Reviewer's report

Title: Alterations in PTEN and PIK3CA in colorectal cancers in the EPIC Norfolk study: associations with clinicopathological and dietary factors

Version: 1 Date: 14 December 2010

Reviewer: Milo Frattini

Reviewer's report:

In this paper the Authors investigated PTEN mutational status (limited to exons 7 and 8), PTEN protein expression and PIK3CA mutational status (exons 8, 9 and 20) in a well characterized series of 186 adenocarcinomas and 16 adenomas of the colon-rectum from the EPIC Norfolk study.

The Authors found PTEN mutations in 2.2% of cases, PIK3CA mutations in 7% of cases, and a loss of PTEN expression in 34% of cases. Statistical analyses revealed that PTEN expression is associated with LDL concentrations, Dukes’ stage and poor differentiation; PIK3CA mutations to gender. In addition, the Authors revealed that PTEN mutations is not the main cause of PTEN loss of expression, at least in colorectal cancer.

The paper is well written, the experiments nicely conceived. The data support the conclusions.

However, all the data and conclusions of the papers can be modified (or confirmed, of course) if the Authors investigate other regions of PTEN mutational status. In fact, according to the literature and to the data included in the COSMIC database, there are other exons of the PTEN gene that are altered in a consistent number of cases: for example, exons 5, 6 and 9. Therefore, the Authors must include the analysis of these additional regions to their data.

Second major point: the frequency of PIK3CA mutation. As the Authors found also a low number of KRAS mutations, it is possible that they did not properly select the tissue for DNA extraction. Can the Authors give much more details about guidelines they followed for DNA extraction? Did they follow the suggestions of Van Krieken and colleagues published on Virchows Archiv?

Last point. the PTEN expression is made by comparing tumoral cells with surrounding endothelial and non-neoplastic cells. In our opinion, it is better to use as comparison the normal mucosa. The Authors should re-evaluate PTEN expression on the basis of this suggestion.

Taken into account all these comments, the paper is, at present stage, not acceptable for publication, pending major compulsory revisions.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests