Reviewer's report

Title: Mutations in APC, CTNNB1 and K-ras genes and expression of hMLH1 in sporadic colorectal carcinomas from the Netherlands Cohort Study

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Reviewer: Sima Salahshor

Reviewer's report:

General

Authors have examined components of different signaling pathways previously reported to be involved in colorectal carcinogenesis in a large series of sporadic colorectal cancer. Tumors have been screened for mutations in APC, b-catenin and K-ras genes and defect in hMLH1 protein expression.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) The number of APC mutation found in this series of sporadic colorectal cancer is very low (37%) compared to previous studies (60%-85%). This is probably due to difficulty to extract good quality DNA from paraffin-embedded tissues. As indicated in the text, 72 cases could not be examined due to PCR amplification problems. However, the same samples could be examined for K-ras gene which indicates that DNA quality has been sufficient for mutational analysis. Is it possible that those samples could not be amplified because there were mutations in the APC primer binding sites? Could you examine those cases for defect in APC by immunohistochemical analysis for either b-catenin or APC protein?

2) Why did you choose to examine tumors that did not express hMLH1 protein for b-catenin mutational analysis (in addition to tumors without APC mutation)? Why do you expect to see defect in b-catenin in cases with defect in hMLH1?

3) What were the criteria for selection of 162 cases for BAT-26 analysis?

4) A figure showing hMLH1 immunohistochemical staining should be included.

5) It is not clear how many samples were examined in the final study- Need to be clarified. Page 5: sufficient DNA could be extracted from 737 samples Page 6: 72 samples could not be examined for APC mutation (737-71= 665) - but the number reported in the abstract is 656.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

6) In 103 cases authors did not find any alterations by the methods used in the selected genes. Recent studies have identified mutations in other component of b-catenin degradation complex such as Axin1 and Axin2 and also other mismatch repair genes. The possibility of defects in other genes in the same pathway could be mentioned in the discussion.
7) A table which compares the mutation frequencies found in this study with other previous reports.

Discretionary Revisions (which the author can choose to ignore)

8) A short discussion on the mutation detection techniques used in this study (or other studies) and how that might affect the results and conclusions.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.