ANSWERING REVIEWERS

January 25, 2014

Dear Editor,

Thank you very much for the consideration of our manuscript "DNA methylation of microRNA genes as diagnostic and prognostic biomarkers in HCC". We are very pleased about the overall positive evaluation of our manuscript described as "well organized, written in a clear way, comprehensive, and informative" and appreciate all comments raised by the reviewers, which gave us possibilities to improve our manuscript. We thank for the opportunity to resubmit a revised version addressing all comments from the reviewers and revised the manuscript accordingly. A point by point-reply describing all changes made in detail is included. All changes in the text are highlighted in yellow.

Please find enclosed the edited manuscript in Word format (file name: 8326_revised_23.01.14).
Title: DNA methylation, microRNA and the crosstalk as potential biomarkers in HCC
Author: Sumadi Lukman Anwar and Ulrich Lehmann
Name of Journal: *World Journal of Gastroenterology*
ESPS Manuscript NO: 8326

The manuscript has been improved according to the suggestions of reviewers:
Reveiwer #1
Comment to author:
DNA methylation and microRNA are epigenetic mechanisms that are widely altered in human liver cancer. DNA methylation and microRNA patterns are regulated in developmental stage specific-, cell type specific- and tissue-specific manner. Although they can potentially serve as biomarkers for specific detection as well as for prognosis, monitoring and predicting therapeutic responses in HCC, it need more work to confirmed in future.
No revision is required.

Reviewer #2

1. Major point 1: The title of this paper emphasizes the "methylation of microRNA genes" in HCC, while the main content gives approximately equal attention to these three topics: methylation of genes, expression of micoRNA, as well as the methylation of microRNA. **The title should be modified to cover these topics addressed thoroughly in the manuscript.**
   Done as requested, with new title: DNA methylation, microRNAs, and their crosstalk as potential biomarkers in HCC
2. Major point 2: **Overview about possibility of certain panels of microRNA methylation to define CIMP in HCC that subsequently might have potential clinical relevance for diagnosis, prognosis and prediction of therapeutic responses in HCC**.
   Done as requested. We add one paragraph at page 21 to explain this.
   Our recent study showed that DNA methylation at microRNA genes was a specific event detectable only in malignant liver cells and tissue samples but not in adjacent liver tissue, benign liver tumours, healthy liver cells, or in hepatocyte lines. These results indicated that

methylation of microRNA genes might represent a new biomarker for specific detection of malignant liver tumors. In addition, concordant DNA methylation at certain microRNA loci correlated with poor HCC survival rendering its potential to be used as prognostic marker in HCC. [166] Differential methylation at these loci appears not to be a random event but highly organized during initiation and progression of HCC. In addition, several microRNAs affected by DNA methylation in HCC are suggested to modulate therapeutic responses upon conventional chemotherapy and or treatment with sorafenib. However, using a panel of DNA methylation aberrations in microRNA genes as a new marker for CIMP in HCC is a great challenge for future research. To evaluate this, quantitative DNA methylation analysis using diverse samples of liver diseases including adenoma, chronic hepatitis, cirrhosis, early and late stage of HCC in a setting of retrospective and prospective studies involving multi-center collaboration are needed. Genome-wide DNA methylation analysis will provide extensive information not only for microRNA loci but also other regions potentially important as a marker for CIMP. Correlating methylation status with clinocopathologic and molecular profiles from large HCC cohort will also strengthen the use of CIMP as a new classifier for HCC samples.

3. Minor points 1: In the "Core tip" part, the authors mentioned "A systematic review of literatures revealed that…" Since the "**systematic** review" is a special article type in evidence-based medicine, the term used here might lead into the misunderstanding that the following described conclusions were drew by author's analysis or cited from other publications.
   We replaced '**systematic**' with '**comprehensive**'
4. Minor points 2: **The list of reference could be shorter, if possible**.
   We would like to include all studies that significantly contribute to building concept and clinical utilities of DNA methylation, microRNA, and their crosstalk for new diagnostic and prognostic markers in HCC therefore we prefer to retain citing them.

Reviewer #3

Aside from a few typographical errors that will need to be amended, I would recommend publication of the review without further revision to the content.

A requested the manuscript was carefully controlled again for any typographical errors.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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