

## OPEN PEER REVIEW REPORT 1

**Name of journal:** Neural Regeneration Research

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**Title:** MicroRNA-670 aggregates cerebral ischemia and reperfusion injury via Yap pathway

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### COMMENTS TO AUTHORS

The authors have submitted a manuscript and described the effects of miRNA760 in vitro as well as in vivo during cerebral ischemia.

Although the results seem to be consistent and interesting, I have some distinct concerns, especially with a lack of method description and language/understanding issues:

#### 1. Abstract

- How can the miRNA regulate cerebral ischemia ? It might play a role, but does not regulate cerebral ischemia per se.
- Refusion = reperfusion
- It is not explained that authors use in vitro and in vivo models of cerebral ischemia, this has to be discriminated and clarified in the abstract
- Degeneration or degradation ?
- Relieve or reduce neurological impairment ?
- "...understanding for..." ? or better "..understanding of.."

#### 2. Introduction

- Cardiovascular diseases are the most common diseases not cerebrovascular diseases ? Please add, for which region this assumption is valid: Asia, USA; Europe, Worldwide ? and add some numbers of cases per year and cost for the healthcare system to underline the importance of this type of research
- Maybe not use intensification, but something like: increase of the proportion of the aged population over 65 years...
- Satisfying instead of satisfied
- Add "subsequent" in "...causes subsequent additional cerebral damages."
- The hypothesis is missing why they have looked at this specific miRNA, this is essential to understand the background of the study and should be explained already in the introduction.

#### 3. Methods

- Approval number for the animal experiments from the local ethic committee is missing. Generally, methods descriptions are not comprehensive enough, much essential information is missing, thus making it impossible to reproduce the experiments, e.g.
- How long took the tMCAO (1 hour) ? This important information is missing.-
- qPCR method has to be described in detail, temperatures, cycle format, etc., composition of mastermix, concentration of primers, how was the cDNA produced etc.
- Which conditions of OGD, cell culture conditions, medium in brief etc. are missing ! description of N2a cells - species, tissue, passage numbers etc. are missing
- generally give product numbers of all kits, antibodies, etc. otherwise it is not reproducible.
- protease ? Authors have added proteases to their RIPA buffer, I would assume they meant protease inhibitor?

- No stripping of western blot membranes was applied to study p-YAP and YAP of the same samples? If not, how the authors can be sure that p-YAP and YAP really corresponds, since WBs are not as reproducible as maybe wished. Is this the reason why no p-YAP/YAP ratios were presented in the results?
- Hopefully the repeated experiments were not only repeated, but also independent from each other?

#### 4. Results

- It is not clear to what the miRNA data were normalized, have the authors used an internal/endogenous control or just compared the data of miRNA expression between the damaged brain half and the brain halves of sham treated mice. Then you would have the problem of individual differences.
- Methods for knockdown/overexpression have to be described in much more detail, unclear in the current version
- Which control and non-sense sequences were used as controls?
- References are missing why YAP was chosen, and which molecules were tested which were not regulated such as ERK, p38, Akt, etc?
- All Figures: n=, N= is unclear, this should be added to each figure legend for each diagram
- Figure 1: Why data were not normalized to contralateral brain halves in order to include individual differences of single mice?
- Figure 2: Methods: it is not clear how many vision fields they have inspected per image/stain and how many independent experiments were accomplished
- Figure 3: It is interesting that total YAP was decreased, the authors could add a diagram relating p-YAP to YAP as usual for MAPK like ERK, p38, etc.

#### 5. Discussion

- How does the increased cell proliferation in cancer cells correspond to the increased apoptosis in N2a cells?
- "degraded proteasomal" change to "degraded by proteasomal enzymes"

In summary, an interesting study with several open questions, especially about the methods, to be answered.