

World Journal of *Gastroenterology*

World J Gastroenterol 2017 May 14; 23(18): 3195-3378



**EDITORIAL**

- 3195** Liver transplantation for intermediate hepatocellular carcinoma: An adaptive approach

Biolato M, Marrone G, Miele L, Gasbarrini A, Grieco A

- 3205** Immune response to vaccines in children with celiac disease

Anania C, Olivero F, Spagnolo A, Chiesa C, Pacifico L

REVIEW

- 3214** Inflammatory bowel disease in liver transplanted patients

Filipek Kanizaj T, Mijic M

- 3228** Platelets in liver disease, cancer and regeneration

Kurokawa T, Ohkohchi N

ORIGINAL ARTICLE**Basic Study**

- 3240** Thiopurine use associated with reduced B and natural killer cells in inflammatory bowel disease

Lord JD, Shows DM

- 3252** Hepatitis B virus X protein induces hepatic stem cell-like features in hepatocellular carcinoma by activating KDM5B

Wang X, Oishi N, Shimakami T, Yamashita T, Honda M, Murakami S, Kaneko S

- 3262** Artificial liver support in pigs with acetaminophen-induced acute liver failure

He GL, Feng L, Cai L, Zhou CJ, Cheng Y, Jiang ZS, Pan MX, Gao Y

- 3269** Effects of sleeve gastrectomy plus trunk vagotomy compared with sleeve gastrectomy on glucose metabolism in diabetic rats

Liu T, Zhong MW, Liu Y, Huang X, Cheng YG, Wang KX, Liu SZ, Hu SY

- 3279** Wall shear stress in portal vein of cirrhotic patients with portal hypertension

Wei W, Pu YS, Wang XK, Jiang A, Zhou R, Li Y, Zhang QJ, Wei YJ, Chen B, Li ZF

Case Control Study

- 3287** Risk of progression of Barrett's esophagus in patients with cirrhosis

Apfel T, Lopez R, Sanaka MR, Thota PN

Retrospective Study

- 3295 Clinical significance of hypoechoic submandibular gland lesions in type 1 autoimmune pancreatitis
Takano S, Fukasawa M, Kadokura M, Shindo H, Takahashi E, Hirose S, Fukasawa Y, Kawakami S, Sato T, Enomoto N
- 3301 Benefit of neoadjuvant concurrent chemoradiotherapy for locally advanced perihilar cholangiocarcinoma
Jung JH, Lee HJ, Lee HS, Jo JH, Cho IR, Chung MJ, Park JY, Park SW, Song SY, Bang S
- 3309 Ling classification describes endoscopic progressive process of achalasia and successful peroral endoscopy myotomy prevents endoscopic progression of achalasia
Zhang WG, Linghu EQ, Chai NL, Li HK

Observational Study

- 3315 Disruptive behavior in the workplace: Challenges for gastroenterology fellows
Srisarajivakul N, Lucero C, Wang XJ, Poles M, Gillespie C, Zabara S, Weinshel E, Malter L
- 3322 Correlation of endoscopic disease severity with pediatric ulcerative colitis activity index score in children and young adults with ulcerative colitis
Kerur B, Litman HJ, Stern JB, Weber S, Lightdale JR, Rufo PA, Bousvaros A
- 3330 Stress and sleep quality in doctors working on-call shifts are associated with functional gastrointestinal disorders
Lim SK, Yoo SJ, Koo DL, Park CA, Ryu HJ, Jung YJ, Jeong JB, Kim BG, Lee KL, Koh SJ

Prospective Study

- 3338 *In vivo* and *ex vivo* confocal endomicroscopy of pancreatic cystic lesions: A prospective study
Krishna SG, Modi RM, Kamboj AK, Swanson BJ, Hart PA, Dillhoff ME, Manilchuk A, Schmidt CR, Conwell DL
- 3349 Chronological age when healthcare transition skills are mastered in adolescents/young adults with inflammatory bowel disease
Stollon N, Zhong Y, Ferris M, Bhansali S, Pitts B, Rak E, Kelly M, Kim S, van Tilburg MAL

Randomized Controlled Trial

- 3356 Low-FODMAP diet reduces irritable bowel symptoms in patients with inflammatory bowel disease
Pedersen N, Ankersen DV, Felding M, Wachmann H, Végh Z, Molzen L, Burisch J, Andersen JR, Munkholm P

EVIDENCE-BASED MEDICINE

- 3367 Antimicrobial susceptibility testing before first-line treatment for *Helicobacter pylori* infection in patients with dual or triple antibiotic resistance
Cosme A, Montes M, Ibarra B, Tamayo E, Alonso H, Mendarte U, Lizasoan J, Herreros-Villanueva M, Bujanda L

CASE REPORT

- 3374** Severe esophageal injury after radiofrequency ablation - a deadly complication

Katz-Agranov N, Nevah Rubin MI

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Ballarin Roberto, PhD, Assistant Professor, Doctor, Surgeon, Hepatobiliopancreatic Oncologic Surgery and Liver Transplant Center, University of Modena, Modena 41100, Italy

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1375 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*WJG*) is now indexed in Current Contents[®]/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch[®]), Journal Citation Reports[®], Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. The 2015 edition of Journal Citation Reports[®] released by Thomson Reuters (ISI) cites the 2015 impact factor for *WJG* as 2.787 (5-year impact factor: 2.848), ranking *WJG* as 38 among 78 journals in gastroenterology and hepatology (quartile in category Q2).

FLYLEAF

I-IX Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Cui-Hong Wang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Yuan Qi*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

Stephen C Strom, PhD, Professor, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach,

CA 90822, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

EDITORIAL OFFICE
Jin-Lei Wang, Director
Yuan Qi, Vice Director
Ze-Mao Gong, Vice Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>

<http://www.wjgnet.com>

PUBLICATION DATE
May 14, 2017

COPYRIGHT
© 2017 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Basic Study

Hepatitis B virus X protein induces hepatic stem cell-like features in hepatocellular carcinoma by activating KDM5B

Xuyang Wang, Naoki Oishi, Tetsuro Shimakami, Taro Yamashita, Masao Honda, Seishi Murakami, Shuichi Kaneko

Xuyang Wang, Naoki Oishi, Tetsuro Shimakami, Taro Yamashita, Masao Honda, Seishi Murakami, Shuichi Kaneko, Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Ishikawa, Japan

Naoki Oishi, Tetsuro Shimakami, Taro Yamashita, Masao Honda, Shuichi Kaneko, Department of Gastroenterology, Kanazawa University Hospital, Kanazawa 920-8641, Ishikawa, Japan

Author contributions: 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.

Supported by Grant-in-Aid for Scientific Research (KAKENHI) (C), No. 15K08992 (to Oishi N); and Core-to-Core Program, B. Asia-Africa Science Platforms, the Japan Society for the Promotion of Science (to Kaneko S).

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Naoki Oishi, MD, PhD, Researcher, Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Ishikawa, Japan. ooishi@m-kanazawa.jp
Telephone: +81-76-2652233
Fax: +81-76-2344250

Received: December 31, 2016
Peer-review started: January 3, 2017
First decision: February 9, 2017
Revised: February 28, 2017
Accepted: March 30, 2017
Article in press: March 30, 2017
Published online: May 14, 2017

Abstract

AIM

To determine the role of hepatitis B virus X protein (HBx), HBx in regulating hepatic progenitor cell (HPC)-like features in hepatocellular carcinoma (HCC) and the underlying molecular mechanisms.

METHODS

We used a retrovirus vector to introduce wild type HBx or empty vector into HepG2 cells. We then used these cells to analyze cell proliferation, senescence, transformation, and stem-like features. Gene expression profiling was carried out on Affymetrix GeneChip Human U133A2.0 ver.2 arrays according to the manufacturer's protocol. Unsupervised hierarchical clustering analysis and Class Comparison analysis were performed by BRB-Array Tools software Version 4.2.2. A total of 238 hepatitis B virus (HBV)-related HCC patients' array data were used for analyzing clinical features.

RESULTS

The histone demethylase KDM5B was significantly

highly expressed in HBV-related HCC cases ($P < 0.01$). In HBV proteins, only HBx up-regulated KDM5B by activating c-myc. Hepatic stem cell (HpSC) markers (EpCAM, AFP, PROM1, and NANOG) were significantly highly expressed in KDM5B-high HCC cases ($P < 0.01$). KDM5B played an important role in maintaining HpSC-like features and was associated with a poor prognosis. Moreover, inhibition of KDM5B suppressed spheroid formation and cell invasion *in vitro*.

CONCLUSION

HBx activates the histone demethylase KDM5B and induces HPC-like features in HCC. Histone demethylases KDM5B may be an important therapeutic target against HBV-related HCC cases.

Key words: Hepatitis B virus X protein; Hepatocellular carcinoma; KDM5B; Progenitor cell; Tumorigenesis

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The role of epigenetic regulation in cancer biology has been the subject of several studies. These chromatin structure modifiers have been increasingly shown to facilitate several steps of cancer progression. However, the epigenetic regulation of hepatocellular carcinoma has not been elucidated. We assumed that multifunctional protein hepatitis B virus X (HBx) protein, may affect epigenetic regulation of Hepatocellular carcinoma. We showed that HBx activated the histone demethylase KDM5B and induced HPC-like features in hepatocellular carcinoma (HCC) in this study. Our results suggested that histone demethylases may be an important therapeutic target against HBV-related HCC cases.

Wang X, Oishi N, Shimakami T, Yamashita T, Honda M, Murakami S, Kaneko S. Hepatitis B virus X protein induces hepatic stem cell-like features in hepatocellular carcinoma by activating KDM5B. *World J Gastroenterol* 2017; 23(18): 3252-3261 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i18/3252.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i18.3252>

INTRODUCTION

Chronic infection by hepatitis B virus (HBV) affects approximately 400 million people worldwide and is a leading cause of hepatocellular carcinoma (HCC), which is considered the sixth most common cancer in the world^[1,2].

HBV encodes the nonstructural hepatitis B virus X (HBx) protein, which is conserved among mammalian hepadnaviruses, suggesting an important function. HBx is the most critical protein for viral replication in hepatocytes and for the development of HCC. HBx is a multifunctional protein that has been shown to regulate

many transcription factors such as nuclear factor-kappa B, ATF-2, activator protein 1, cAMP response element binding protein^[3], Ras-Raf mitogen-activated protein kinase, extracellular signal-regulated kinase, phosphoinositol-3-kinase-protein kinase B/Akt^[4], and Wnt/beta-catenin pathway^[5]. These pathways are involved in a range of cellular functions including apoptosis, cell proliferation, cell cycle progression, and cytokine production. Therefore, it is proposed that HBx manipulates these cellular signaling pathways, resulting in the transformation of HBV-infected hepatocytes. Although extensive studies have focused on the roles of HBx in malignant transformation^[5-10], the molecular mechanisms underlying this process are not well elucidated.

KDM5B, which is also known as JARID1B or PLU1, is an H3K4me3 histone demethylase that is overexpressed in many types of cancer including breast^[11], prostate^[12], bladder^[13], and lung cancer^[14]. Histone modifiers, such as KDM5B, and their associated post-translational modifications are thought to be central to the determination of embryonic stem cell fate^[15,16]. Embryonic stem cell pluripotency and differentiation depend on the interactions between an array of chromatin modifiers and several downstream transcription factors. This stem cell epigenetic landscape has been implicated in tumor progression and chemoresistance.

The central hypothesis we explored here is that KDM5B maintains cancer stem cells in HCC.

MATERIALS AND METHODS

Human subjects

For gene expression analysis, we assessed three already published cohorts. Cohort 1 was 139 caucasian HCC cases. KDM5B expression data were derived from these specimens as described. Survival data linking to this cohort were kindly provided by Dr. Snorri Thorgeirsson at NCI^[17]. Cohort 2 was 238 cases who received surgical resection of HBV-related HCC at the Liver Cancer Institute of Fudan University. Their clinicopathologic characteristics and prognostic data were kindly provided by Dr. Xin Wei Wang at NCI^[16]. Cohort 3 was 89 Japanese HCC cases who received surgical resection at the Kanazawa University Hospital^[18].

Cell lines

Human HCC cell lines (HepG2, Huh7 and Hep3B) were maintained in Dulbecco's modified Eagle's medium (Gibco BRL, Gaithersburg, MD) containing 10% fetal bovine serum and 1% penicillin-streptomycin. We used two immortalized human hepatocyte cell lines, TTNT16 cells (in which hTERT was introduced)^[19,20] and T5B cells [in which the SV40 large T antigen (LT) was introduced]^[21,22]. These cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco BRL, Gaithersburg, MD) containing 10% fetal bovine serum

Table 1 Real-time reverse-transcription polymerase chain reaction primers and probes designed and used in the experiments

PC-C (core)	FW	5'	CGAGGCAGGTCCCCTAGAA	3'
	RV	5'	CGGCGATTGAGACCTTCGT	3'
	probe	5'-FAM	AGAACTCCCTCGCCTCG	MGB-3'
PS-S (surface)	FW	5'	ACCCCAACAAGGATCATTGG	3'
	RV	5'	CGAATGCTCCCGCTCCTA	3'
	probe	5'-FAM	CAGAGGCAAATCAG	MGB-3'
HBx	FW	5'	TGTCAACGACCGACCTTGAG	3'
	RV	5'	CCCAACTCCTCCAGTCCTT	3'
	probe	5'-FAM	CATACTTCAAAGACTGTTTGT	MGB-3'
Pol (polymerase)	FW	5'	TCCGCTGCCGATCCAT	3'
	RV	5'	GCTGCGAGCAAAACAAGCT	3'
	probe	5'-FAM	CTGCGGAACCTCT	MGB-3'
Pg RNA (Pregenomic RNA)	FW	5'	GCTCTGTATCGGGAGGCCTTA	3'
	RV	5'	TGAGTGCTGTATGGTGAGGAGAA	3'
	probe	5'-FAM	AGTCTCCGGAACATT	MGB-3'

Primers and probes for hepatitis B virus (HBV) type C.

and 1% penicillin-streptomycin.

Real-time reverse-transcription polymerase chain reaction analysis

Total RNA was subjected to quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR). mRNAs were analyzed using TaqMan Gene Expression Assays in accordance with the manufacturer's instructions (Applied Biosystems, Foster City, CA). All RT reactions were run in a GeneAmp PCR 9700 Thermocycler (Applied Biosystems). Probes used for the analyses were as follows: KDM5B, Hs00981910_m1; MYC, Hs00905030_m1 (Applied Biosystems), HBV proteins, shown in Table 1. The experiments were performed in triplicate. The TaqMan gene assay for actin was used to normalize the relative abundance of mRNA.

RNA interference

A small interfering RNA (siRNA) specific to KDM5B and a control siRNA were obtained from Nippon Gene. siRNA specific for c-Myc and a control siRNA were obtained from Invitrogen. Transfection was performed with Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions.

Invasion and cell migration assays

Cell migration and invasion were assessed using a Cytoselect 24-Well Cell Migration and Invasion Assay (CELL BIOLABS). For migration and invasion measurements, transfected Huh7 and Hep3B cells were seeded at 5.0×10^5 cells/well. The cells that invaded or migrated to the underside of the chamber were harvested and measured according to the manufacturer's protocol. Results were determined from triplicate wells in three independent experiments and expressed as a percentage relative to control.

Spheroid formation assays

For spheroid formation assays, single cell suspensions

of 2.0×10^3 cells were seeded in 6-well Ultra-Low Attachment Microplates (Corning, Corning, NY). The number of spheroids was measured at 10-14 d after seeding. Invasion assays were performed using BD BioCoat™ Matrigel Matrix Cell Culture Inserts and Control Inserts (BD Biosciences, San Jose, CA).

Immunohistochemistry analyses

Immunohistochemistry (IHC) was performed using Envision+ kits according to the manufacturer's instructions. An anti-KDM5B monoclonal antibody was used for detecting KDM5B. The staining area and intensities were evaluated in each sample and graded from 0-3 (0, 0-5%; 1, 5%-25%; 2, 25%-50%; 3, > 50%) and 0-2 (0, negative; 1, weak; 2, strong), respectively. The sum of the area and intensity scores of each marker (IHC score) were calculated. Samples were defined as marker-positive (IHC score ≥ 3) or -negative (IHC score ≤ 2).

Statistical analysis

Mann-Whitney, χ^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).

RESULTS

KDM5B expression in HBV-related HCC

We performed transcriptomic analysis of 139 retrospectively collected HCC (cohort 1) patients to assess whether KDM5B was associated with hepatocarcinogenesis. We evaluated and compared the expression of KDM5B in HCC cases developed from various backgrounds: chronic hepatitis B, chronic hepatitis

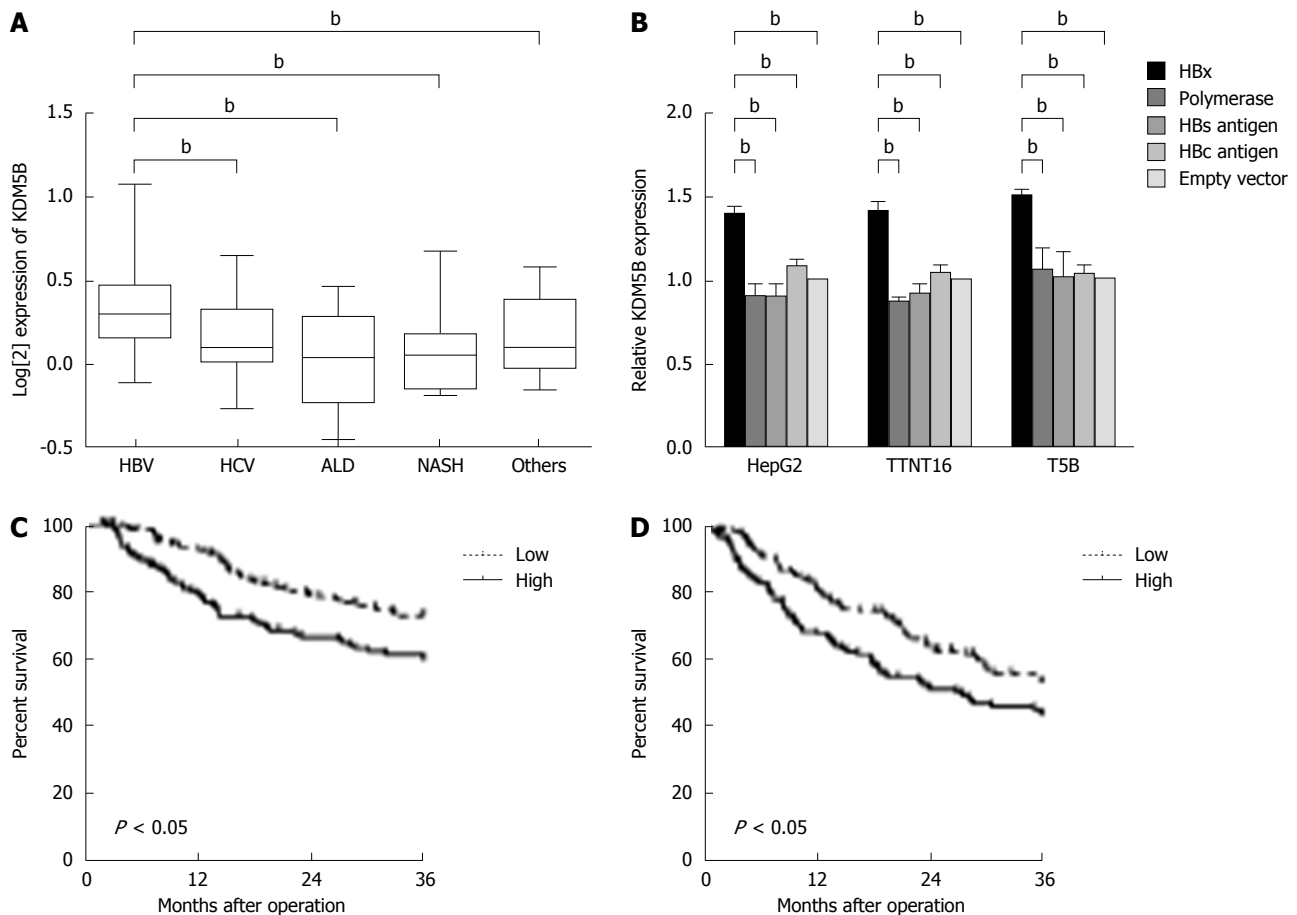


Figure 1 Heterogeneity of KDM5B expression in various hepatocellular carcinoma cases. **A:** Expression analysis of KDM5B-based quantitative RT-PCR data in 139 HCC samples. The horizontal lines in the boxplots represent the median, the boxes represent the interquartile range, and the whiskers represent the 10th and 90th percentiles. A nonparametric test was used to compare the 5 patient groups of hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease (ALD), nonalcoholic steatohepatitis (NASH) and others respectively as indicated by *P* values; **B:** Expression of KDM5B gene in HepG2, TTNT16, and T5B cells. HBV proteins were introduced into these cells by a retroviral system; **C:** Kaplan-Meier plot of the overall survival of 238 hepatocellular carcinoma (HCC) cases based on their classification by KDM5B expression; **D:** Kaplan-Meier plot of recurrence-free survival of HCC cases based on their classification by KDM5B expression.

C, alcoholic liver disease, and nonalcoholic steatohepatitis. As shown in Figure 1A, KDM5B was significantly highly expressed in HBV-related HCC cases compared to other etiological-related HCC cases. Moreover, for HBV proteins, only HBx up-regulated KDM5B in HepG2 cells and immortalized human hepatocytes (TTNT16 and T5b cells) (Figure 1B). These results indicated that HBx was associated with HBV-related hepatocarcinogenesis through the activation of KDM5B.

To understand the effect of KDM5B on the survival of HBV-related HCC cases, we analyzed the microarray data of 238 HCC cases (cohort 2). Kaplan-Meier survival analysis revealed that the KDM5B high expression group had a significantly shorter overall survival ($P < 0.05$) and recurrence-free survival ($P < 0.05$) than the KDM5B low expression group (Figure 1C and 1D).

KDM5B regulates hepatic stem cell-like features in HCC

Previously, we identified two HCC subgroups, one resembling the gene expression signatures of hepatic stem cells (HpSCs) (referred to as HpSC-HCC) and the other similar to mature hepatocytes (referred to

as MH-HCC). HpSC-HCC had stem cell-like features and a poorer prognosis than MH-HCC cases. We hypothesized that KDM5B induced hepatic progenitor-like features in HBV-related HCC cases.

To determine whether KDM5B was functionally linked to the HpSC-like phenotype, we analyzed representative expression levels of HpSC markers in cohort 2 HCC cases. Consistently, HpSC markers, such as EpCAM, AFP, PROM1, and NANOG, were more abundantly expressed in KDM5B high expression HCC cases as compared to KDM5B low expression cases (Figure 2A). Moreover, KDM5B levels were positively correlated with EpCAM or AFP levels in 238 HCC cases (Figure 2B and C).

Next, to confirm that HBx upregulated KDM5B and HpSC markers in HCC cells, we introduced HBx-wt in HepG2 and Huh7 cells and evaluate the expression of KDM5B and HpSC markers. Introduction of HBx up-regulated the expression of KDM5B, EpCAM, AFP, PROM1 and NANOG in these cells (Figure 3A and B).

In addition, inhibition of KDM5B suppressed cell invasion (Figure 4A and B) and spheroid formation

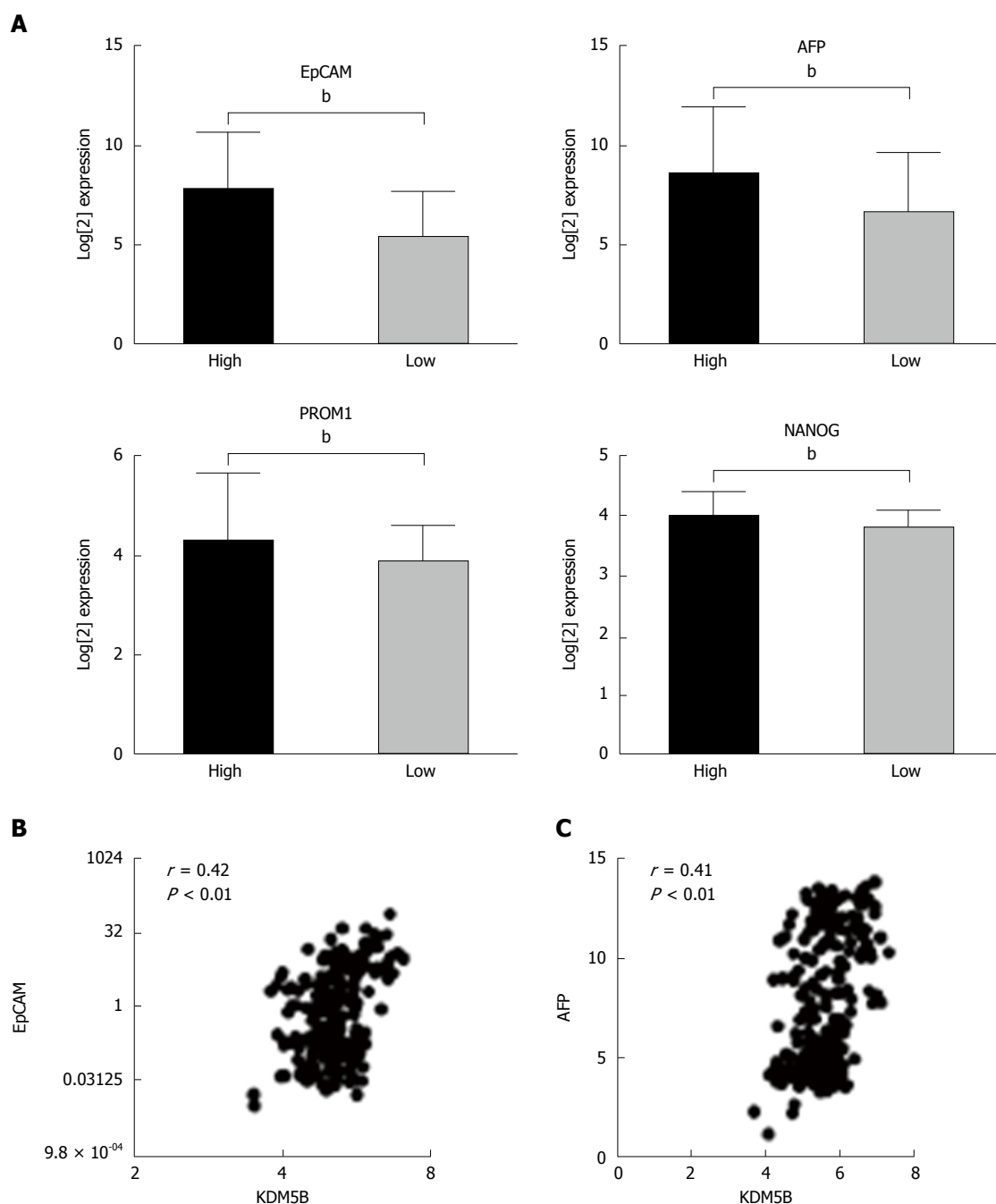


Figure 2 KDM5B is associated with HpSC markers in hepatocellular carcinoma cases. A: Expression analysis of HpSC markers (EpCAM, AFP, PROM1, and NANOG) in cohort 2 cases based on their classification by KDM5B expression; B: Spearman correlation analysis of KDM5B and EpCAM expression data as determined by mRNA arrays of cohort 2 cases; C: Spearman correlation analysis of KDM5B and AFP expression in 238 HCC cases.

(Figure 4C) in Hep3B and Huh7 cells. However, KDM5B did not affect cell proliferation or apoptosis in these cells as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide and transferase-mediated dUTP nick-end labeling assays (data not shown). These data indicated that KDM5B is an important molecule for maintaining HpSC-like features in HBV-related HCC cases.

The available Chip-Seq data (<http://genome.ucsc.edu/>) revealed that several transcriptional factors, such as c-Myc and TCF, preferentially bound to the immediate 5' upstream sequence of the predictive transcription

initiation site of KDM5B. Moreover, c-Myc is one of the transcription factors known to be activated by HBx. To determine whether c-Myc regulates KDM5B expression, we silenced c-Myc expression with a c-Myc-specific siRNA in Hep3B and Huh7 cells. Consistently, we found that inhibition of c-Myc resulted in the suppression of KDM5B and EpCAM expression (Figure 4D-F).

KDM5B predicts poor prognosis in HCC cases

We performed IHC analysis of cohort 3 cases. We confirmed the nuclear accumulation of KDM5B stained by an anti-KDM5B antibody (Figure 5A). After

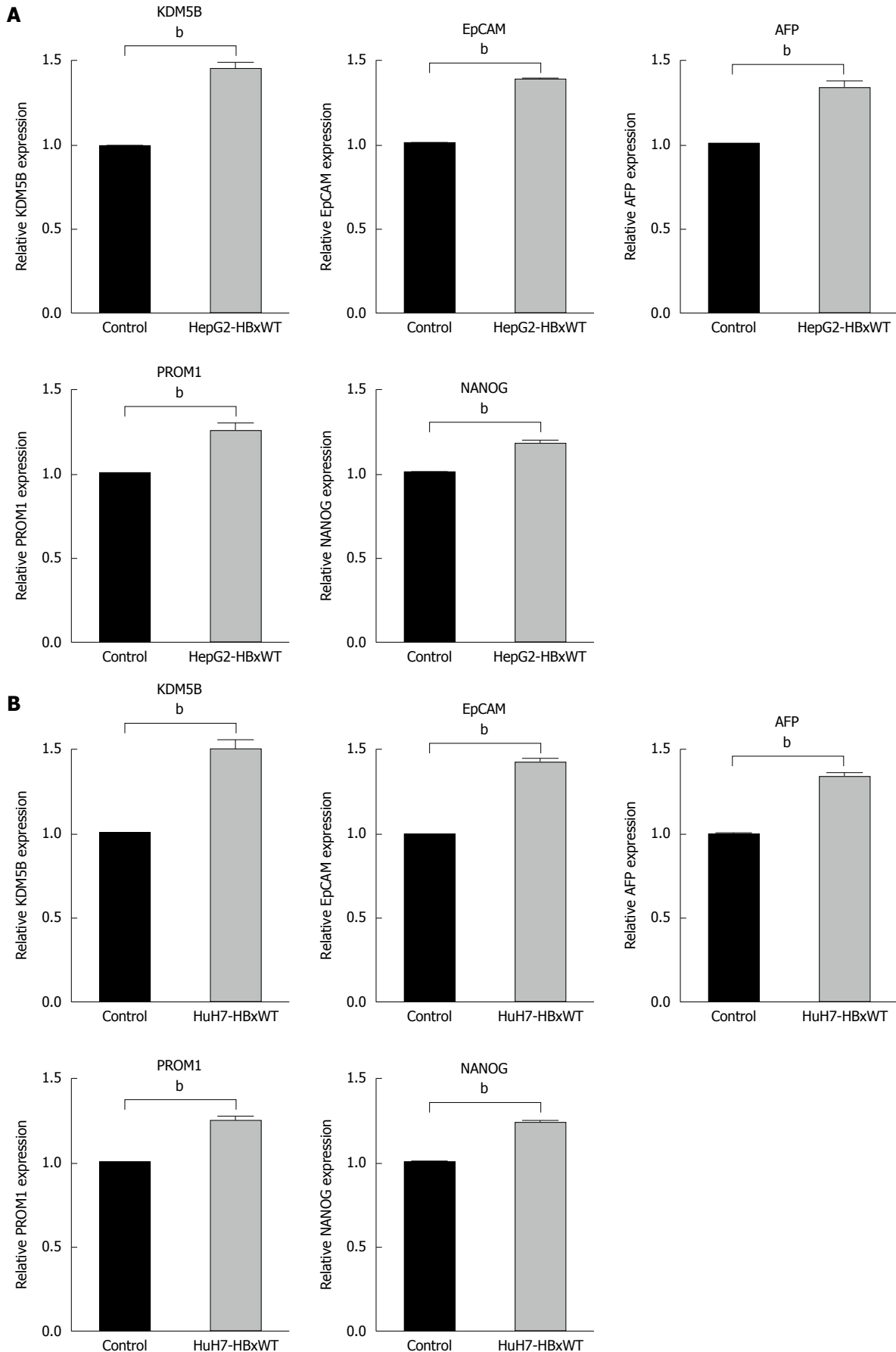


Figure 3 Introduction of hepatitis B virus X protein is associated with KDM5B and HpSC markers expression. A: Expression analysis of KDM5B and HpSC markers (EpCAM, AFP, PROM1, and NANOG) in HepG2 cells; B: Expression analysis of KDM 5B and HpSC markers in Huh7 cells.

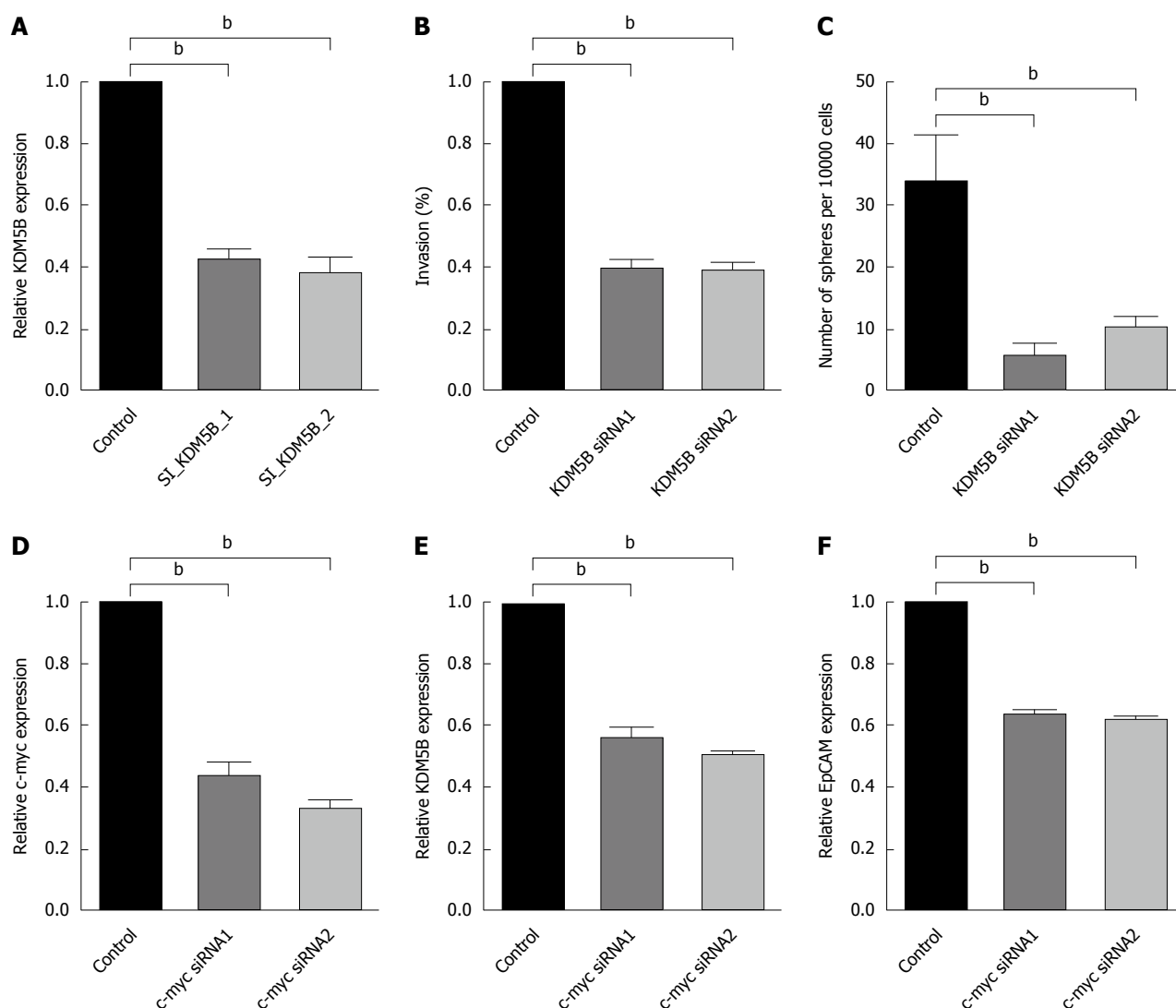


Figure 4 Inactivation of KDM5B is associated with stem-like features. Effect of KDM5B siRNA on KDM5B expression (A); Cell invasion of Huh7 cells transduced with KDM5B siRNA as determined by the Boyden chamber cell invasion assay (B); Spheroid formation of Huh7 cells transduced with KDM5B siRNA. A vertical axis indicates a total number of spheroids from 1000 cells (C); c-Myc mediated silencing of KDM5B and HsSC markers (D). Effect of c-Myc siRNA on the endogenous levels of c-Myc (D), KDM5B (E), and EpCAM (F) in Huh7 cells.

evaluating the clinicopathological characteristics of EpCAM-positive and -negative HCC cases, we found that EpCAM-positive HCCs were associated with a significantly high frequency of KDM5B-positive cases (Figure 5B). We further identified the survival outcome of these cases by Kaplan-Meier survival analysis. KDM5B-positive HCCs were associated with significantly lower overall survival outcomes within three years compared with KDM5B-negative HCCs ($P < 0.01$) (Figure 5C).

DISCUSSION

HBV is the smallest human hepatotropic DNA virus, which mainly infects host hepatocytes and causes a spectrum of pathological processes from acute hepatitis and chronic hepatitis, to serious end-stage liver diseases such as hepatic cirrhosis and primary HCC. Studies of its epidemiology and natural history

have shown that approximately 25% of chronic hepatitis B patients will develop HCC^[23,24]. Although the pathogenesis of HBV-related HCC has not been identified, many studies have suggested that HBx is one of the risk factors and is strongly implicated in hepatocarcinogenesis.

The role of epigenetic regulation in cancer biology, especially that of the histone lysine demethylases (KDMs), has been the subject of several studies^[25-27]. These chromatin structure modifiers have been increasingly shown to facilitate several steps of cancer progression^[28-30]. Several KDMs have been implicated in tumor growth, angiogenesis, invasion, metastasis, and tumor-related chemoresistance. KDM5B specifically removes methyl residues from methylated lysine 4 of histone 3, consequently repressing gene transcription. The cancer stem-like cell (CSC) hypothesis has drawn much attention^[31]. CSCs possess stem cell characteristics, including self-

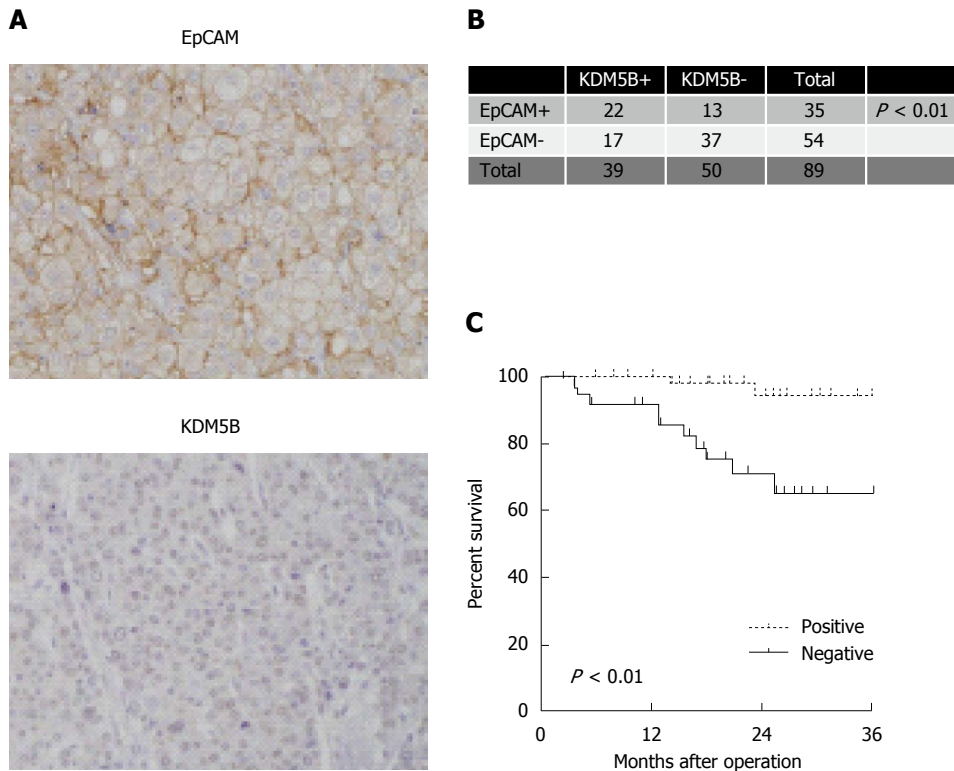


Figure 5 KDM5B protein expression is associated with EpCAM protein expression and poor prognosis. A: Photographs of immunohistochemistry staining with anti-KDM5B and EpCAM antibodies in 89 Japanese hepatocellular carcinoma (HCC) cases; B: Summary of KDM5B and EpCAM expression; C: Kaplan-Meier plot of overall survival of cohort 3 cases based on their classification by KDM5B expression.

renewal, chemotherapy resistance, and metastasis. On the basis of the role of KDM5B in cancer stem-like features, we hypothesized that KDM5B is actively involved in the poor prognosis of HCC patients.

In this study, we showed that KDM5B were associated with poor prognosis of HBV-related HCC. In various HBV proteins, HBx strongly up-regulated KDM5B expression in HBV-related HCC cases. Moreover, KDM5B expression is associated with the increased presence of CSC features, including enhanced tumor sphere formation, cell migration, and invasion, and is related with poor prognosis. The administration of a KDM5B inhibitor suppressed HpSC-like features in HBV-related cases. Therefore, we consider that KDM5B could be a potential therapeutic target against HBV-related HCC. Many researchers reported that HBx-MYC interaction was associated with HBV-related hepatocarcinogenesis^[32-34]. However, its mechanism is not yet clear. Our results indicated that KDM5B was a central molecule of HBx-MYC introduced hepatocarcinogenesis.

Recently, Wang *et al.*^[35] reported that KDM5B affected the poor prognosis of HCC cases through regulating p15 and p27. Similarly, in the present study, KDM5B was related to the poor prognosis of HCC patients. 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. Histone demethylases KDM5B may be an important therapeutic target against HBV-related HCC cases.

In conclusion, HBx induced HpSC-like features in hepatocellular carcinoma by activating KDM5B. Moreover, HBx and KDM5B interaction related poor prognosis of hepatocellular carcinoma cases.

ACKNOWLEDGMENTS

We thank Drs. Xin Wei Wang and Snorri Thorgeirsson at NCI for clinical data of HCC cases. We also thank Dr. Hikari Okada and Takayoshi Shirasaki for help on cell analysis.

COMMENTS

Background

The role of epigenetic regulation in cancer biology has been the subject of several studies. These chromatin structure modifiers have been increasingly shown to facilitate several steps of cancer progression. However, the epigenetic regulation of hepatocellular carcinoma has not been elucidated.

Research frontiers

Previous study showed that KDM5B affected the poor prognosis of hepatocellular carcinoma (HCC) cases through regulating p15 and p27. However, the relation between HCC background and KDM5B has not yet clear.

Innovations and breakthroughs

This is the first study evaluating that KDM5B, which is activated by HBx, a

useful marker for poor prognosis in HBV-related HCC cases. Moreover, the authors demonstrated the possibility that suppression of KDM5B may improve the poor phenotype of HBV-related HCCs.

Applications

HBx activated the histone demethylase KDM5B and induced hepatic progenitor cell (HPC)-like features in HCC. Presented results suggested that histone demethylases may be an important therapeutic target against HBV-related HCC cases.

Peer-review

Authors demonstrated that HBx activates the histone demethylase KDM5B and induces HPC-like features in HCC. This is the first study evaluating that KDM5B, which is activated by HBx, a useful marker for poor prognosis in HBV-related HCC cases. Moreover, they demonstrated the possibility that suppression of KDM5B may improve the poor phenotype of HBV-related HCCs.

REFERENCES

- Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]
- Hirata A**, Hirata T, Takahashi Y, Nakayama T. Surveillance rates for hepatocellular carcinoma among patients with cirrhosis, chronic hepatitis B, and chronic hepatitis C based on Japanese claims database. *Hepatol Res* 2017; **47**: 283-292 [PMID: 27027417 DOI: 10.1111/hepr.12714]
- Zhang X**, Zhang H, Ye L. Effects of hepatitis B virus X protein on the development of liver cancer. *J Lab Clin Med* 2006; **147**: 58-66 [PMID: 16459163 DOI: 10.1016/j.lab.2005.10.003]
- Matsuda Y**, Ichida T. Impact of hepatitis B virus X protein on the DNA damage response during hepatocarcinogenesis. *Med Mol Morphol* 2009; **42**: 138-142 [PMID: 19784739 DOI: 10.1007/s00795-009-0457-8]
- Levrero M**, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 2016; **64**: S84-101 [PMID: 27084040 DOI: 10.1016/j.jhep.2016.02.021]
- Zhang Y**, Liu H, Yi R, Yan T, He Y, Zhao Y, Liu J. Hepatitis B virus whole-X and X protein play distinct roles in HBV-related hepatocellular carcinoma progression. *J Exp Clin Cancer Res* 2016; **35**: 87 [PMID: 27255760 DOI: 10.1186/s13046-016-0366-3]
- Zhang W**, Lu Z, Kong G, Gao Y, Wang T, Wang Q, Cai N, Wang H, Liu F, Ye L, Zhang X. Hepatitis B virus X protein accelerates hepatocarcinogenesis with partner survivin through modulating miR-520b and HBXIP. *Mol Cancer* 2014; **13**: 128 [PMID: 24886421 DOI: 10.1186/1476-4598-13-128]
- Oishi N**, Shilagardi K, Nakamoto Y, Honda M, Kaneko S, Murakami S. Hepatitis B virus X protein overcomes oncogenic RAS-induced senescence in human immortalized cells. *Cancer Sci* 2007; **98**: 1540-1548 [PMID: 17760951 DOI: 10.1111/j.1349-7006.2007.00579.x]
- Tang H**, Oishi N, Kaneko S, Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. *Cancer Sci* 2006; **97**: 977-983 [PMID: 16984372 DOI: 10.1111/j.1349-7006.2006.00299.x]
- Hu XM**, Yan XH, Hu YW, Huang JL, Cao SW, Ren TY, Tang YT, Lin L, Zheng L, Wang Q. miRNA-548p suppresses hepatitis B virus X protein associated hepatocellular carcinoma by downregulating oncoprotein hepatitis B x-interacting protein. *Hepatol Res* 2016; **46**: 804-815 [PMID: 26583881 DOI: 10.1111/hepr.12618]
- Lu PJ**, Sundquist K, Baeckstrom D, Poulosom R, Hanby A, Meier-Ewert S, Jones T, Mitchell M, Pitha-Rowe P, Freemont P, Taylor-Papadimitriou J. A novel gene (PLU-1) containing highly conserved putative DNA/chromatin binding motifs is specifically up-regulated in breast cancer. *J Biol Chem* 1999; **274**: 15633-15645 [PMID: 10336460]
- Xiang Y**, Zhu Z, Han G, Ye X, Xu B, Peng Z, Ma Y, Yu Y, Lin H, Chen AP, Chen CD. JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. *Proc Natl Acad Sci USA* 2007; **104**: 19226-19231 [PMID: 18048344 DOI: 10.1073/pnas.0700735104]
- Hayami S**, Yoshimatsu M, Veerakumarasivam A, Unoki M, Iwai Y, Tsunoda T, Field HI, Kelly JD, Neal DE, Yamaue H, Ponder BA, Nakamura Y, Hamamoto R. Overexpression of the JmJc histone demethylase KDM5B in human carcinogenesis: involvement in the proliferation of cancer cells through the E2F/RB pathway. *Mol Cancer* 2010; **9**: 59 [PMID: 20226085 DOI: 10.1186/1476-4598-9-59]
- Xie L**, Pelz C, Wang W, Bashar A, Varlamova O, Shadle S, Impey S. KDM5B regulates embryonic stem cell self-renewal and represses cryptic intragenic transcription. *EMBO J* 2011; **30**: 1473-1484 [PMID: 21448134 DOI: 10.1038/emboj.2011.91]
- Stalker L**, Wynder C. Evaluation of histone-modifying enzymes in stem cell populations. *Methods Mol Biol* 2012; **809**: 411-426 [PMID: 22113291 DOI: 10.1007/978-1-61779-376-9_27]
- Jia HL**, Ye QH, Qin LX, Budhu A, Forgues M, Chen Y, Liu YK, Sun HC, Wang L, Lu HZ, Shen F, Tang ZY, Wang XW. Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. *Clin Cancer Res* 2007; **13**: 1133-1139 [PMID: 17317821 DOI: 10.1158/1078-0432.CCR-06-1025]
- Lee JS**, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens F, Roskams T, Thorgeirsson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006; **12**: 410-416 [PMID: 16532004 DOI: 10.1038/nm1377]
- Yamashita T**, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, Zeng SS, Hayashi T, Kondo M, Takatori H, Yamashita T, Mizukoshi E, Ikeda H, Zen Y, Takamura H, Wang XW, Kaneko S. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. *Hepatology* 2013; **57**: 1484-1497 [PMID: 23174907 DOI: 10.1002/hep.26168]

- 19 **Okitsu T**, Kobayashi N, Jun HS, Shin S, Kim SJ, Han J, Kwon H, Sakaguchi M, Totsugawa T, Kohara M, Westerman KA, Tanaka N, Leboulch P, Yoon JW. Transplantation of reversibly immortalized insulin-secreting human hepatocytes controls diabetes in pancreatectomized pigs. *Diabetes* 2004; **53**: 105-112 [PMID: 14693704]
- 20 **Shirasaki T**, Honda M, Shimakami T, Murai K, Shiimoto T, Okada H, Takabatake R, Tokumaru A, Sakai Y, Yamashita T, Lemon SM, Murakami S, Kaneko S. Impaired interferon signaling in chronic hepatitis C patients with advanced fibrosis via the transforming growth factor beta signaling pathway. *Hepatology* 2014; **60**: 1519-1530 [PMID: 24962339 DOI: 10.1002/hep.27277]
- 21 **Shinoda M**, Tilles AW, Kobayashi N, Wakabayashi G, Takayanagi A, Totsugawa T, Harada H, Obara H, Suganuma K, Berthiaume F, Shimazu M, Shimizu N, Tanaka N, Kitajima M, Tompkins RG, Toner M, Yarmush ML. A bioartificial liver device secreting interleukin-1 receptor antagonist for the treatment of hepatic failure in rats. *J Surg Res* 2007; **137**: 130-140 [PMID: 17081566 DOI: 10.1016/j.jss.2006.08.009]
- 22 **Pfeifer AM**, Cole KE, Smoot DT, Weston A, Groopman JD, Shields PG, Vignaud JM, Juillerat M, Lipsky MM, Trump BF. Simian virus 40 large tumor antigen-immortalized normal human liver epithelial cells express hepatocyte characteristics and metabolize chemical carcinogens. *Proc Natl Acad Sci USA* 1993; **90**: 5123-5127 [PMID: 7685115]
- 23 **Buendia MA**, Neuveut C. Hepatocellular carcinoma. *Cold Spring Harb Perspect Med* 2015; **5**: a021444 [PMID: 25646384 DOI: 10.1101/cshperspect.a021444]
- 24 **Saitta C**, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, Raffa G, Sangiovanni A, Navarra G, Raimondo G, Pollicino T. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. *Liver Int* 2015; **35**: 2311-2317 [PMID: 25677098 DOI: 10.1111/liv.12807]
- 25 **Secombe J**, Eisenman RN. The function and regulation of the JARID1 family of histone H3 lysine 4 demethylases: the Myc connection. *Cell Cycle* 2007; **6**: 1324-1328 [PMID: 17568193 DOI: 10.4161/cc.6.11.4269]
- 26 **Yamamoto S**, Wu Z, Russnes HG, Takagi S, Peluffo G, Vaske C, Zhao X, Moen Volla HK, Maruyama R, Ekram MB, Sun H, Kim JH, Carver K, Zucca M, Feng J, Almendro V, Bessarabova M, Rueda OM, Nikolsky Y, Caldas C, Liu XS, Polyak K. JARID1B is a luminal lineage-driving oncogene in breast cancer. *Cancer Cell* 2014; **25**: 762-777 [PMID: 24937458 DOI: 10.1016/j.ccr.2014.04.024]
- 27 **Wang Z**, Tang F, Qi G, Yuan S, Zhang G, Tang B, He S. KDM5B is overexpressed in gastric cancer and is required for gastric cancer cell proliferation and metastasis. *Am J Cancer Res* 2015; **5**: 87-100 [PMID: 25628922]
- 28 **Li X**, Liu L, Yang S, Song N, Zhou X, Gao J, Yu N, Shan L, Wang Q, Liang J, Xuan C, Wang Y, Shang Y, Shi L. Histone demethylase KDM5B is a key regulator of genome stability. *Proc Natl Acad Sci USA* 2014; **111**: 7096-7101 [PMID: 24778210 DOI: 10.1073/pnas.1324036111]
- 29 **Shen X**, Zhuang Z, Zhang Y, Chen Z, Shen L, Pu W, Chen L, Xu Z. JARID1B modulates lung cancer cell proliferation and invasion by regulating p53 expression. *Tumour Biol* 2015; **36**: 7133-7142 [PMID: 25877751 DOI: 10.1007/s13277-015-3418-y]
- 30 **Kuo YT**, Liu YL, Adebayo BO, Shih PH, Lee WH, Wang LS, Liao YF, Hsu WM, Yeh CT, Lin CM. JARID1B Expression Plays a Critical Role in Chemoresistance and Stem Cell-Like Phenotype of Neuroblastoma Cells. *PLoS One* 2015; **10**: e0125343 [PMID: 25951238 DOI: 10.1371/journal.pone.0125343]
- 31 **Tan BT**, Park CY, Ailles LE, Weissman IL. The cancer stem cell hypothesis: a work in progress. *Lab Invest* 2006; **86**: 1203-1207 [PMID: 17075578 DOI: 10.1038/labinvest.3700488]
- 32 **Shukla SK**, Kumar V. Hepatitis B virus X protein and c-Myc cooperate in the upregulation of ribosome biogenesis and in cellular transformation. *FEBS J* 2012; **279**: 3859-3871 [PMID: 22889122 DOI: 10.1111/j.1742-4658.2012.08745.x]
- 33 **Lakhtakia R**, Kumar V, Reddi H, Mathur M, Dattagupta S, Panda SK. Hepatocellular carcinoma in a hepatitis B 'x' transgenic mouse model: A sequential pathological evaluation. *J Gastroenterol Hepatol* 2003; **18**: 80-91 [PMID: 12519229]
- 34 **Terradillos O**, Billet O, Renard CA, Levy R, Molina T, Briand P, Buendia MA. The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* 1997; **14**: 395-404 [PMID: 9053836 DOI: 10.1038/sj.onc.1200850]
- 35 **Wang D**, Han S, Peng R, Jiao C, Wang X, Yang X, Yang R, Li X. Depletion of histone demethylase KDM5B inhibits cell proliferation of hepatocellular carcinoma by regulation of cell cycle checkpoints p15 and p27. *J Exp Clin Cancer Res* 2016; **35**: 37 [PMID: 26911146 DOI: 10.1186/s13046-016-0311-5]

P- Reviewer: Ohkoshi S, Qin Y **S- Editor:** Yu J **L- Editor:** A
E- Editor: Wang CH





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgooffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



ISSN 1007-9327

