

Cerebral autoregulation refers to the inherent cerebrovascular physiological mechanisms that keep CBF relatively constant despite wide variation in arterial blood pressure levels. These mechanisms act to protect the brain from the harmful effects of oligoemia and hyperaemia due to decreased and increased perfusion pressure, respectively [2–3]. Loss of CA in FHF can be explained by the dysfunction of cerebral arterioles, leading to vasodilation, associated with metabolic derangement and toxic substances released from the necrotic liver [4], but the actual pathophysiological mechanism remains unknown. Both loss of CA and hyperaemia have been considered important factors for the worsening of hepatic encephalopathy, brain swelling, and unfavourable outcomes. The present case raises the possibility that CBF dynamics and CA capacity can be restored shortly after recovery of liver function [4]. TCD can be a useful method for real-time monitoring of FHF patients in terms of CBF velocity and CA. Improvement of CA can be directly associated with recovery of liver function; for this reason, CA may be a marker of liver function, as suggested by this case. Future studies should determine the dynamic behaviour of CA. Such evaluation can help elucidate the pathophysiology of FHF and enable better therapeutic management of patients.

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No expression of HBV-human chimeric fusion transcript (HBx-LINE1) among Vietnamese patients with HBV-associated hepatocellular carcinoma



Dear Editor,

Hepatitis B virus (HBV) related hepatocellular carcinoma (HCC) is the most common malignancy among males in Vietnam and annually approximately 500,000 new HCC cases are diagnosed worldwide [1]. The rates of progression to liver cirrhosis (LC) in chronic HBV carriers are estimated to be 10% per year [2], and the risk of HCC development in chronic HBV carriers is significantly higher compared to that in uninfected individuals [1]. Chronicity of HBV infection results from persistence of the viral minichromosome (cccDNA) and the integration of HBV DNA into human genome contributing to pathogenesis/carcinogenesis of HBV infection has been widely described [3]. HBx-LINE1 is believed to be a chimeric viral and human long non-coding RNA (lncRNA) that induces tumor formation by promoting Wnt signaling and promotes HCC-related injury by sequestering liver-specific microRNA-122 expression [4–6]. Nevertheless, integration of HBV in the cell genome in HCC is still poorly understood and the exact mode of HBx-LINE1 in HCC development needs to be defined in more detail [7]. Recently, a whole-transcriptome sequencing study utilized HBV-positive HCC cell lines and indicated common transcription of a viral-human chimera in response to HBV genome integration (HBV-human chimeric fusion transcript; HBx-LINE1) [8]. In this study, HBx-LINE1 was described to occur in 23.3% of HBV-associated HCC tumors and the authors also suggested that HBx-LINE1 might be an independent prognostic factor predicting shorter survival of HCC patients [8]. However, a later study conducted in France did not confirm these findings and found complete absence of these fusion transcripts in 50 HBV-associated HCC patients [9]. The reasons for such a discrepancy in detecting HBx-LINE1 chimeric transcripts remain to be explained.

Vietnam has a high prevalence of hepatitis B and 8.6 million people are reported to be HBsAg positive. An estimated 8.8% of women and 12.3% of men are chronically infected with HBV [10]. In recent years, there is a growing incidence of HBV-associated HCC and increasing mortality [10]. In the context of the growing incidence of HBV-related HCC mortality, we tried to determine if HBx-LINE1 might be used as a prognostic marker for HCC.

Fresh HCC tumor tissue and matched serum samples from 119 unrelated Vietnamese HBV-infected patients with HCC in the 108 Military Central Hospital, Hanoi, Vietnam, between 2014 and 2016 were screened for the presence of the HBx-LINE1 transcript. Patients were characterized based upon pathohistological findings and clinical and laboratory manifestations (hepatomegaly, splenomegaly, hyperbilirubinemia, elevated levels of aspartate aminotransferase (AST) and alanine transaminase (ALT), HBV-specific serology, alpha-fetoprotein). All HCC patients were positive for HBsAg and negative for antibodies against HCV and HIV. Blood samples were collected from all HCC patients and serum was immediately separated and stored at –70 °C until further analyses.

In brief, total RNA was isolated from 200 µl serum and from matched dyads of liver biopsy tissues with Trizol reagent (Life Technologies). 500 ng of total RNA were used for reverse transcription (RT) by RevertAid First Strand cDNA Synthesis Kit (ThermoFisher

Scientific Inc., Singapore). Reverse-transcribed cDNA was subsequently amplified by hemi-nested PCR using the primer pairs as described by Lau et al. [8]. A synthesized plasmid sequence as described containing the fusion HBx-LINE1 transcript was utilized as positive control [8]. The GAPDH cDNA gene transcript (496 bp) was used as an internal control. cDNA was amplified using a first set of primer pairs (HBx-LINE1-1F: 5'-GGACTCTACCGTCCCTTCT-3'; HBx-LINE1-1R: 5'-AGTAGGGGACTGCTGGATCA-3'). Subsequently, the first-round PCR products were utilized as templates for the hemi-nested PCR, which, compared to the first round, utilizes a different forward primer (HBx-LINE1-2F: 5'-CCGTCTGTGCCTTCTCATCT-3') and a similar reverse primer (HBx-LINE1-1R: 5'-AGTAGGGGACTGCTGGATCA-3'). In both PCR reactions, the annealing temperatures were 60°C and products were amplified in 30 cycles. The first and second round of PCR should yield amplicons of 283 bp and 211 bp, respectively. PCR products were analyzed on 1.5% agarose gel together with the positive plasmid control.

In the 119 tumor and matched serum samples, HBx-Line1 transcripts were not detected in any of the samples examined. The positive control remained positive and the negative control was negative in all assays. HBx-LINE1, a chimerical transcript recently identified in a Chinese population of HBV-related HCC was claimed to be associated with tumor formation and poor patient survival [8]. While ethnic variation might in part account for such a discrepancy, we, at this moment, must definitely conclude that, based on our results and the previous study of Amaddeo et al. [9], any usefulness of the HBx-Line1 transcript as a prognostic biomarker must clearly be rejected. Further studies are warranted to assess any prognostic potential of HBx-Line1 in HBV-associated HCC development.

Ethics approval and consent to participate

The study was approved by the institutional review board and an Independent Ethics Committee of the 108 Military Central Hospital, Hanoi, Vietnam. Informed written consent was obtained from all study patients.

Consent to publish

All authors read the manuscript and have provided their consent to publish.

Availability of data and materials

Data and supporting materials associated with this study will be shared upon request.

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Conflict of interest

All authors declare no conflict of interests in this study.

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