Reviewer's report

Title: Irresponsiveness of two retinoblastoma cases to conservative therapy correlates with up-regulation of the VEGF-A pathway.

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Reviewer: Rossella Rota

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Fortunato and colleagues report 2 Cases of sporadic bilateral retinoblastoma that they present as irresponsive to therapy. They analyze the expression of the angiogenic factor VEGF-A, its receptors VEGFR1 and KDR along with HIF-1alpha and the two hERG1A and B K+ channels by real time RT-PCR in tumour tissues vs normal ocular tissues. Fortunato and coworkers also evaluate the secreted amounts of VEGF in the vitreous of the enucleated left eye for each patient and its expression in tumour tissues by immunohistochemistry. Authors show that the expression of the genes of interest is higher in retinoblastoma tissues in both Cases vs normal control samples. They conclude that patients with aggressive tumours could benefit from an anti-angiogenic therapy.

The novelty of the study is the finding of a hyper-expression of the two hERG1 channels previously found associated to VEGF and invasive phenotype in another malignancy. This should be highlighted in the title of the manuscript. This result opens the way to deeper investigations in the field.

However, the work needs to be implemented with other data and revised for the significance of the finding.

MAJOR Compulsory Revisions
1. It is not clear on which tissue Authors did the genetic analysis for RB1 gene in both Cases. It should be specified.
2. Authors report on the Abstract and Cases Presentation Sections “very aggressive RB…….classified at Stage II of the Reese-Elsworth Classification”. The term “aggressive” seems not to be suitable in this case. Please Authors should explain better the term “aggressive” or replace it.
3. References about angiogenesis on retinoblastoma are often old. There are several new reports on the argument.
4. Due to the amount of data in the literature correlating VEGF secretion to tumour growth and a very recent work of Areán C et al. (Arch Ophthalmol 2010) in which VEGF staining intensity is shown to be correlated to mitotic and apoptotic indexes, Authors should report these parameters.
5. Histology and immunohistochemistry of the Case 2 should be inserted in Figure 3C.
6. Authors should show larger fields in Figure 3C in order to highlight differentiated tumour structures, or report a larger portion of the tumours together
with higher Magnifications.

7. Is there sufficient tissue to assess the microvessel density (MVD) on tissue specimens? This could be interesting.

8. The number and intensity of stained cells for VEGF should be semi-quantitatively assessed.

MINOR essential Revisions

1. Authors should carefully check for the reference numbering appearance.
2. Authors should check manuscript for slips.
3. The tumour size for the Case 1 is 7.5 mm in diameter? Explain better please.
4. In the Figure 1A the ecographic profile of the tumour mass should be indicated with arrows.
5. It is not clear if the Case 2 is a responder to therapy for the right eye. Maybe the time of observation is too short but the Authors could clarify this aspect.

**Level of interest:** An article of importance 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 not competing interests