Reviewer's report

**Title:** miR-24 contributes to retinoblastoma development by compromising the p53 response to RB1 loss in retina

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**Reviewer:** Erik Vassella

**Reviewer's report:**

The authors demonstrated that ARF is activated in developing retinas of mice with Rb-inactivation, but not in wt retinas. In contrast, ARF protein was low in retinoblastoma cell lines, but in some cell lines ARF mRNA was high, suggesting a translational or post-translational control. However, p14ARF was normally degraded by proteasomes suggesting that the observed effect is not due to an altered protein turnover. The authors investigated a miRNA (miR-24). From previous experiments it was known that this miRNA targets p14ARF. They concluded that miR-24 may be implicated in the translational repression of ARF in retinoblastoma cells.

This is an interesting work, showing that miR-24 contributes to tumorigenesis of retinoblastoma. However, more experiments are required to support the conclusions made by the authors.

**Major points:**

- The authors suggested that miR-24 is implicated in translational control of ARF. This is based on the finding that silencing of miR-24 in WERI-Rb cells resulted in higher ARF protein. However, the proposed mechanism may only apply for RB381, 383 and 247 cells (in which ARF mRNA was disproportionally high), but not necessarily WERI-Rb cells (low ARF mRNA level). Why did the authors perform the experiment in WERI cells?

- The authors proposed that miR-24 is implicated in translational repression. However, mRNA stability may also be affected. It would be informative to show the mRNA level of ARF in miR-24-silenced cells. This information is important since it would allow the authors to conclude, if the observed disproportion of mRNA and protein is indeed due to miR-24.

- The authors used mock-transfected cells as a control. However, it would be more appropriate to use a scrambled control to exclude non-specific effects of the siRNA.

**Minor points:**

- Materials and methods: what assay was used for cDNA synthesis and quantification miR-24?

- How was the quantification of the protein level in Fig. 4D performed?
**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests’