Overexpression of lysine specific demethylase 1 predicts worse prognosis in primary hepatocellular carcinoma patients

Ze-Kun Zhao, Hai-Feng Yu, Dao-Rong Wang, Ping Dong, Lei Chen, Wen-Guang Wu, Wen-Jun Ding, Ying-Bin Liu

Ze-Kun Zhao, Ping Dong, Lei Chen, Wen-Guang Wu, Wen-Jun Ding, Ying-Bin Liu, Department of General Surgery, Xinhua Hospital Affiliated to Shanghai Jiaotong University, Shanghai 200092, China
Hai-Feng Yu, Department of General Surgery, Yixing People's Hospital, Yixing 214200, Jiangsu Province, China
Dao-Rong Wang, Department of General Surgery, Northern Jiangsu People's Hospital, Yangzhou 225000, Jiangsu Province, China
Author contributions: Zhao ZK and Liu YB designed the study; Zhao ZE, Yu HF, Dong P and Ding WJ performed the majority of the experiments; Wang DR performed the data analysis; Chen L and Wu WG supervised the experimental work; Liu YB wrote the manuscript and is fully responsible for the management of the study; and all authors have read and approved the final manuscript.
Correspondence to: Ying-Bin Liu, Professor, Department of General Surgery, Xinhua Hospital Affiliated to Shanghai Jiaotong University, No. 1665 Kongjiang Rd., Shanghai 200092, China. yingbinliu02@yahoo.com.cn
Telephone: +86-20-8234245 Fax: +86-20-83647362
Received: June 23, 2012 Revised: August 10, 2012
Accepted: August 25, 2012
Published online: December 7, 2012

Abstract

AIM: To investigate the clinicopathological features and prognostic value of lysine specific demethylase 1 (LSD1) in hepatocellular carcinoma (HCC).

METHODS: We examined LSD1 expression in 60 paired liver cancer tissues and adjacent noncancerous tissues by quantitative real time polymerase chain reaction (qRT-PCR) and Western blotting. In addition, we analyzed LSD1 expression in 198 HCC samples by immunohistochemistry. The relationship between LSD1 expression, clinicopathological features and patient survival was investigated.

RESULTS: Immunohistochemistry, Western blotting, and qRT-PCR consistently confirmed LSD1 overexpression in HCC tissues compared to adjacent non-neoplastic tissues ($P < 0.01$). Additionally, immunostaining showed more LSD1-positive cells in the higher tumor stage (T3-4) and tumor grade (G3) than in the lower tumor stage (T1-2, $P < 0.001$) and tumor grade (G1-2, $P < 0.001$), respectively. Moreover, HCC patients with high LSD1 expression had significantly lower 5-year overall survival rates ($P < 0.001$) and lower 5-year disease-free survival rates ($P < 0.001$), respectively. A Cox proportional hazards model further demonstrated that LSD1 over-expression was an independent predictor of poor prognosis for both 5-year disease-free survival [hazards ratio (HR) = 1.426, 95%CI: 0.672-2.146, $P < 0.001$] and 5-year overall survival (HR = 2.456, 95%CI: 1.234-3.932, $P < 0.001$) in HCC.

CONCLUSION: Our data suggest for the first time that the overexpression of LSD1 protein in HCC tissues indicates tumor progression and predicts poor prognosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, especially in Asia[1]. In China, HCC ranks behind gastric and esophageal cancer with the third highest mortality rate among all malignant carcinomas, leads to approximately 110 000 deaths every year, and accounts for 45% of total HCC deaths worldwide[2]. Multiple risk factors have been associated with the initiation and development of HCC; these include chronic infection with hepatitis B, C or D virus, aflatoxin, alcohol abuse, hereditary metabolic liver diseases, and diabetes mellitus[1,3]. Like most other cancers, hepatocarcinogenesis is a multistep process that involves multiple genetic alterations that may activate oncogenes and/or inactivate tumor suppressor genes, ultimately leading to the malignant transformation of hepatocytes.

Histone demethylase lysine specific demethylase 1 (LSD1), the first histone demethylase that was discovered as a nuclear homolog of amine oxidases, removes the methyl groups from mono- and dimethylated lysine (Lys) 4 of histone H3 (H3K4me1/2) and Lys9 of histone H3 (H3K9me1/2). LSD1 is essential for mammalian development and is involved in many biological processes, including cell-type differentiation, gene activation and gene repression[4]. A recent study indicated that LSD1 might promote cell phase transition (deficiency in LSD1 led to partial cell cycle arrest in G2/M) and cell proliferation, suggesting that its over-expression might promote tumorigenesis[5]. The expression of LSD1 has been associated with tumor recurrence during therapy in various cancers, further implicating LSD-1 as a tumor promoter[6-8]. A tissue cDNA microarray analysis also demonstrated the presence of LSD1 transactivation in lung and colorectal carcinomas[7]. LSD1 knockdown with small interfering (si) RNAs resulted in the suppression of proliferation of various bladder and lung cancer cell lines[7]. To the best of our knowledge, there is little available data regarding the involvement of *LSD1* genes in hepatic tumorigenesis. In this study, we investigated LSD1 expression in HCC and its correlation with the clinicopathological features of patients with HCC, including patient survival.

MATERIALS AND METHODS

Patients and tissue samples

The study was approved by the Research Ethics Committee of Xinhua Hospital, which is affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China. Informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to accepted ethical and legal standards.

A total of 198 patients who presented with primary HCC and later underwent curative liver resection at Xinhua Hospital affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China, were included in this retrospective study. The tissue samples used in this study were retrieved from the tissue bank of the Depart-

Table 1 Clinicopathological features and the expression of lysine specific demethylase 1 in 198 hepatocellular carcinoma patients

Characteristics	*n*	LSD1 (%)		*P* value
		High expression	Low expression	
Gender				
Male	101	58	43	
Female	97	54	43	NS
Age (yr)				
⩾ 50	112	61	51	
< 50	86	51	35	NS
Tumor stage				
T1	39	5	34	
T2	42	21	21	
T3	76	47	29	
T4	41	39	2	< 0.001
Tumor grade				
G1	45	9	34	
G2	114	63	51	
G3	39	38	1	< 0.001
Growth pattern				
Trabecular	147	84	63	
Nontrabecular	51	28	23	NS
Cirrhosis				
Yes	151	87	64	
No	47	25	22	NS
Underlying liver disease				
Alcoholic	21	15	6	
Hepatitis B	136	74	62	
Hepatitis C	30	17	13	
Unknown	11	6	5	NS

LSD1: Lysine specific demethylase 1; NS: Not significant.

ment of Pathology in the Xinhua Hospital affiliated with Shanghai Jiaotong University School of Medicine. The patients had been diagnosed with HCC between 2001 and 2006. None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. HCC diagnosis was based on World Health Organization criteria. Tumor differentiation was defined according to the Edmondson grading system. Liver function was assessed using the Child-Pugh scoring system. Tumor staging was determined according to the sixth edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer. The clinicopathological features of 198 patients are summarized in Table 1. In addition, 60 self-pairs of HCC specimens (10 TNM stage Ⅰ, 16 TNM stage Ⅱ, 24 TNM stage Ⅲ, and 10 TNM stage Ⅳ) and adjacent non-neoplastic liver tissues were snap frozen in liquid nitrogen and stored at -80 ℃ following surgery for quantitative real time polymerase chain reaction (qRT-PCR) assay and western blot analysis. The median follow-up period was 8.6 years. Postoperative surveillance included routine clinical and laboratory examinations every third month, computed tomography scans of the abdomen, and radiographs of the chest every third month. After 5 years, the examination interval was extended to 12 mo.

Immunohistochemistry analysis

Immunohistochemical staining was carried out follow-

ing the protocol of our previous study[9-11]. The primary antibody against LSD1 was a rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., United States) at a dilution of 1:50. The specificity of the primary antibody has been validated by the previous studies of Müller *et al*[12] and Lü *et al*[13]. The secondary antibody for the detection of primary antibody was anti-rabbit immunoglobulin G (Sigma, St. Louis, MO, United States). The negative controls were processed in a similar manner with phosphate-buffered saline instead of primary antibody. Further, positive LSD1 expression, as confirmed by western blotting, was used as a positive control for immunostaining. Following hematoxylin counterstaining, immunostaining was scored by two independent experienced pathologists who were blinded to the clinicopathological parameters and clinical outcomes of the patients. The scores of the two pathologists were compared, and any discrepant scores were re-examined for staining by both pathologists until a consensus score was obtained. The number of cells that stained positive for nuclear LSD1 in ten representative microscopic fields was counted, and the percentage of positive cells was calculated. The percentages of cells that were immunoreactive were converted to scores as follows: 0 (0%), 1 (1%-10%), 2 (11%-50%) and 3 (> 50%). Staining intensity was visually scored and stratified as follows: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). A final score was obtained for each case by multiplying the percentage score and the intensity score. Multiplied scores exceeding 5 (median of total scores for LSD1) were considered to indicate low levels of LSD1 expression, while all other scores were considered to indicate high levels of LSD1 expression.

Western blotting
The Western blotting protocol and semiquantitative analysis were carried out following the protocol of Xu *et al*[14]. LSD1 antibody (rabbit polyclonal antibody, dilution 1:50, Santa Cruz Biotechnology, Inc., United States) was used, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (CW0266, dilution 1:1000, CoWin Biotech) was used as an internal control.

Quantitative RT-PCR
To measure the mRNA expression levels of LSD1, total RNA was extracted from frozen liver tissues using TriZol reagent (Invitrogen) according to the manufacturer's instructions. The extraction was followed by RT-PCR using the TransStart Green qPCR SuperMix (TransGen Biotech). The primer sequences for LSD1 amplification were 5'-CGAACGCACATCAAGACGA-3' for the forward primer and 5'-AGGTGAAGGTGGAGTAGAGGC-3' for the reverse primer. The transcription of GAPDH was used as an internal control for normalization. LSD1 expression levels were calculated relative to GAPDH using the delta-delta computed tomography method[15].

Statistical analysis
SPSS version 13.0 for Windows (SPSS Inc, IL, United
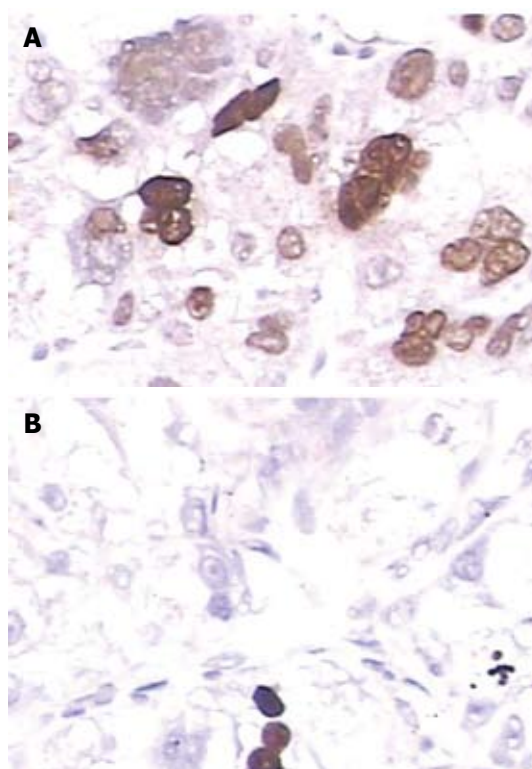
Figure 1 Lysine specific demethylase 1 expression in hepatocellular carcinoma and adjacent non-neoplastic liver tissues. A: High expression of lysine specific demethylase 1 (LSD1) in hepatocellular carcinoma (HCC) samples; B: Low expression of LSD1 in HCC samples (original magnification, 200×).

States) and SAS 9.1 (SAS Institute, Cary, NC) were used for statistical analysis. A Fisher's exact test and χ^2 test were performed to assess associations between LSD1 expression and clinicopathological parameters. A Kaplan-Meier method was used for survival analysis, and differences in survival were estimated using the log-rank test. A multivariate survival analysis was performed for all parameters that were significant in the univariate analyses using a Cox regression model. Differences were considered statistically significant when P was less than 0.05.

RESULTS

Expression of LSD1 protein and mRNA in HCC
Immunohistochemical analysis revealed that LSD1 staining was mainly localized to the nucleus of noncancerous and malignant epithelial cells (Figure 1). In addition, we found that 112 (56.6%) of 198 HCC tissues had high LSD1 expression, and 86 (43.4%) of 198 HCC tissues had low LSD1 expression. Additionally, 52 (26.3%) of 198 adjacent non-neoplastic liver tissues had high LSD1 expression, and 146 (73.7%) of 198 adjacent nonneoplastic liver tissues had low LSD1 expression. Thus, the LSD1 immunostaining in HCC tissues was significantly higher than the staining in the adjacent non-neoplastic liver tissues ($P < 0.01$). To confirm LSD1 protein expression by an independent method, Western blotting analysis was performed using 60 self-pairs of HCC and adjacent
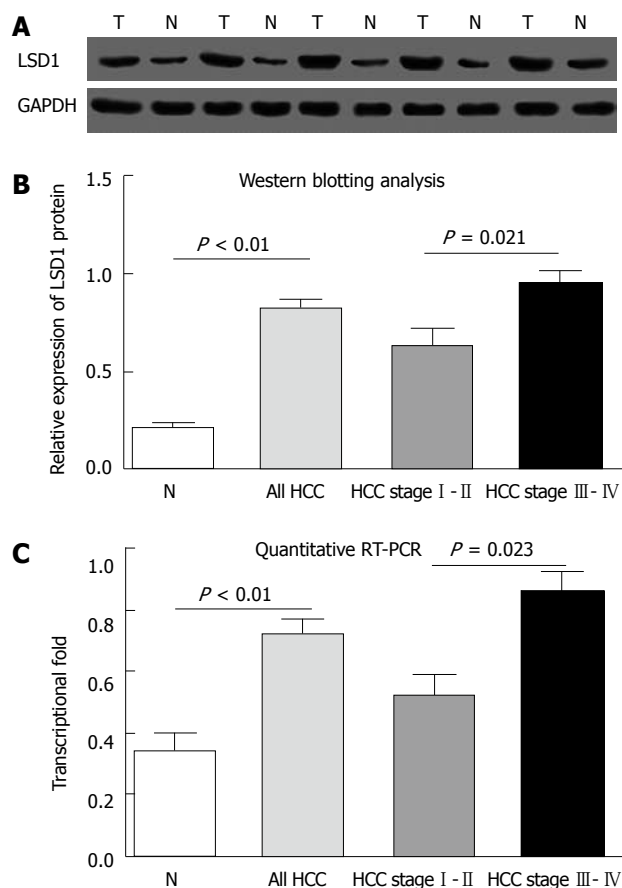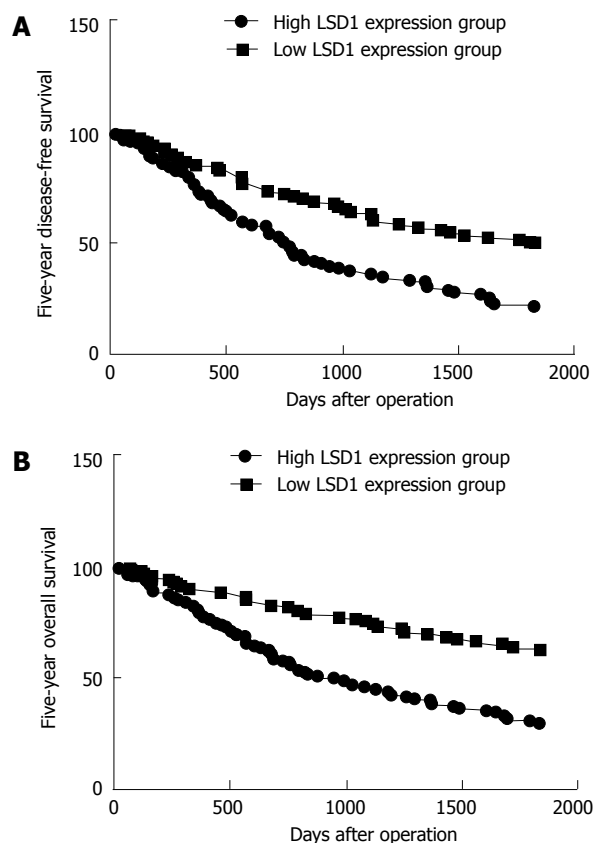
Figure 2 Increased lysine specific demethylase 1 protein and mRNA levels in hepatocellular carcinoma with different tumor node metastasis stages and adjacent non-neoplastic liver tissues. A: Representative Western blotting of lysine specific demethylase 1 (LSD1) protein levels in hepatocellular carcinoma (HCC) tissues (T) and adjacent non-neoplastic liver tissues (N); B: Semiquantitative Western blotting showed that the expression levels of LSD1 protein were significantly higher than those in adjacent non-neoplastic liver tissues ($P < 0.01$). Additionally, the expression levels of LSD1 protein increased with ascending tumor node metastasis (TNM) stages. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. *P* values, mean and SD were given (*t* test); C: Quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay showed significantly increased LSD1 mRNA levels in HCC tissues compared with adjacent non-neoplastic liver tissues ($P < 0.01$). Additionally, the expression levels of LSD1 mRNA were increased with ascending tumor TNM stages. GAPDH was used as the internal control. *P* values, mean and SD were given (Mann-Whitney test).

non-neoplastic liver tissues. Overexpression of LSD1 protein in HCC tissues was compared with adjacent non-neoplastic liver tissues ($P < 0.01$, Figure 2A and B) using this method, and significantly increased LSD1 mRNA levels were detected by qRT-PCR ($P < 0.01$, Figure 2C). The expression levels of LSD1 protein and mRNA in high stage (Ⅲ-Ⅳ) HCC tissues were both significantly higher than the levels of LSD1 protein and mRNA in low stage (Ⅰ-Ⅱ) HCC tissues (for protein: $P = 0.021$; for mRNA: $P = 0.023$; Figure 2B and C).

Association of LSD1 expression with the clinicopathological features of HCC

To evaluate whether LSD1 protein expression was associated with clinicopathological features of patients with

Figure 3 Kaplan-Meier survival curves for lysine specific demethylase 1 expression in hepatocellular carcinoma patients. A: The hepatocellular carcinoma patients with high lysine specific demethylase 1 (LSD1) expression showed significantly shorter disease-free survival ($P < 0.001$); B: Overall survival ($P < 0.001$) rates than those with low LSD1 expression.

HCC, we correlated immunohistochemical LSD1 staining results with T stage, tumor grade, presence of cirrhosis, underlying liver disease, including alcohol abuse, viral hepatitis B and C, sex and age (Table 1). We found more LSD1 positive cells in tissues with higher tumor stages (T3-4) and higher tumor grades (G3) than in the lower tumor stages (T1-2, $P < 0.001$) and tumor grades (G1-2, $P < 0.001$), respectively.

Prognostic values of LSD1 expression in HCC

The 5-year disease-free survival in the group with high LSD1 expression was significantly poorer than the disease-free survival in the group with low LSD1 expression ($P < 0.001$, log-rank test; Figure 3A). A Kaplan-Meier plot of 5-year overall survival curves stratified by LSD1 expression is shown in Figure 3B. There was a significant relationship between LSD1 expression and 5-year overall survival ($P < 0.001$, log-rank test; Figure 3B). In a multivariate Cox model that included tumor size, tumor stage, tumor grading, presence of cirrhosis, gender, age, and LSD1 staining, we found that LSD1 expression independently indicated poor prognosis for both 5-year disease-free survival [hazards ratio (HR) = 1.426, 95%CI: 0.672-2.146, $P < 0.001$; Table 2] and 5-year overall survival (HR = 2.456, 95%CI: 1.234-3.932, $P < 0.001$; Table

Table 2 Multivariate survival analysis of five-year overall and disease-free survival in 198 patients with hepatocellular carcinoma

Parameter	Five-year overall survival			Five-year disease-free survival		
	P value	HR	95%CI	*P* value	HR	95%CI
Age	0.775	0.867	0.463-1.452	0.714	1.174	0.883-1.853
Gender	0.456	1.121	0.569-1.867	0.634	1.126	0.684-1.846
Tumor stage	< 0.001	1.634	1.142-2.537	< 0.001	1.423	0.784-2.161
Tumor grade	< 0.001	1.154	0.647-1.893	< 0.001	1.023	0.456-1.638
Presence of cirrhosis	0.542	1.143	0.647-1.784	0.427	1.321	0.824-1.917
LSD1 expression	< 0.001	2.456	1.234-3.932	< 0.001	1.426	0.672-2.146

LSD1: Lysine specific demethylase 1; HR: Hazards ratio.

2) in patients with HCC.

DISCUSSION

Genetic alterations are a hallmark of human cancer. In recent years, the field of cancer genomics has made significant advances in the area of cancer-associated genetic lesion identification. Furthermore, the importance of epigenetic changes that occur during HCC development has also been recognized[16]. Epigenetic changes can take the form of DNA methylation or histone modification[17]. Histone modifications in the form of selective acetylation, phosphorylation, and methylation serve as switches that alter chromatin structure, allowing posttranscriptional activation or repression of downstream proteins[18]. Understanding these epigenetic changes will lead to the identification of novel cancer-related genes that may represent attractive targets for cancer treatment and provide new insights into the biology of hepatic cancers. Thus, an integrative approach to hepatic cancer research that combines epidemiological, genetic and epigenetic information has emerged as an important paradigm for cancer therapy[19]. The methylation status of histone methyltransferases and histone demethylases plays a pivotal role in the regulation of gene expression[20]. LSD1, the first histone demethylase that was discovered as a nuclear homolog of amine oxidases, removes methyl groups from mono- and dimethylated H3K4me1/2 and H3K9me1/2[21].

Epigenetic changes in LSD1 have been shown to play a key role in carcinogenesis[22]. LSD1 can prevent the accumulation of the dimethyl groups of p53, repressing p53-mediated transcriptional up-regulation, preventing apoptosis, and contributing to human carcinogenesis *via* a chromatin modification mechanism. To date, a few studies have indicated that LSD1 may promote cell phase transition (deficiency in LSD1 led to partial cell cycle arrest in G2/M) and cell proliferation, suggesting that LSD1 over-expression might promote tumorigenesis[5]. The expression of LSD1 has been associated with tumor recurrence during therapy in various cancers, further implicating LSD-1 as a tumor promoter[6-8]. Tissue cDNA microarray analysis also revealed LSD1 transactivation in lung and colorectal carcinomas[7]. Knocking down LSD1 with small interfering RNAs resulted in suppression of proliferation of various bladder and lung cancer cell lines[7]. However, the association between LSD1 and the survival of HCC patients was not well defined. In this study, we investigated the associations between LSD1 expression levels and clinical features of HCC patients.

In order to demonstrate that the epigenetic changes were associated with genetic changes in lung cancer, we first investigated the expression of LSD1 in HCC clinical samples. Previous studies have demonstrated that LSD1 protein and mRNA levels could act as biomarkers for the identification of patients with more aggressive breast cancer, prostate cancer, lung cancer and neuroblastoma[23-26]. In our study, we detected LSD1 by immunohistochemistry analysis, Western blotting, and qRT-PCR. Our results showed that LSD1 immunoreactivity was significantly increased in HCC compared with adjacent non-neoplastic liver tissue in a substantial proportion of cases. The over-expression of LSD1 was observed in tumor tissues with higher tumor stage and higher tumor grade. Additionally, our investigation revealed that high LSD1 expression is associated with a significant trend toward both poorer disease-free survival and poorer overall survival. Our study further confirms that high LSD1 expression independently predicts a higher risk of disease relapse or death after multivariate adjustment for other prognostic factors. These observations support the hypothesis that LSD1 may function as an oncogene in HCC and suggest that LSD1 may play an important role in the tumorigenesis of HCC. However, the role of LSD1 in HCC remains to be elucidated. Our data may offer new insight into LSD1 as a potentially important contributor to the progression of HCC and as a new prognostic factor for HCC. As the 198 cases of the present study were all obtained from the Chinese population, the results reported here should be further confirmed in other populations.

In conclusion, our study suggests that LSD1 is over-expressed in HCC tissues compared with their benign counterparts. To the best of our knowledge, this is the first study evaluating the expression levels of LSD1 mRNA and protein in HCC tissues and the association between these expression levels and clinicopathologic parameters. The most important finding of this study is that LSD1 expression may predict a poorer prognosis for HCC patients after surgery. Further studies are needed to investigate the precise function of LSD1 in the progression of HCC.

COMMENTS

Background
Lysine specific demethylase 1 (LSD1) is essential for mammalian development and is involved in many biological processes, such as cell-type differentiation, gene activation and gene repression. Knocking down LSD1 with small interfering RNAs suppressed the proliferation of various bladder and lung cancer cell lines. To be known, little data has been generated with regard to the involvement of *LSD1* genes in hepatic tumorigenesis. In this study, the authors investigated LSD1 expression in hepatocellular carcinoma (HCC) and its correlation with clinicopathological features, including the survival of patients with HCC.

Research frontiers
The data suggest for the first time that the overexpression of LSD1 protein in HCC tissues may help predict tumor progression and poor prognosis.

Innovations and breakthroughs
The authors examined LSD1 expression in 60 paired liver cancer tissues and adjacent noncancerous tissues by quantitative real time polymerase chain reaction and Western blotting. In addition, authors analyzed LSD1 expression in 198 HCC samples by immunohistochemistry. The relationships between LSD1 expression and both clinicopathological features and patient survival were investigated.

Applications
These findings provide evidence that the overexpression of LSD1 serves as a biomarker for poor prognosis in HCC. Thus, authors speculate that LSD1 may be a potential target of anti-angiogenic therapy for HCC.

Terminology
LSD1, the first histone demethylase that was discovered as a nuclear homolog of amine oxidases, removes the methyl groups from mono- and dimethylated Lysine (Lys) 4 of histone H3 and Lys9 of histone H3.

Peer review
The authors investigated the expression of LSD1 in HCC and determined its correlation with tumor progression and prognosis. The authors claim that the expression levels of LSD1 protein in HCC tissues correlates with tumor progression and prognosis.

REFERENCES

1   **Schütte K**, Bornschein J, Malfertheiner P. Hepatocellular carcinoma--epidemiological trends and risk factors. *Dig Dis* 2009; **27**: 80-92

2   **Li D**, Mallory T, Satomura S. AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. *Clin Chim Acta* 2001; **313**: 15-19

3   **Blum HE**, Spangenberg HC. Hepatocellular carcinoma: an update. *Arch Iran Med* 2007; **10**: 361-371

4   **Wang J**, Scully K, Zhu X, Cai L, Zhang J, Prefontaine GG, Krones A, Ohgi KA, Zhu P, Garcia-Bassets I, Liu F, Taylor H, Lozach J, Jayes FL, Korach KS, Glass CK, Fu XD, Rosenfeld MG. Opposing LSD1 complexes function in developmental gene activation and repression programmes. *Nature* 2007; **446**: 882-887

5   **Scoumanne A**, Chen X. The lysine-specific demethylase 1 is required for cell proliferation in both p53-dependent and -independent manners. *J Biol Chem* 2007; **282**: 15471-15475

6   **Lim S**, Janzer A, Becker A, Zimmer A, Schüle R, Buettner R, Kirfel J. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. *Carcinogenesis* 2010; **31**: 512-520

7   **Hayami S**, Kelly JD, Cho HS, Yoshimatsu M, Unoki M, Tsunoda T, Field HI, Neal DE, Yamaue H, Ponder BA, Nakamura Y, Hamamoto R. Overexpression of LSD1 contributes to human carcinogenesis through chromatin regulation in various cancers. *Int J Cancer* 2011; **128**: 574-586

8   **Chen PP**, Li WJ, Wang Y, Zhao S, Li DY, Feng LY, Shi XL, Koeffler HP, Tong XJ, Xie D. Expression of Cyr61, CTGF, and WISP-1 correlates with clinical features of lung cancer. *PLoS One* 2007; **2**: e534

9   **Guo X**, Xiong L, Zou L, Zhao J. Upregulation of bone morphogenetic protein 4 is associated with poor prognosis in patients with hepatocellular carcinoma. *Pathol Oncol Res* 2012; **18**: 635-640

10   **Schmilovitz-Weiss H**, Tobar A, Halpern M, Levy I, Shabtai E, Ben-Ari Z. Tissue expression of squamous cellular carcinoma antigen and Ki67 in hepatocellular carcinoma-correlation with prognosis: a historical prospective study. *Diagn Pathol* 2011; **6**: 121

11   **Hong H**, Patonay B, Finley J. Unusual reticulin staining pattern in well-differentiated hepatocellular carcinoma. *Diagn Pathol* 2011; **6**: 15

12   **Müller P**, Crofts JD, Newman BS, Bridgewater LC, Lin CY, Gustafsson JA, Ström A. SOX9 mediates the retinoic acid-induced HES-1 gene expression in human breast cancer cells. *Breast Cancer Res Treat* 2010; **120**: 317-326

13   **Lü B**, Fang Y, Xu J, Wang L, Xu F, Xu E, Huang Q, Lai M. Analysis of SOX9 expression in colorectal cancer. *Am J Clin Pathol* 2008; **130**: 897-904

14   **Xu MZ**, Yao TJ, Lee NP, Ng IO, Chan YT, Zender L, Lowe SW, Poon RT, Luk JM. Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. *Cancer* 2009; **115**: 4576-4585

15   **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408

16   **Piperi C**, Vlastos F, Farmaki E, Martinet N, Papavassiliou AG. Epigenetic effects of lung cancer predisposing factors impact on clinical diagnosis and prognosis. *J Cell Mol Med* 2008; **12**: 1495-1501

17   **Seligson DB**, Horvath S, McBrian MA, Mah V, Yu H, Tze S, Wang Q, Chia D, Goodglick L, Kurdistani SK. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 2009; **174**: 1619-1628

18   **Shi Y**. Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* 2007; **8**: 829-833

19   **Risch A**, Plass C. Lung cancer epigenetics and genetics. *Int J Cancer* 2008; **123**: 1-7

20   **Ueda R**, Suzuki T, Mino K, Tsumoto H, Nakagawa H, Hasegawa M, Sasaki R, Mizukami T, Miyata N. Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J Am Chem Soc* 2009; **131**: 17536-17537

21   **Shi Y**, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**: 941-953

22   **Lim S**, Metzger E, Schüle R, Kirfel J, Buettner R. Epigenetic regulation of cancer growth by histone demethylases. *Int J Cancer* 2010; **127**: 1991-1998

23   **Zhu Q**, Huang Y, Marton LJ, Woster PM, Davidson NE, Casero RA. Polyamine analogs modulate gene expression by inhibiting lysine-specific demethylase 1 (LSD1) and altering chromatin structure in human breast cancer cells. *Amino Acids* 2012; **42**: 887-898

24   **Schulte JH**, Lim S, Schramm A, Friedrichs N, Koster J, Versteeg R, Ora I, Pajtler K, Klein-Hitpass L, Kuhfittig-Kulle S, Metzger E, Schüle R, Eggert A, Buettner R, Kirfel J. Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 2009; **69**: 2065-2071

25   **Suikki HE**, Kujala PM, Tammela TL, van Weerden WM, Vessella RL, Visakorpi T. Genetic alterations and changes in expression of histone demethylases in prostate cancer. *Prostate* 2010; **70**: 889-898

26   **Lv T**, Yuan D, Miao X, Lv Y, Zhan P, Shen X, Song Y. Overexpression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer. *PLoS One* 2012; **7**: e35065

S- Editor Gou SX   **L- Editor** A   **E- Editor** Li JY