Title: HBx protein induces HpSC-like features in hepatocellular carcinoma by activating KDM5B

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1 What did this study explore?

We explored the role of epigenetic regulation of hepatocellular carcinoma by assuming that multifunctional protein HBx may affect epigenetic regulation of Hepatocellular carcinoma. We showed that HBx activated the histone demethylase KDM5B and induced HPC-like features in HCC in this study.

2 How did the authors perform all experiments?

The experiments were performed according to the manufacturer's instructions. All in vitro experiments were performed in triplicate wells for each condition and repeated at least twice with similar results.

3 How did the authors process all experimental data?

Mann-Whitney, $\chi 2$, Fisher's exact, and Kruskal-Wallis tests were used to compare the clinicopathologic characteristics and gene expression data. The correlation of the gene expression data was evaluated by Spearman's rank correlation coefficient. Kaplan-Meier survival analysis with the log-rank test was performed to compare patients' survival. All analyses were performed using GraphPad Prism software 5.0.1 (GraphPad Software, San Diego, CA).

4 How did the authors deal with the pre-study hypothesis?

Wang X, Oishi N and Shimakami T designed the study and contributed to acquisition of data; Wang X, Oishi N, and Yamashita T contributed to analysis and interpretation of data; Wang X and Oishi N contributed to drafting of the manuscript; Honda M, Murakami S, and Kaneko S contributed to critical revision of the manuscript for important intellectual content; Wang X and Oishi N contributed to statistical analysis; Murakami S and Kaneko S are the guarantors of this study.

5 What are the novel findings of this study?

Our data show that KDM5B, which is activated by HBx, is a useful marker for poor prognosis in HBV-related HCC cases. Moreover, we demonstrated the possibility that suppression of KDM5B may improve the poor phenotype of HBV-related HCCs. Our new knowledge is useful for the diagnosis of severe HCC patients.