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: Roland Wolf

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This is a primarily a descriptive study, documenting mutations in APC, CTNNB1 (b-catenin), K-Ras and hMLH1 expression in a series of sporadic colorectal tumours from the Netherlands Cohort Study.

This manuscript follows a series of similar publications from the same study group, where mutations in APC (Carcinogenesis, 25, 1219, 2004), hMLH1 expression (Am J Epidemiol, 161, 806, 2005) and K-Ras mutations (Br J Cancer, 92, 1310, 2005; Int J Cancer 114, 824, 2005; Carcinogenesis, 25, 1619, 2004) have been previously described. As such, only the b-catenin data presented in this manuscript is novel, although the analysis of co-occurrence of the various mutations is of obvious interest.

The aims of this study and hypothesis to be tested are not obvious, however, and should be more clearly defined. The authors should also comment on their decision to exclude p53 mutations from their analysis – as they state in the first sentence of their abstract “The majority of colorectal tumours are thought to be driven by a sequential accumulation of mutations in the APC, K-ras and TP53 genes” it is not clear why detailed analysis of mutations in APC and K-Ras is included, but p53 is not, particularly as the APC and K-Ras data has been previously published. It is also not clear why only tumours without a truncating APC mutation or lack of hMLH1 expression were selected for b-catenin mutation analysis. Similarly, it is not obvious why BAT-26 analysis was performed on only 162 patients. The basis for selection of this patient group should be provided and the statement “mutation analysis of other samples was abandoned since this was deemed irrelevant” should be justified.

Specific points to be addressed:

1. There are discrepancies in the number of tumours analysed described in the various sections of the manuscript. For example, the abstract states that tumours from 656 unselected colorectal cancer patients were analysed, Methods refers to 737 patients (665 following quality exclusion) and mutation analysis data is presented on 646 tumours for CTNNB1. This should be corrected or justified by the authors.
2. The phosphorylation sites in CTNNB1 should be defined in the abstract.
3. The relevance of the Food Frequency Questionnaire described in the Methods section is not clear, as no dietary information is included in the analysis.
4. It is not clear why the authors report a greater than 10% error rate in replicate sequencing analyses of APC and K-Ras mutations.
5. Description of appropriate positive controls for WAVE analysis of b-catenin mutations should be provided. The authors suggest that mutations “may have escaped detection” using this technique. As only a limited number of phosphorylation sites in b-catenin were analysed, it is not clear why an additional technique e.g. PCR-RFLP analysis was not used to validate the WAVE screening approach.
6. The Cramér's V statistical test is not described in the Methods section.
7. The same data appears to be duplicated in the final sentences of paragraphs 2 and 3 on page 10 of the manuscript.
8. The conclusion that APC and K-Ras mutations occur at similar frequencies in colorectal tumours should be discussed in relation to the majority of previously published data, where APC mutations are significantly more frequent that K-Ras mutations.
9. The authors suggest that their analysis is compromised by incomplete analysis of the APC mutation cluster region (missing in 72 cases). As tissue is available and methods are in place, it is not clear why this additional data was not obtained before the data set was prepared for publication. A similar justification should be provided for the incomplete hMLH1 data set.
10. K-Ras “non-activating” mutations are described in the legend to Figure 1, but are not introduced elsewhere in the manuscript. The basis for “non-activation” should be provided.
11. The appropriate statistical analysis and p-values should be provided for Table 2.