Reviewer's report

Title: Gene Expression Profiling of Laterally Spreading Tumors

Version: 2

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Reviewer: Jordi Camps

Reviewer's report:

In the present manuscript, Minemura and colleagues attempted to generate the transcriptional profiling of colorectal laterally spreading tumors (LST) using PCR-based array analysis by comparing 17 adenomas, 12 carcinomas and 12 pedunculated adenomas as a control. Immunohistochemistry results confirmed and validated that overexpression of BCL2L1 is associated with laterally spreading tumors, and potentially indicate worse prognosis. Functionally, the authors attempted to inversely correlate the expression of the anti-tumor BCL2L1 with the presence of apoptosis in tissue sections. Thus the authors conclude that BCL2L1 is upregulated and blocks apoptosis in laterally spreading tumors.

The manuscript is well structured and very straightforward; however, there are some suggestions this reviewer would like to make in order to give some strength to the author's conclusions.

Major revisions:

1. Some of the conclusions the authors make are only observational so there are some statements that there should be downsized.
2. The inverse correlation between expression of BCL2L1 and apoptosis is highly speculative unless some functional assays are performed. There is no biological rationality to claim that the inverse relation between expression of BCL2L1 and apoptosis happens specifically in LTS.
3. If possible, the correlation between mRNA and the protein should be performed in the same set of samples.
4. The methodological description of the immunohistochemistry should include each dilution factor utilized for each antibody.
5. In Figure 1, some L-Can cluster within the groups of adenomas, both LTS and non-LTS. Given the fact that cancers should actually be the most well separated entities in the hierarchical clustering, the authors should attempt to find an explanation about their results.
6. Is the overexpression of BCL2L1 a marker of high-grade dysplasia in LTS only or it is also true for other tumor subtypes?
7. The results of the qRT-PCR would be much easier to follow by the reader if they are presented in a plot (e.g. box plot).
8. Although it is mentioned by the authors in the Discussion, it would be informative to know whether BCL2L1 upregulated cells are Ki-67 positive. This
could give some insights in the mechanism of action.

9. The rationale as to why the authors dropped off the follow-up of TNFRSF25 is not clear.

Minor revisions:
1. The name of the genes must be in italics.
2. Last paragraph in the abstract needs to be rewritten.
3. In Line 251 "claim" should "replace" declare.
4. In Line 258 "to" should replace "with".

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I do not have competing interests.