Author's response to reviews

Title: A novel method for detection of HBVcccDNA in hepatocytes using rolling circle amplification combined with in situ PCR

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Author's response to reviews: see over
Dear Editor:

On behalf of all authors, I would like to submit our manuscript entitled “A novel method for detection of HBV cccDNA in hepatocytes using rolling circle amplification combined with in situ PCR” to BMC infectious disease for consideration of publication as an original paper. I state that the manuscript has not been considered for publication elsewhere. I declare that there is no potential competing interest of this article.

HBV cccDNA is the original template for HBV replication. In vitro studies have shown that the persistence of cccDNA is responsible for the recurrence of HBV infection. The detection of cccDNA will help people to develop new antiviral drug against HBV replication links, to reduce the resistance and recurrence, as well as to discover extrahepatic HBV infection.

Although the development of highly selective real-time polymerase chain reaction (PCR) assays has provided sensitive tools to investigate the replicative activity and the effectiveness of antiviral therapy in HBV infected patients by quantification of the cccDNA. However, those methods can not observe the distribution and localization of HBV cccDNA in liver tissues and further analyze
the relationship between cccDNA and pathological characteristics.

So far, the technique for specific and efficient locating detection of HBV cccDNA in situ remains immature, the methodology for detecting HBV cccDNA is suggested being unreliable because existence of non-specific amplification of other HBV DNA form and low sensitivity.

In this study, we developed a new sensitive and specific method for the locating detection of HBV cccDNA in sections from formalin fixed paraffin-embedded (FFPE) liver tissues using RCA combined with in situ polymerase chain reaction (IS-PCR). We investigated 26 patients with HBV infection, including 10 chronic hepatitis B (CHB), 6 liver cirrhosis (LC) and 10 hepatocellular carcinoma (HCC). HBV cccDNA was expressed and located in hepatocyte nucleus in 19 patients (73.07%). Compared with the IS-PCR, introduction of RCA increased the limit of detection. RCA combined with IS-PCR yielded strong positive signals in HCC liver tissue despite of low copy number cccDNA (2 copies of target sequence per cell), whereas no positive signal was detected in negative control. RCA combined with IS-PCR is an effective and practicable method that could detect the presence of low copy number cccDNA sensitively and specifically.

Enclosed please find the text and 2 tables and 4 figures of the
manuscript, which were organized according to requested format. The text contains 2,585 words (not including abstract, acknowledgements, references and tables and figures) and the abstract comprises 300 words. I hope that it fulfills the requirement of BMC infectious disease.

I appreciate very much for your consideration and time.

Best personal regards

Yours sincerely,

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