Reviewer's report

Title: Protective effect of 14-3-3 antibodies on stressed neuroretinal cells via the mitochondrial apoptosis pathway

Version: 2  Date: 4 January 2015

Reviewer: Rahul Modak

Reviewer's report:

The paper titled “Protective effect of 14-3-3 antibodies on stressed neuroretinal cells via the mitochondrial apoptosis pathway” by Bell et. al. reports the neuroprotective effect of 14-3-3 autoantibody in POAG patients. Authors show that POAG patient serum has low level of antibody and it alters expression of several genes in RGC-5 cells. They further show that 14-3-3 proteins are significantly overexpressed and its major regulator, CALM is downregulated upon serum treatment thus triggering apoptosis. Treatment with 14-3-3 antibody reverts the induction of apoptosis through suitable modulation of pathway proteins. In summery, the authors claim that higher level of 14-3-3 autoantibody helps in protection RGCs in normal individuals. Although it is an important observation and relevant study in the field there are several questions that need to be addressed before the study could be published.

Major Compulsory Revisions

1. Authors show 10ug/ml antibody treatment gives protection to H2O2 stress & ROS production, whereas authors used up to 5ug/ml antibody for other two stress inducers. It is important to show the protective effects up to 10ug/ml antibody for better understanding of the data.

2. It is often observed that when cells are treated with increasing concentration of any modulator, there is slight drop of cell viability at certain lower concentration followed by significant increase at higher concentration. What is the effect of various concentrations antibody alone on the cells? Is it possible that 10ug/ml antibody induces cell proliferation even in absence of H2O2?

3. Did the authors observe significant difference in 14-3-3 expression pattern and/or localization upon POAG serum stimulation? It is important to compare the localization of 14-3-3 after serum (normal vs. POAG) stimulation.

4. The authors used myoglobin antibody as control. Does the level of same changes in POAG patients? What is the expression profile of myoglobin in RGC-5 cells?

5. What other autoantibodies present in POAG patients? What are their known effects on RGC-5 cells?

6. Discussion: Difficult to understand in many places, e.g. Page 8 line 5-8. It needs to be suitably modified.

7. Figure-3 A: Why antibody is showing ~10% increased viability compared to
10% decrease in viability in Figure-2? Considering the range of error bar, why do the authors think the change in cell viability is significant?

8. Figure-3 B: Control+ glutamate sample shows very high variation in viability and hence it is difficult to compare other lanes with the same. It will of great interest to understand how the treatment with antibody reduced such huge variation.

Minor essential revisions

9. Figure-1: Please clearly mention the Y-axis. In the figure legend it says ‘fold change’ but it is difficult to decipher the same from the figure.

10. Figure-2: Please include the missing error bar for each data point. Why there is 10% drop in viability at 0.5ug/ml antibody?

Discretionary Revisions

11. Please comment on which serum factor(s) is altering the 14-3-3 and other proteins expression in cell?

12. Authors may compare the effect of any other autoantibody whose target is expressed in RGC-5 cells with the effect of 14-3-3 antibody to decipher whether it is specific phenomenon related to 14-3-3 antibody.

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests