Reviewer's report

**Title:** Early onset MSI-H colon cancer with MLH1 promoter methylation, is there a genetic predisposition

**Version:** 1  **Date:** 27 November 2009

**Reviewer:** Richard Hamelin

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Microsatellite unstable tumors (MSI tumors) have a defect in their mismatch repair (MMR) system. They can be either hereditary (Lynch syndrome) occurring generally at a young age and mostly due a MMR gene germline mutation, or sporadic, occurring generally at a more advanced age, and mostly due to the methylation of the promoter of MLH1, one of the MMR genes.

In this work, van Roon et al. analyzed a series of 46 MSI colon cancer cases with MLH1 promoter methylation, of which 13 cases were with an age of onset below 50 years.

The authors compared genetic and epigenetic alterations between MSI tumor series subsets occurring before or after the age of 50. To my knowledge, this comparison has never been investigated and is interesting, although the authors did no find important differences between both subsets, and these differences were not so unexpected.

**Minor essential revisions**

- It is not said, and it is important, whether this series was enriched for young patients or whether it was a non-selected series, indicating thus that about 28% of MSI tumors with MLH1 methylation occur in patients younger than 50 years.

- GADD45A. It is reported in detail in the introduction that GADD45A could play a role in aberrant DNA methylation, but shortly that other reports disputed this fact. No GADD45A mutation has been reported in the literature for human cancers, and it is the same here, with the exception of a SNP in 5 cases, and a neutral amino acid change in one case. So, these negative results complicate table 1 (where it is written mutation instead of SNP) and should be removed.

- The importance of the MLH1 -93G>A polymorphism has already been reported. The authors should emphasize what is new here.

- Since all tumors were selected because they had methylation on the MLH1 promoter, it is not surprising that MLH1 is more methylated than all other CIMP genes. The methylation mechanism being (at least partly) age related, and probably progressive, a similar selection of tumors methylated on MINT1 for example, would have also shown more frequent methylation on MINT1 than other CIMP genes, including MLH1, and occurring in tumors not reaching yet the CIMP level of 4 or 5/9 methylated genes. It would also probably show a progressive methylation and CIMP appearance according to age as shown on
table 2. This possibility is not enough discussed.
- The part concerning genomic profiling should be condensed.
- BRAF data are more convincing. The relationship between BRAF/KRAS mutations and DNA hypermethylation should be discussed further with the help of the published literature on the subject.

Discretionary revisions
- The two cases with a germline MLH1 epimutation (ID60 and ID36) should be removed from the following analysis since these cases probably follow a different pathway.

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

**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