Reviewer’s report

Title: DNA methylation alterations of AXIN2 in sessile serrated adenomas and colon carcinomas with microsatellite instability

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Reviewer: Keith Robertson

Reviewer’s report:

Previous research has demonstrated genetic and epigenetic alterations in sessile serrated adenomas (SSA) and colon carcinomas with (MSI) (Maeda T, et al, 2011). The mechanism by which adenoma transitions to MSI remains unknown. In this paper the authors analyzed that genome-wide surveillance of hyper- and hypo-methylation alterations in NotI sites by MSFLP-array in tubular adenoma (TA), SSA, colon carcinomas with (MSI) and without microsatellite instability (MSS). The authors show that 1) between these four groups, 56 probes were differentially altered in analysis of DNA methylation. 2) These four groups fell into two clusters: Group 1, TAs and MSS cancers with KRAS mutations. Group 2 was composed of the cells with BRAF mutations. Group 2 was further divided into two subgroups; cancer with MSI and MHL1 mutation (Group 2A) and SSAs without MHL1 mutation (Group 2B). 3) The authors also revealed that aberrant methylation of AXIN2 is observed in SSA and MSI. From these results, the authors suggest that repression of AXIN2 by DNA methylation might trigger a transition of SSA adenoma cells with BRAF mutation, into MSI cancers.

The authors describe convincing results but there are several concerns.

Major point

1) The authors categorized genetic and epigenetic changes of TA, SSA, MSI and MSS. The categorized data is very similar to their own previously reported data (Maeda T, et al, 2011). This is most likely because the authors used same specimens that they had used in previous reports (Maeda T, et al, 2011). So there is some question as to how much these new findings add to our understanding of SSAs and their progression.

2) The authors have shown for the first time that aberrant DNA methylation of AXIN2 in SSA and the methylation status was apparently increased in MSI. From these results, the authors hypothesized silencing of AXIN2 might be a trigger of transition of SSA adenoma cells with BRAF mutation, into MSI. But, the author’s group previously reported that AXIN2 transcripts were suppressed in most of MSI colorectal carcinoma specimens. (Koinuma T, et al, 2006). Therefore while the finding of AXIN methylation is new, it is not unexpected given the expression patterns already reported.

Minor point

1) The numbers in the table 1 and 2 are difficult to understand. The numbers
should be written as a ratio. For example, in table 1, KRAS mutation in TA should be written as 4/8 (mutant/total). hMLH1 methylation in TA also should be written as 0/8 (methylation +/-total).

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

**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