Author's response to reviews

Title: Promoter methylation of CDKN2A and the lack of p16 expression characterize patients with hepatocellular carcinoma

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Author's response to reviews: see over
To  
Rachel Neilan, MSc  
Assistant Scientific Editor  
BMC Cancer  
BioMed Central  
Editorial Board

Title: Promoter Methylation of CDKN2A and Lack of p16 Expression Characterize Patients With Hepatocellular Carcinoma.

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Dear Mrs Neilan,

we reconsider to publish our experiences on changes of promoter methylation of CDKN2A gene and expression of p16 in patients with different liver tumors after extensively copyediting the manuscript.

The content of the manuscript is original and it has not been published or accepted for publication, either in whole or in part, in any form. No part of the manuscript is currently under consideration for publication elsewhere. Informed consent was not given because the vast majority of patients had died before our study started. The study was approved by the Ethics Committee of Otto-von-Guericke University of Magdeburg.

With kind regards,
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Referee 1

We could not show any association between p16 expression and CpG island methylation. This is probably due to further mechanisms leading to silencing of CDKN2A gene. Further mechanisms of gene silencing were not investigated in our study. Lack of p16 expression represents a marker of HCC in our study and can differentiate metastatic liver lesions from HCC.
Referee 2

Major comments:

1. Annotations were not recorded.
2. The tables contain all information are needed to understand results and discussion.
3. “Patients and methods” has been completely edited.

Minor comments:

1. Annotations were not recorded.
2. “Mistakes about data” and “carelessness of the author” remain unclear defined and therefore can not be discussed.
3. The references are updated.
Referee 3

1. In this study, two different methods were applied to detect und study methylation of promoter region of p16 gene in patients with liver tumors. Primers used for MSP and MethyLight amplify different sequences containing different CpG islands. Therefore, analysis using each method results in methylation of different CpG islands. In our study, MethyLight analysis using probes and primers defined in the manuscript seems to be specific for HCC compared to MSP. That means that methylated CpG islands detected by MethyLight seem to be markers of HCC and are not present in non-tumor liver tissues. We have not found a clear association between p16 expression and CpG island methylation. This is probably due to further mechanisms leading to inactivation or activation of CDKN2A gene. These mechanisms were, however, not investigated in our study. Lack of p16 expression is the second marker of HCC and can differentiate metastatic liver lesions from HCC. mRNA and Western blot were not used as methods in our experiments. Unfortunately, we do not have further tissue samples from these patients in order to further clarify these issues.

2. The manuscript has been extensively edited