Author’s response to reviews

Title: Early diagnostic value of Bcl-3 localization in colorectal cancer

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Author’s response to reviews:

Point-to-point letter

Editor’s comment:
The manuscript is well written. There are few minor comments and revisions from reviewers.

Editorial Request: Conclusions section. This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

The authors would like to thank you the Editor for the suggestion.

We have included the main conclusions and relevance of our study in the revised version of our manuscript (Page13).

Reviewer #1 (Ruaidhri J. Carmody):
The authors would like to thank the reviewer for the suggestions.

1. The authors stain for caspase-3 (Figure 4) which they use as a proxy for cell death. I am presuming that the antibody is specific for active caspase-3 rather that total caspase-3. The authors need to clarify this and provide specific details for the antibody used including supplier, clone and catalogue number.

The reviewer is correct. We used antibodies against active caspase-3. This mistake is corrected and we have provided details information about the active (cleaved) form of caspase-3 in the revised version of our manuscript (Page 6).

2. Anti-bodies against Bcl-3 have been notoriously unreliable and so the use of the Bcl-3 blocking peptide in Figure 1 is welcome. However, the authors need to provide more details on the antibody used in this study including the clone and
We have included additional information about the Bcl-3 antibody in the revised version of our manuscript (Page 6).

Reviewer #1 (Alain Chariot):
The authors would like to thank the reviewer for the suggestions.

1. Some breast cancer cell lines (MCF7, ...) show elevated levels of BCL-3. The authors could use them as a control for nuclear localization of BCL-3. The goal would then be to see whether this cytoplasmic localization of BCL-3 is specific to transformed intestinal epithelial cells.

- We have followed the reviewer’s suggestion and used MCF7 as control for nuclear localization of Bcl-3. As expected, Bcl-3 was mainly localized in the nuclei of these cells (Figure 5C).

2. The authors previously showed that TPA triggers the nuclear localization of BCL-3 in keratinocytes. What is happening if they treat CACO-2 and HCT116 cells with TPA? Do they trigger the nuclear important of BCL-3? The goal would be to see whether the signaling pathway known to trigger BCL-3 nuclear import is specifically disrupted in colon cancer cells.

- We stimulated CACO-2 and HCT-116 cells with 100 nM TPA for 30 minutes. This treatment did not show any differences in the localization of Bcl-3 compared with non-stimulated cells. This suggests that mechanism for Bcl-3 translocation in primary mouse keratinocytes by TPA is not similar to human colon cancer cell lines (Figure 5C).

3. All tested colon cancer-derived cell lines show constitutive Wnt signaling due to #beta-catenin or Apc mutations. The RKO cell line does not show constitutive Wnt signaling. Therefore, it would be interesting to see whether BCL-3 expression is enhanced and if so, whether BCL-3 is also found in the cytoplasm of those cells. Similarly, does TPA trigger the nuclear import of BCL-3 (assuming that it is detectable)?

- We purchased the RKO colon cell line from ATCC (Page 7) and compared the levels of Bcl-3 in RKO with CACO-2 and SW-48 cell lines. Unfortunately, since Bcl-3 levels were undetected in RKO cells we could not test the localization of Bcl-3 in this cell line. (Figure 5D, Page 10-11).