To and colleagues propose a novel mechanism by which the p53-mediated response to RB1 loss may be compromised during retinoblastoma tumorigenesis.

Retinoblastomas are thought to arise due to RB1 inactivation in a specific retinal cell type. In most cells, aberrant proliferation accompanying RB1 loss provokes a tumor suppressor response through induction of ARF, inhibition of MDM2, and activation of p53. In turn, this necessitates genetic changes in the ARF-MDM2-p53 pathway in nearly all human tumors. Retinoblastomas generally lack genetic aberrations in ARF, MDM2, or TP53, suggesting that they may derive from a cell type that has an intrinsic defect in this pathway. It was recently suggested that the p53 response to Rb loss is defective in human cone precursors (which resemble retinoblastoma cells and are thus a potential cell of origin), due to their intrinsic high MDM2 expression (Xu et al, 2009). The study by To et al now suggests that the p53 pathway might be compromised through intrinsic miR-24 expression and repression of ARF mRNA translation.

The evidence for a role of miR-24 in retinoblastoma includes:

Figure 1: Inactivation of RB1 in the peripheral mouse retina induced expression of ARF RNA and protein, as well as p53 and some but not all of the expected p53 target genes. This is interpreted as suggesting that ARF-induced p53 signaling is sub-optimal in retinal cells.

Figure 2: Three retinoblastoma cell lines have a much lower ARF protein:RNA ratio than HeLa cells, suggesting that these lines have an impediment to ARF protein production and/or stability, whereas three other retinoblastoma lines expressed low levels of ARF protein “at least in part due to low ARF mRNA.” This is interpreted as evidence that a post-transcriptional mechanism impairs ARF expression.

Figure 3: Ectopic expression of ARF activated p53 in a cell line (WERI-1) that was previously shown to express high levels of MDM2 and MDM4. This implies that sufficiently high levels of ARF may overcome high levels of MDM2 and MDM4 to activate p53 (and thus ARF is a limiting component of the pathway).
Figure 4. Treatment of retinoblastoma cells with a proteasome inhibitor did not restore ARF expression to the level found in HeLa cells, despite that the RNA levels were similar, implying that ARF destabilization via the proteasome could not fully explain the difference in ARF expression in retinoblastoma vs. HeLa. Furthermore, siRNA directed against miR-24 enhanced ARF expression, implying that miR-24 was in part responsible for the low level of ARF protein.

While the idea that intrinsic miR-24 expression compromises the p53 response in retinoblastoma is interesting, further substantiation is needed before such an hypothesis would be warranted, as follows:

Major Compulsory Revisions:

1. The notion that “intrinsic” miR-24 activity compromises the p53 response during retinoblastoma tumorigenesis would seem to require that the retinoblastoma cell of origin has unusually high levels of miR-24 activity. However, the authors provide no evidence that higher-than-average levels of miR-24 are intrinsic to any retinal cell, nor (more specifically) to a cell that could plausibly be the retinoblastoma origin. In the absence of such evidence, it could be argued that miR-24 expression is acquired during tumorigenesis, rather than being an intrinsic cell-of-origin feature.

2. The notion that miR-24 underlies the lack of ARF protein in retinoblastoma, would seem to require that miR-24 expression or activity correlates with impaired ARF expression in retinoblastoma cell lines and tumors. However no such evidence is proided. In the absence of such evidence, it could be concluded that ARF is primarily regulated by other factors, and that miR-24 has only a minor effect that is no more significant in retinoblastoma than in any of the hundreds of other cancers. Accordingly, the relationship between miR-24 and ARF RNA and protein levels should be compared for retinoblastoma tumors, retinoblastoma cell lines, and other cancers in which there is abundant ARF expression (for example, those with TP53 mutations), prior to concluding that miR-24 has a significant role.

3. In the opening of the “Conclusions” section, the authors state that their Figure 1 data indicated that “p53-tumor surveillance in response to RB loss may be suboptimal in the developing retina, since expression of key p53-downstream targets remain unchanged upon Rb inactivation.” However, this interpretation does not seem warranted, because the ARF-MDM2-p53 pathway is generally induced ONLY when Rb loss is accompanied by aberrant proliferation, and the vast majority of mouse retinal cells do not aberrantly proliferate (or re-enter the cell cycle) upon Rb inactivation. Thus, the lack of a strong p53 response may relate to the lack of a proliferative stimulus, rather than an intrinsic defect in pathway activation. A more appropriate setting to evaluate pathway activation would be the Rb/p107 double mutants, in which there is more widespread aberrant proliferation. Alternatively, the data should not be interpreted as evidence of a p53 pathway defect.

4. The p53 pathway expression profiles in Figure 1C-D are of uncertain
relevance to retinoblastoma because a) the profiles reflect the behavior of the major retinal cell types at P0-P15, but give no information about minor cell populations from which retinoblastomas could arise; and b) mice do not develop retinoblastoma in response to Rb inactivation, and thus may not display responses that underlie retinoblastoma (in either the major or the minor cell populations). The p53 pathway response would be more appropriately evaluated in human retinas (where Rb mutation is thought to initiate tumorigenesis), and stratified according to retinal cell type. These concerns, along with those in #3, brings into question whether Figure 1 provides any insight into ARF regulation that is relevant to retinoblastoma, and warrant reconsideration of the inclusion of this data.

5. Figure 2 shows there is a low ARF protein:RNA ratio in three retinoblastoma lines as compared to HeLa cells, but no other lines were evaluated for both ARF RNA and protein. To assess whether retinoblastoma lines generally have a low ARF protein: RNA ratio (rather than HeLa having an unusually high ratio), both ARF RNA and ARF protein should be evaluated in each of the retinoblastoma lines, as well as in a series of other tumor cell lines (as per point 2, above).

Minor essential revision:

6. Tumor cell lines known to have high ARF expression ought not to be referred to as “controls” for the retinoblastoma cell lines (p. 11, top), as they have many differences beyond not being retinoblastoma cells.

Level of interest: An article whose findings are important to those with closely related research interests

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.