are often assumed to be secondary to seizure activity and the role of Tsc2 in astrocytes

has been debated due to lack of specificity in vivo using cre driver lines in mice. The expression of C3 also appears to increase ROS, at least in a different neuroblastoma cell line. Moreover, the C3 and GFAP staining also confirms the changes in mRNA levels seen in transcriptomic analyses confirms the up-regulation of C3. Taken together, the data are consistent and reliable, but it is unclear as to the legitimacy of using these additional manuscripts without the authors consent or names on the paper. Finally, no ethical statements were seen regarding consent to use these data from patients for these additional purposes and no approval is provided for C3 staining of patient samples.

The responds to the reviewers' comments are as following:

Comment 1: The reviewers doubted that whether the GEO dataset could ethically be re-analyzed and published. And suggested that this data, could potentially go in supplemental data and results could be described, but with greater emphasis on the fact that this work is from a different group.

Reply 1: Thank you for your comment that allows us to greatly improve the quality of our manuscript. We respect the work of others and emphasis it on Method section of Abstract. We have transferred the Figure 1 and Figure 2 to Supplementary Materials. We also checked and re-submitted all the other figures in order.

Changes in the text: see line 22-23, line 238, line 242, line 245, line246, line248.

Comment 2: the reviewers doubted that legitimacy of using these additional manuscripts without the authors consent or names on the paper.

Reply 2: I appreciated your drawing our attention to it. Initially, we cited this data to reinforce our evidence, and we are sorry for your misunderstanding. We found that deleting this section did not cause fundamental problems. Hence, we have decided to remove the figure 5B from the manuscript.

Changes in the text: see line 122-124, line 274.

Comment 3: The reviewers concerned that ethical approval we supplied is not in accordance with the purposes of this study, and no approval is provided for C3 staining of patient samples.

Reply 3: Our ethical approval is "the mechanisms of adenosine in drug-resistant epilepsy caused by focal cortical dysplasia". Tuberous sclerosis complex is an important cause of focal cortical dysplasia. Adenosine dysfunction in epilepsy is tightly linked to astrocyte pathophysiology. Astrocyte-mediated inflammation can promote epileptogenesis and seizure recurrence. We found that the complement C3 mediates immune injury during exploring the mechanisms of tuberous sclerosis complex. In the further, we will explore the relationship between complement C3 and adenosine. for the above-mentioned reasons, the ethical approval we offered is accordance with the purposes of this study.