Reviewer's report

Title: DNA hypermethylation and upregulated miRNA-21 expression leads to decreased mRNA expression of MAL, PRIMA1, PTGDR and SFRP1 in left-sided colorectal adenoma and cancer

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Reviewer: Alexander Link

Reviewer's report:

The study by Molnar et al. is another step on the way to understand the role of DNA methylation in the CRC. In the present study, the authors perform another way of data analyses with the aim to identify additional genes that may be involved in process of colon carcinogenesis and correlate them to DNA methylation.

Although the paper is well written and includes various pieces of valuable descriptive data, there are several comments that may be helpful to improve the manuscript.

1) Methods part in the abstract are overloaded with information. The authors may wish to shorten it substantially providing the global overview of tissues and techniques.

2) As authors point out, the study is based at least partly on the results that have been previously published. It may be helpful to include the clear statement in methods to what extent the data has been previously published (Ref. 6, 7)? Why the selection gives a different set of genes?

3) In the both previous publication (6,7) I was unable to find clinical information regarding the clinical samples which may provide valuable information from the clinical perspective.

4) As the HT29 is a chromosomally instable (CIN) cell line with multiple chromosomal aberrations limiting DNA methylation analyses, do the authors expect different results in other CRC cell lines?

5) One of the shortcomings is the microRNA expression analyses. Unfortunately, the authors use only each 3 samples from each group to evaluate or validate results. The information regarding the used normalizer miR-423-5p may be helpful. The authors may wish to explain why they include the miRNA analyses. Did the authors confirm the array expression analyses for miR-21-3p, miR-181c or let-7i-3p using qPCR? Please correct the labeling in Figure 6?

6) In the present work the authors do not provide mechanistical or functional analyses therefore the current title is misleading. “DNA hypermethylation correlates with decreased mRNA expression...”. The study relates to 4 microRNAs in very limited subset of subjects and may be not scientifically powered for the main message in title.
7) Selection of 18 transcripts differs from previously selected targets from previous publication (7). The authors may wish to include the selection criteria (Table 1).

8) Expression of microRNAs is shown on the Figure 6 with visually reduced expression.

9) Line 250 and 251: does “altering expression” and “gene alternation” refers to down regulation, please, specify. Are those 18 genes show all reduced expression?

10) Figure 1 and Figure 4 are too busy. Because of the great number of data sets, I would recommend to use a table (exp. N, Mean or median +/- SD and p-value) or alternatively heat-map.

11) Could you please provide the information if any expression profiling data were validated by exp. PCR?

12) Description of statistical analyses is partly missing.

**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 know Dr. Molnar personally, however, I have no other competing interests.