Reviewer’s report

Title: pRb2/p130 protein expression and RBL2 mutation analysis in Burkitt lymphoma from Uganda

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Reviewer: Rocio Hassan

Reviewer’s report:

This is a well written report designed to validate a previously proposed pathogenic model for endemic Burkitt lymphoma (eBL). For that aim, a pediatric series of eBL from Uganda was studied, with respect to pRB2 expression in tumor cells, as well as RBL2 specific mutations.

The methodology used is appropriate for the planned objectives, and the limitations of the study are relatively well explained.

In this study, two relevant findings are reported. First, the observation that pRb2 is overexpressed/accumulated in more than 50% of endemic BL cases. The second one, is the lack of mutations in RB2, which does not conform to a previous pathogenic model proposed for endemic BL.

Discretionary Revisions

Background

5th paragraph

Authors state, when referring to the study of Klumb et al, that no RBL2 mutations were detected in a series of endemic BL. To be precise, BL from Southeastern Brazil cannot be considered endemic, and about half of the cases are EBV-.

Material and Methods

Since the monoclonal antibody used in this study was different than the one used in the original report showing cytoplasmic staining of Rb2/p130 in eBL, and the present study drains different conclusions, it would be important to describe the epitope (carboxy-terminus vs. N-terminus) recognized by the clone 130P215 from Abcam used in this study.

Results

Histopathological characterization of BL series: although clinical presentation and demographic characteristics of patients in this series are well characterized, and this seems to be a homogeneous childhood BL collection, little attention has been paid to de histopathological features. Since WHO classification takes into account morphological variability and atypias, whose clinical/biological importance are still an open question, it would be interesting to add some information on this issue, for instance, to describe if all cases were classified as classical BL.
Second paragraph
As to the immunohistochemical characterization, even when the primary data about pRb2 expression levels are shown in table 2, it seems worthwhile to include in the text the percentages of cases included in each expression category: i.e. 12/51 cases (23.50%) expressed low levels while 31/51 cases (60.7%) expressed high levels pRb2.

In the abstract, results sections, it would be also important to include the percentage of cases expressing high levels of pRb2.

Discussion
It is my impression that discussion will be strengthened if, in the first part, you rest more clearly on your finding of a high expression observed in most of the BL cases, uncoupled to cell cycle arrest, suggesting a putative functional impairment of Rb2 pathway. In that case, do you think that pRb2 accumulates? Is its expression misregulated along the cell cycle? It would be also important to more clearly compare your cases with AIDS-associated BL, not only mentioning a possible viral link (see below), but also focusing on probable common mechanisms. For instance, even when you did not assess p105 expression in your BL series, is it possible that p105 were highly expressed in eBL over-expressing pRb2, as observed in AIDS-BL? And then, you can put forward the differences with NHL in immunocompetent patients and with BL bearing NSL mutations in RBL2.

Regarding the proposed viral link, it is well recognized that viral oncogenesis mediated by DNA virus, such as polyoma- (in in vitro oncogenic models) and HPV, is based on the targeting of suppressor genes (i.e. p53 and pRb) via protein-protein interactions. However, this does not seem to be the case of EBV, particularly in type I latency, characteristic of BL (even when EBV expression, latent and lytic, in eBL showed to be less restricted!). In fact, some proteins such as EBNA3C and EBNALP were described to interact with Rb and p53 in type III latency, but they seem to be important for the lymphoblastic transforming properties in primoinfection or in lymphoproliferations in immunocompromised hosts (not necessarily AIDS-related, where latency is not clearly of type III). Then, since an underlying molecular mechanism cannot be claimed on the basis of present knowledge, it is my feeling that the authors' mention to a viral link common to eBL and AIDS-BL is at present a bit overestimated (even when future research give reason to this claim!). Otherwise, Kenyan BL are also EBV-associated, and the molecular results obtained, at least in the original work by Cinti et al (2000), is quite different. It is my suggestion that, if authors decide to maintain the mention to a viral link (which perhaps is worthwhile), they may also decide to include some references to the present limitation of their statement.

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 no competing interests'