Reviewer’s report

Title: The role and prognostic value of apoptosis in colorectal carcinoma.

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Reviewer: Prashant Bavi

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Major concerns

- Prognostic significance of apoptotic index in colorectal carcinoma is controversial, as apoptotic index has been reported to be associated with both good and bad outcome in different studies. The authors should discuss this issue in more detail with reference to lack of uniformity in pre analytic processing variables especially cold ischemic times and fixation in formalin. Although the prognostic significance of increasing apoptotic rates and proliferation do lean towards poorer prognosis, it would be interesting to support this with additional data of additional markers of apoptosis like M30, cleaved caspase3 and maybe proliferative marker Ki-67 in the stepwise normal to adenoma to cancer sequence.

- In the Materials and Methods section the authors should specify the anatomical site and the timeframe of these 103 colorectal cancers. Were any rectal cancers included? If yes, why did they not receive neoadjuvant chemo/radiotherapy? Did the presence of some preexisting conditions prevent them from receiving treatment or were these patients excluded based on other factors? Another factor that the authors should discuss is the poor survival observed in distal tumors with lower apoptotic indices,(Sinicrope FA et al. Clinical Cancer Research Vol. 5, 1793–1804, July 1999.)

- Terminal deoxyribonucleotidyl transferase mediated nick end labelling (TUNEL) and in-situ end labelling (ISEL) are the methods most often used to demonstrate and quantify apoptosis in histological tissue sections, and the interpretation and specificity of these techniques have been controversial. Another major issue is why TUNEL assay was not validated using the M30 antibody for immunohistochemistry that has proved useful in assessing apoptotic indices in colorectal carcinomas.

- Please elaborate if any steps were taken to avoid false positive staining in the TUNEL assay with regard to inhibiting endogenous endonucleases and endogenous alkaline phosphatase in the intestine. What is lacking is the need for supplementary data that would have complemented and added value to their findings as using a single method of detection fails to discriminate between different types of cell death.

- Apoptotic indices were determined in grouped stages (I&II and III&IV) and it is necessary to to evaluate them in each stage individually and calculate OS and DFS in subgroups later. Similarly, AI should be correlated with age groups as it
would be of interest to see if there was clustering of higher AI in the older age group (above 70yrs).

Minor Essential Revisions

• The authors need to define the 20 “normal” colon tissues studied with regards to the following: Were they normal colonic tissue adjacent to cancer tissue or were they normal colonic tissues from non cancerous specimens? If yes, what was the distance from the cancer?

• Apoptotic counts are higher in normal mucosa obtained from resection margins than in truly normal mucosa. The authors should comment on field change of inhibited apoptosis in mucosa adjacent to colorectal carcinomas.

• The authors should provide photomicrographs that illustrate the differential AI across the colorectal carcinogenesis spectrum.

Discretionary Revisions

An added suggestion would have been to try double labeling protocols for the TUNEL assay.

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