Biochemical and Biophysical Research Communications 437 (2013) 192–198

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

High expression of lysine-specific demethylase 1 correlates with poor prognosis of patients with esophageal squamous cell carcinoma

Yanyan Yu [a], Bin Wang [a], Kun Zhang [b], Zengjie Lei [a], Yan Guo [a], Hualiang Xiao [c], Jun Wang [a], Lilin Fan [a], Chunhui Lan [a], Yanling Wei [a], Qiang Ma [c], Li Lin [c], Chengyi Mao [c], Xin Yang [c], Xiaodi Chen [a], Yan Li [a], Yun Bai [b,*], Dongfeng Chen [a,*]

[a] Department of Gastroenterology, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China
[b] Department of Medical Genetics, College of Basic Medical Science, The Third Military Medical University, Chongqing 400038, China
[c] Department of Pathology, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China

ARTICLE INFO

Article history:
Received 25 May 2013
Available online 6 June 2013

Keywords:
LSD1
Esophageal squamous cell carcinoma
Invasion

ABSTRACT

Recent studies have elucidated the role of lysine-specific demethylase 1 (LSD1), a member of the histone demethylases, in epigenetic regulation of tumor suppressing/promoting genes and neoplastic growth. However, the expression of LSD1 in patients with esophageal squamous cell carcinoma (ESCC) is still unknown. Here, we reported that LSD1 expression was elevated in cancerous tissue and correlated with lymph node metastasis and poorer overall survival in patients with ESCC. Compared to EC109 cells, LSD1 expression was unregulated in aggressive cancer cell lines KYSE450 and KYSE150. Knockdown of LSD1 using lentivirus delivery of LSD1-specific shRNA abrogated the migration and invasion of ESCC cells *in vitro*. Further, a LSD1 inhibitor, tranylcypromine, suppressed H3K4me2 demethylation and attenuated cellular motility and invasiveness in a dose-dependent manner. Taken together, these data suggested that LSD1 was a potential prognostic maker and may be a molecular target for inhibiting invasion and metastasis in ESCC.

1. Introduction

Esophageal cancer is the eighth most common cancer worldwide [1,2], with advanced esophageal cancer being predictive of significantly poorer prognosis [3]. The 5-year survival rate for esophageal squamous cell carcinoma (ESCC) is only 5–12.3% in Europe [4,5]. Therefore, novel prognostic and molecular targets for therapeutic intervention are rapidly needed for patients with ESCC.

A wide range of genetic and epigenetic modifications have been shown to play a pivotal role in the development and tumorigenesis of esophageal cancers. These epigenetic changes are associated with DNA methylation and histone modifications [6–9]. Understanding epigenetic changes may help to identify a novel cancer-related network that may represent attractive targets for ESCC treatment and provide new insights into the biological characteristics of ESCC. Chromatin with histone tailing is defined as a critical regulator of gene transcription [6,8]. Histone demethylase lysine specific demethylase 1 (LSD1) was the first histone demethylase that was discovered, and is a nuclear homolog of amine oxidases. LSD1 removes methyl groups from mono- and dimethylated Lysine (Lys) 4 of histone H3 (H3K4me1/2), and Lys9 of histone H3 (H3K9me1/2) [10]. A recent study uncovered the role of LSD1 in cell phase transition, suggesting that its over-expression may promote tumorigenesis [11]. The expression of LSD1 has been associated with tumor recurrence during therapy in various human cancers, implicating LSD1 as a tumor promoter. It is of note that LSD1 is involved in embryonic differentiation [12], proliferation of pluripotent stem cells [13], HIV infection [14], as well as in the development and metastasis of cancers [15,16]. Furthermore, LSD1 is highly expressed in prostate cancer [17,18], bladder cancer [15], breast cancer [16,19], hepatoma [3], non-small-cell lung cancer [20] and hematopoietic tumors [21]. All of these studies imply that LSD1 may be associated with the pathogenesis of ESCC, however the expression and significance of LSD1 in ESCC is obscure.

In our study, we investigated the expression of LSD1 in ESCC tissues. Then, we tested the role of LSD1 in inducing tumor cell invasion in ESCC cells *via* genetic elimination or pharmacological inhibition. Our results provided a novel insight that LSD1 may

* Corresponding authors. Fax: +86 23 68757741 (D. Chen).
E-mail addresses: baiyungene@gmail.com (Y. Bai), chendf1981@126.com (D. Chen).

0006-291X
http://dx.doi.org/10.1016/j.bbrc.2013.05.123

serve as a prognostic indicator and potential molecular target in the pathogenesis of ESCC.

2. Materials and methods

2.1. Patients and tissue microarray

This study enrolled a total of 134 histopathologically-confirmed ESCC patients who underwent a resection of esophageal carcinoma in the Institute of Surgery Research, Daping Hospital affiliated to the Third Military Medical University between 2002 and 2007. All patients received no previous chemotherapy or radiotherapy before surgery. A multi-disciplinary team, including an oncosurgeon, an oncologist, and a radiologist, determined the therapeutic regimen. The clinicopathological information and patients' medical history were documented during postoperative follow-up. The histologically-confirmed ESCC tissues were investigated by micro-array. With the exception of cancerous tissues, we also obtained 23 cases of esophageal precancerous lesions, and 29 cases of esophageal normal mucosal tissue. We obtained written consent from all participants. Our study was approved by ethical review board of the Third Military Medical University.

2.2. Immunohistochemistry (IHC)

Paraffin tissue sections (4 μm) were incubated overnight with an antibody against LSD1 (1:400; Abcam, USA). The slides were incubated for 30 min with goat anti-rabbit immunoglobulin (E0432, Abcam) after being washed with Tris-buffered NaCl solution for 30 min. The percentage of positive cells was determined by counting 500 cells within five high-resolution fields. Immunohistochemical staining was evaluated using the semi-quantitative Remmele scoring system [22], which links the IHC staining intensity (SI) with the percentage of positive cells (PP). SI was scored according to the following criteria: (0) no positive nuclei, (1) all the positive nuclei display weak staining, (2) the most stained nuclei display moderately positive, (3) the nuclei display intensive staining. PP was scored between 0 and 4 (0: no positive cells, 1: less than 10% nuclei display intense staining, 2: 11–50% nuclei, 3: 51–80%, 4: more than 80%). The IHC score was finally calculated by SI × PP. According to the IHC score, all patients were classified into two groups: low expression level (0–4) and high expression level (4–12).

2.3. Cell culture and protein extraction

Esophageal cancer cell lines, KYSE150, KYSE450 and EC109, were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). At the indicated time points, total cell protein was extracted using cell lysis buffer (Beyotime Biotechnology, China).

2.4. Knock-down of LSD1 via shRNA-delivered by lentivirus

KYSE450 cells were cultured at a concentration of 3×10^5 cells/well for 24 h in RPMI 1640 medium supplemented with 10% FCS. According to the protocol, lentivirus with green fluorescent protein (GFP; Sunbio, China) was added to the 6-well plates. In addition, the lentiviral transfection enhancer (Sunbio, China) was applied at a concentration of 5 μg/ml. The medium was refreshed after 12-h. Notably, puromycin, a cytotoxic agent, eliminated the unstably infected cells. The shRNA sequences we used in this study are listed below: Control: TTC TCC GAA CGT GTC ACG T; LSD1-shRNA1: GCA GCT CGA CAG TTA CAA A; LSD1-shRNA2: CCA CCT GAC AGT AAG GAA T.

2.5. Western blotting

Cellular proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransfered to a nitrocellulose membrane (Bio-Rad, USA). Western blotting was carried out by sequential incubation in 5% non-fat milk blocking buffer at room temperature for 60 min, using the following antibodies: LSD1 (Abcam, USA), 1:2000; H3K4me1 (Millipore, USA), 1:2000; H3K4me2 (Millipore, USA), 1:500; H3K9me1 (Millipore, USA), 1:1000; H3K9me2 (Millipore, USA), 1:2000. Following overnight incubation at 4 °C, the secondary antibodies were added and incubated for 60 min at 4 °C. HRP-GAPDH (Kangchen, China) was used as the loading control.

2.6. RNA isolation, reverse transcription and quantitative reverse transcription (RT)-PCR

Total RNA from cell culture was extracted using the Trizol reagent, according to the manufacturer's protocol (Takara, Japan). The RNA samples were digested with DNase I (Takara, Japan), then reverse-transcribed into cDNAs and sequence amplified by Quantitative RT-PCR (Eco™ Real-Time PCR system, USA). According to the manufacturer's protocol, qRT-PCR was performed using the FastStart Universal SYBR Green Master Mix kit (Roche, USA). Relative mRNA levels of LSD1 were normalized to levels of the housekeeping gene, GAPDH, and results were calculated using the $2^{-\Delta\Delta Ct}$ method. All samples were measured in triplicate. The following primers were used: GAPDH, forward: 5′-GAA GGT GAA GGT CGG AGT CA-3′; reverse 5′-TTG AGG TCA ATG AAG GGG TC-3′. LSD1, forward: 5′-TTC TGG AGG GTA TGG AGA CG-3′; reverse: 5′-CCT TCT GGG TCT GTT GTG GT-3′.

2.7. Cell migration and invasion assay

Using a scratch assay, the cultured cells were incubated for 24 h in 6-well plates (Costar, USA) with RPMI 1640 plus 10% FCS until 90–100% confluence. A 1 mm-wide linear scratch was applied across the each well to evaluate cell migration. Subsequently, 2 ml of RPMI 1640 medium without FCS was introduced to repress cell proliferation after washing. Tranylcypromine (Sigma–Aldrich, USA) was added at a final concentration of 0 μM, 50 μM and 250 μM in the treatment groups. 48 h after application of the scratch, the width of the scratch was measured by CorelDRAW 9 (Corel Software Company, Canada).

Cell invasiveness was evaluated using a Transwell chamber assay (Costar, USA). Chamber membranes (8 μm, BD Falcon) were pre-coated with 6 μl matrigel at 4 °C overnight, and seeded with 1×10^5 cells. RPMI 1640 with 2% FCS supplement was added to the upper chamber and 600 μl of RPMI 1640 (containing 20% FCS) was added to the lower chamber. Cells were incubated for 48 h with or without treatment. The cells on the top of membranes were removed, and the cells that penetrated the membrane were fixed in ethanol, followed by crystal violet staining. The number of cells on the opposite side of the membrane was counted under the microscope in four random fields of vision.

2.8. Statistical analysis

Quantitative data is expressed as mean ± SD in figures, and multiple comparisons between the groups was performed using SNK method. Using SPSS 13.0 (SPSS Software Company, USA), one-way ANOVA and student's *t*-test were performed to determine the significance of the relationship between LSD1 expression and the clinicopathological factors in patients with ESCC, including sex, age, and pathological differentiation, infiltration, lymphatic metastasis, and the overall survival. A Kaplan–Meier survival curve

was drawn using Graphpad Prism 5 (Graphpad Software Company, USA), and the significance was calculated with the log-rank value. The comparisons were considered statistically significant when the *P* value was less than 0.05.

3. Results

3.1. LSD1 expression was up-regulated in the ESCC tissues

To determine the association of LSD1 with the clinicopathological characteristics of patients with ESCC, we examined and compared expression levels of LSD1 in ESCC tissues, precancerous lesions and paired normal tissues. The normal esophageal epithelial cells demonstrated slight positive LSD1 expression, while precancerous lesions presented a higher LSD1 expression level. In the normal and precancerous tissues, 51.7% and 73.9% of the cells were LSD1-positive, respectively (Fig. 1A, $P < 0.05$). Nuclear LSD1 was observed in 75.4% of LSD1-positive ESCC, of which, 43.7% of LSD1-positive cells were associated with strengthened staining (Fig. 1B), indicating that LSD1 was expressed at a relatively higher level in ESCC relative to normal or precancerous tissues.

As shown in Table 1, LSD1 expression was not correlated with any of the following clinicopathologic characteristics: sex, age, infiltration, differentiation, and histological type ($p > 0.05$). However, we detected a significant correlation between LSD1 expression and regional lymph node metastasis (LNM) in patients with ESCC. ESCC patients with LNM expressed LSD1 (56.8%) at a higher level than those without LNM (32.6%) ($p < 0.05$, Fig. 1C and D).

Table 1
Correlation between LSD1 expression and clinical pathological data in the tissue micro-array of 126 ESCC cases.

	LSD1		*P* value
	Low level	High level	
Sex			
Male	57	41	0.44
Female	14	14	
Age			
⩽60	35	34	0.16
>60	36	21	
Pathological type			
Medullary	5	7	0.19
Ulcerative	43	41	
Cavity	5	2	
Narrow	4	2	
Fungating	13	3	
Differentiation			
Well	27	19	0.80
Moderately	33	32	
Poorly	11	4	
Infiltration			
T1–2	37	29	0.94
T3–4	34	26	
Lymphatic metastasis			
Negative	52	30	0.03
Positive	19	25	
Life-time			
⩽36 m	29	37	0.03
>36 m	42	18	

3.2. The over-expression of LSD1 predicts poor prognosis in patients with ESCC

We evaluated the relationship between the LSD1 expression and overall survival after surgery in patients with ESCC. The results were similar in both cohorts between micro-assay results and tissue IHC staining (Fig. 2A and C). The ESCC patients with lower LSD1 expression were associated with a survival benefit, suggesting LSD1 expression was significantly associated with the prognosis of patients with ESCC. According to Kaplan–Meier curves, the

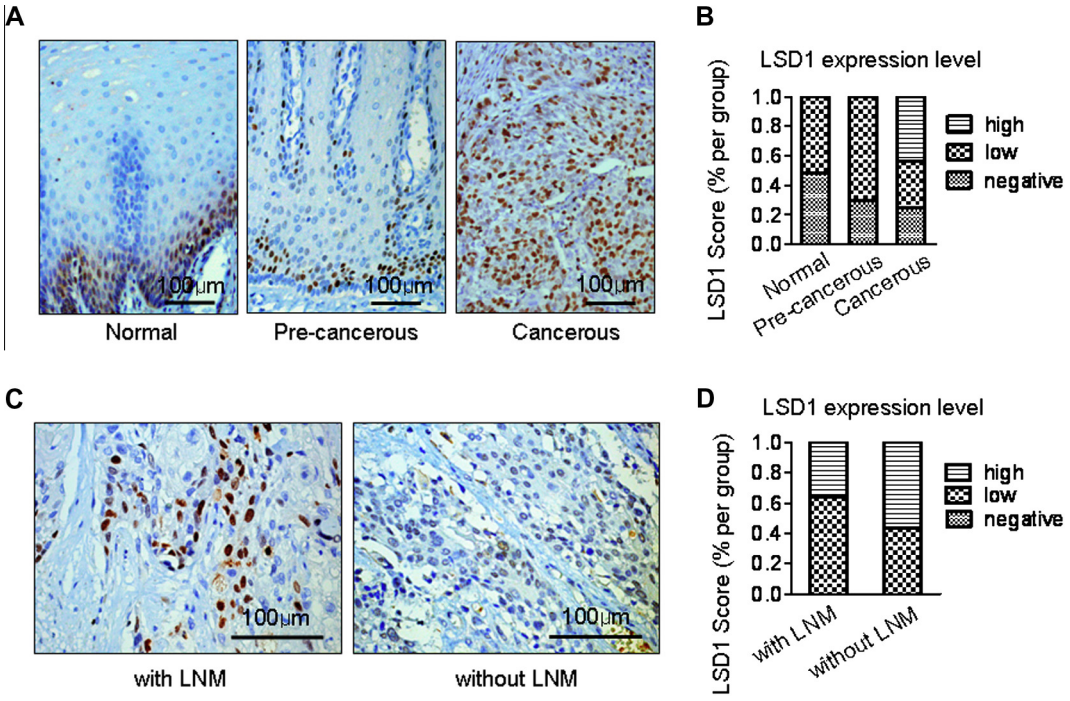

Fig. 1. LSD1 expression in normal esophageal epithelium, precancerous lesions and ESCC tissues. (A) LSD1 expression was investigated by immunohistochemistry (IHC) in normal esophageal, precancerous, and cancerous tissues. (B) There were significant differences of LSD1 expression between cancerous and non-cancer tissues (including normal and precancerous tissues) ($p < 0.05$). (C) Representative IHC images showing the expression of LSD1 in the ESCC cases with lymph node metastasis (LNM), and those without LNM. (D) The expression level of LSD1 was higher in the cases with LNM than those without LNM ($p < 0.05$).

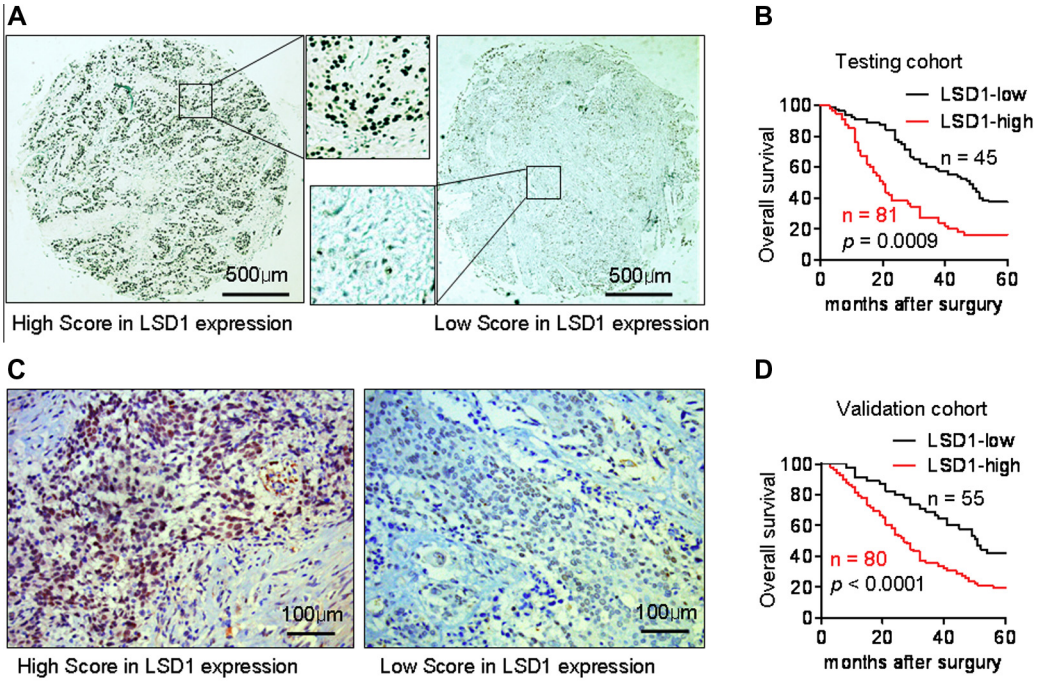

Fig. 2. LSD1 expression was predictive of prognosis in patients with ESCC. (A) The discrepancy of LSD1 expression was detectable using ESCC micro-array. (B) Kaplan–Meier curves showed the survival benefit in ESCC patients with low LSD1 expression relative to that in ESCC patients with high LSD1 expression from the micro-array. (C) LSD1 was expressed in ESCC tissue sections. (D) The survival curve derived from ESCC tissue slices was established similar to (B).

log-rank analysis indicated that patients with a higher expression of LSD1 were significantly inferior to overall survival as compared to ESCC patients with lower expression of LSD1 ($p < 0.05$, Fig. 2B and D).

3.3. Endogenous up-regulation of LSD1 promotes ESCC cell motility and migration in vitro

We investigated LSD1 expression by RT-PCR and Western blot in ESCC cell lines, including KYSE150, KYSE450 and EC109. We observed a discrepancy in LSD1 expression between the three cells lines (Fig. 3A and B). We explored the effects of endogenous LSD1 on the migration and invasion of tumor cells. In the scratch assay, KYSE450 cells migrated to the wounded area more efficiently than other two cell lines at 48 h (Fig. 3C). Similar results were confirmed in the transwell assay (Fig. 3D).

To further test whether LSD1 expression was required for ESCC cell migration and invasion, ESCC cells were transfected with a lentivirus carrying shRNA specific for LSD1 (Fig. 3E and F). As shown in Fig. 3G and H, knockdown of LSD1 significantly inhibited migration and invasion of LSD1-positive KYSE450 cells as compared with cells transfected with control shRNA.

3.4. LSD1 inhibitor, tranylcypromine, suppressed ESCC cells migration and invasion via regulating demethylation level of H3K4me2/H3K4me1

We discovered that the cells treated with tranylcypromine (TCP), which acts as an inhibitor of LSD1, induced a similar effect as knockdown of LSD1 in KYSE450 cells. With increasing concentrations of TCP, H3K4me1 expression decreased, while H3K4me2 expression increased. However, there was not a significant change in levels of H3K9me1 or H3K9me2 (Fig. 4A). Moreover, TCP suppressed the migration and invasion of KYSE450 cells (Fig. 4B–E) in a dose-dependent manner.

4. Discussion

Recently, epigenetics has become a focus of cancer research. The balance of methylation and demethylation in epigenetic modification affects gene expression and cellular activity. Previous studies have demonstrated that aberrant histone lysine methylation in cancer is associated not only with the repression of chromatin, but also with the repression of large chromosomal regions [23,24]. Epigenetic modulation of LSD1 has been shown to play a key role in carcinogenesis, in which LSD1 can prevent the accumulation of the dimethyl groups of p53 from inducing apoptosis and contributing to human carcinogenesis *via* a chromatin modification mechanism [25].

Previous reports have suggested that LSD1 could act as a biomarker for patients with aggressive phenotypes of breast cancer [16], prostate cancer [18], and neuroblastoma [26], in which LSD1 is frequently found at higher expression levels. However, only few studies have implicated LSD1 with ESCC [9]. In the current study, we found that LSD1 expression was relatively higher in ESCC tissue compared with normal esophageal epithelial or atypical epithelial hyperplastic tissues. Moreover, among the clinicopathological parameters, LSD1 expression was significantly correlated with lymph node metastasis. This finding suggests that LSD1 may be predictive of poorer prognosis in ESCC patients on the basis of the observation that ESCC patients with up-regulated LSD1 expression were associated with lymph node metastasis and inferiority for postoperative overall survival. Similarly, Lv and colleagues found that the patients with a higher LSD1 expression had a poorer prognosis in non-small cell lung cancer [20]. LSD1 has been shown to function as an androgen receptor (AR)-dependent transcription cofactors and promotes expression of androgen-dependent target genes in prostate cancer cells [16,18]. Furthermore, high expression level of LSD1 is associated with a poorer survival and is predictive of increased risk of postoperative local or metastatic recurrence in prostate cancer [16]. Therefore, these results suggested that aberrant overexpression of LSD1 may be predictive of

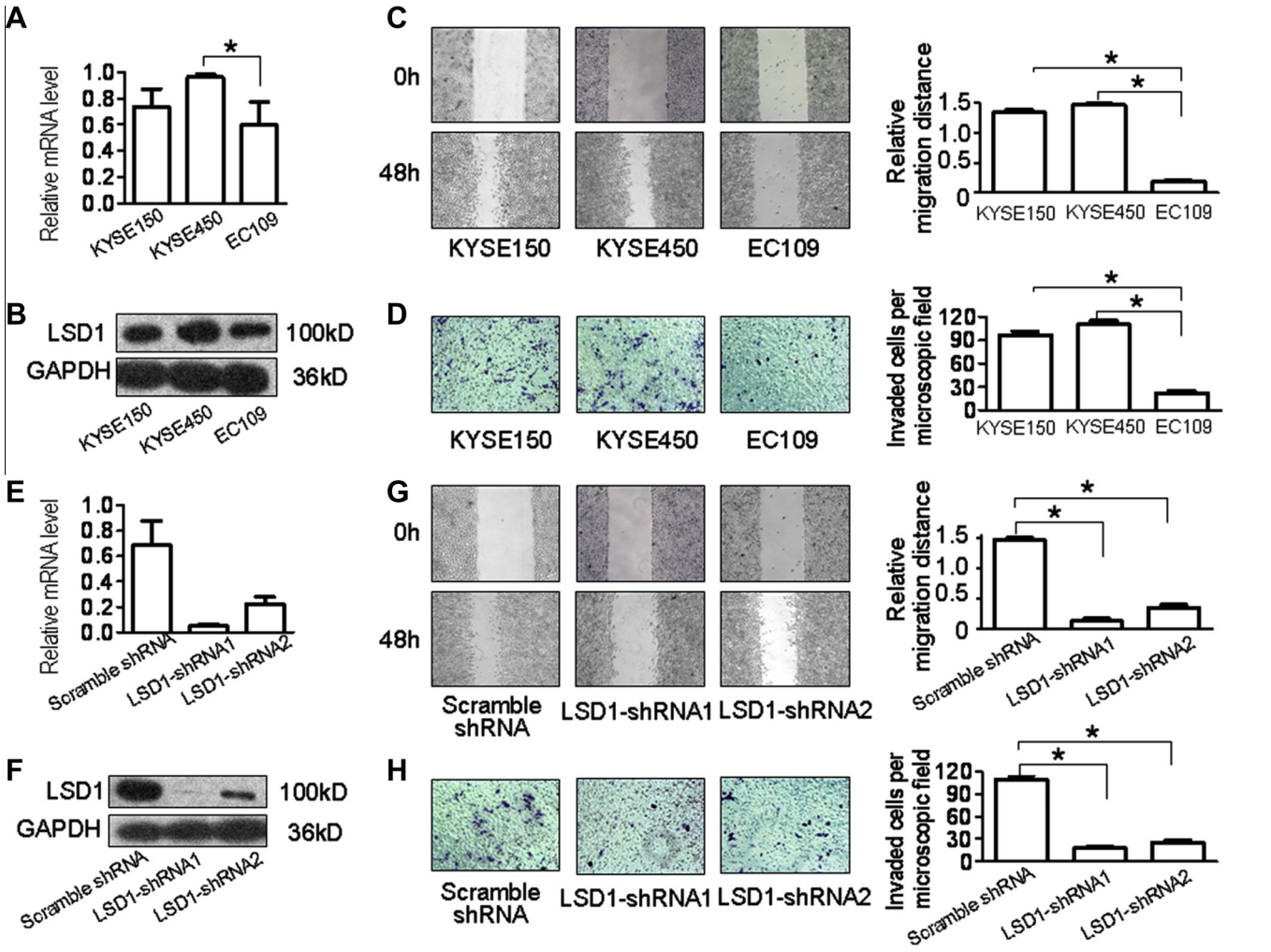

Fig. 3. LSD1 was associated with cell migration and invasion. (A and B) LSD1 was detected in KYSE150, KYSE450 and EC109 ESCC cell lines at both the RNA and protein level. (C) The scratch within KYSE450 and KYSE150 monolayers healed more rapidly compared to EC109 monolayers. (D) A transwell assay was performed in the three cell lines, and the histogram show that invaded EC109 cells were significantly fewer compared to the other two cell lines. (E and F) The expression of LSD1 was knocked down in KYSE450 cells by LSD1-shRNA1 and LSD1-shRNA2 on the basis of RT-PCR and Western blot. (G) The migration of the cells transfected with LSD1-shRNA1 and LSD1-shRNA2 compared to the control cell line. (H) According to a transwell assay, it was shown that the cells transfected with LSD1-shRNA1 and LSD1-shRNA2 featured significantly weakened invasion ability relative to control cells.

tumor metastasis and prognosis in a large variety of solid tumors, including ESCC.

We investigated the relationship between LSD1 expression and ESCC *in vitro*. In the wound and transwell assays, LSD1 knockdown lead to a sharp decrease in the motility and invasion of KYSE450 compared to control shRNA treated cells. We detected methylation of histone H3K4 in cells treated with the LSD1 inhibitor tranylcypromine. Additionally, Western blot analysis uncovered an increase in H3K4me2 and a decrease in H3K4me1 methylation, reducing the chance of transformation from H3K4me2 to H3K4me1 in a dose-dependent manner. However, H3K9 methylation was dramatically blocked in KYSE450 cells. These results implied that LSD1 may regulate downstream genes *via* demethylating H3K4me2. The invasion of KYSE450 cells was significantly attenuated with increasing concentrations of tranylcypromine. This is consistent with results previously observed in other tumors [20]. Therefore, it is likely that the demethylation of LSD1 is important in promoting tumor invasion, and tranylcypromine may be of therapeutic value for ESCC patients.

The aberrant over-expression of LSD1 in ESCC may be a good candidate for a novel therapeutic molecular target [12]. First-generation drugs targeting the relatively promiscuous DNA methylation and histone acetylation modifiers have had success in the treatment of hematological tumors. If LSD1 inhibition leads to significant derepression of some genes, LSD1 may be an important alternative target for therapy. In addition, development of histone methyltransferase and demethylase inhibitors has recently been reported [27]. In ESCC, over-expression of LSD1 should contribute to gene repression that promotes cellular growth and malignant progression. We plan to investigate this in future studies. Currently, there is vast knowledge concerning the roles of histone demethylation in regulating biology of esophageal cancers. Our study demonstrated that high LSD1 expression may modulate expression of downstream genes through promoting demethylation of H3K4me2 in ESCC cells. This function of LSD1 may facilitate metastasis, leading to poor prognosis of patients with ESCC. LSD1-targeting shRNAs and the LSD1 inhibitors may reduce cell invasion. However, the specific downstream target genes of LSD1 in esophageal cancer cells remain unclear.

A potential limitation of our study is that the sample size is relatively small, which affects the ability of this study to represent a wider population. However, this study suggests that LSD1 may have predictive prognostic value in patients with ESCC. A randomized clinical trial (RCT) is necessary to detect the potential

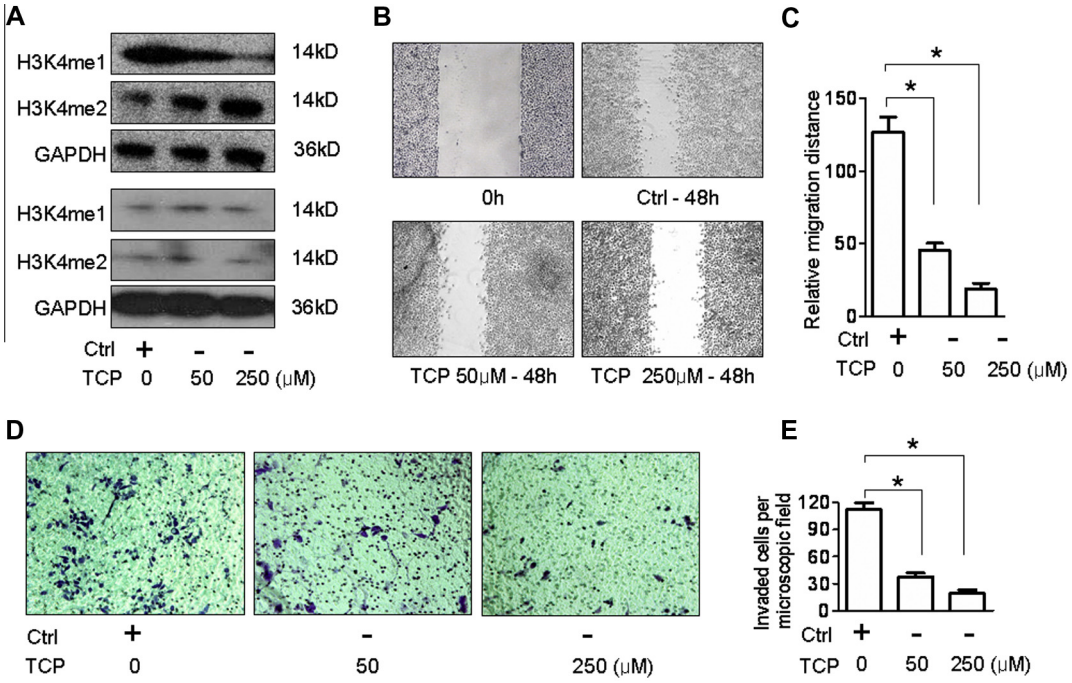

Fig. 4. The effects of LSD1 inhibitor, tranylcypromine on the migration and invasiveness of ESCC cells. (A) After treating the cells with 50 μM and 250 μM tranylcypromine (TCP) for 24 h we found H3K4me1 was decreased in parallel with the increasing TCP concentration, while H3K4me2 was increased. But there were no significant difference in H3K9me1 and H3K9me2 expression. (B and C) The scratch assay was performed in KYSE450 cells treated with vehicle control or with TCP (50 μM or 250 μM). The relative width of the trace was measured at 48 h. (D and E) According to the transwell assay, the invasiveness of KYSE450 cells after 24 h was inhibited by TCP in a dose-dependent manner.

mechanism by which LSD1 induces tumorigenesis. Further studies are designed to reveal the regulatory mechanisms of LSD1 in ESCC, and to find novel molecular targets that reduce postoperative recurrence and metastasis in patients with ESCC.

Acknowledgments

We would like to thank Miss Xiewan Chen and Ms. Xiaoqing Zhan (Medical English Department, Third Military Medical University, China) for a critical reading of the manuscript. This project was supported by the National Natural Science Foundation of China (NSFC Nos. 81101631 and 81201949), the Natural Science Foundation Project of CQ CSTC (CSTC2012JJA10124), and the Science Foundation of the Third Military Medical University (Nos. 2012XJQ22 and 2012XJQ21).

References

[1] R. Siegel, C. DeSantis, K. Virgo, K. Stein, A. Mariotto, T. Smith, D. Cooper, T. Gansler, C. Lerro, S. Fedewa, C. Lin, C. Leach, R.S. Cannady, H. Cho, S. Scoppa, M. Hachey, R. Kirch, A. Jemal, E. Ward, Cancer treatment and survivorship statistics, CA Cancer J. Clin. 62 (2012) (2012) 220–241.

[2] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, CA Cancer J. Clin. 62 (2012) (2012) 10–29.

[3] Z.K. Zhao, P. Dong, J. Gu, L. Chen, M. Zhuang, W.J. Lu, D.R. Wang, Y.B. Liu, Overexpression of LSD1 in hepatocellular carcinoma: a latent target for the diagnosis and therapy of hepatoma, Tumour Biol. 34 (2013) 173–180.

[4] R. Lambert, Endoscopy in screening for digestive cancer, World J. Gastrointest Endosc. 4 (2012) 518–525.

[5] S. Kolahdoozan, A. Sadjadi, A.R. Radmard, H. Khademi, Five common cancers in Iran, Arch. Iran Med. 13 (2010) 143–146.

[6] A.M. Kaz, W.M. Grady, Epigenetic biomarkers in esophageal cancer, Cancer Lett. (2012) [Epub ahead of print].

[7] R.A. Varier, H.T. Timmers, Histone lysine methylation and demethylation pathways in cancer, Biochim. Biophys. Acta 2011 (1815) 75–89.

[8] Y. Fang, F. Xiao, Z. An, L. Hao, Systematic review on the relationship between genetic polymorphisms of methylenetetrahydrofolate reductase and esophageal squamous cell carcinoma, Asian Pac. J. Cancer Prev. 12 (2011) 1861–1866.

[9] W.Y. Cheung, G. Liu, Genetic variations in esophageal cancer risk and prognosis, Gastroenterol Clin. North Am. 38 (2009) 75–91. viii.

[10] Y. Shi, F. Lan, C. Matson, P. Mulligan, J.R. Whetstine, P.A. Cole, R.A. Casero, Histone demethylation mediated by the nuclear amine oxidase homolog LSD1, Cell 119 (2004) 941–953.

[11] J. Wang, S. Hevi, J.K. Kurash, H. Lei, F. Gay, J. Bajko, H. Su, W. Sun, H. Chang, G. Xu, F. Gaudet, E. Li, T. Chen, The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation, Nat. Genet. 41 (2009) 125–129.

[12] S. McGraw, C. Vigneault, M.A. Sirard, Temporal expression of factors involved in chromatin remodeling and in gene regulation during early bovine in vitro embryo development, Reproduction 133 (2007) 597–608.

[13] Q. Wang, X. Xu, J. Li, J. Liu, H. Gu, R. Zhang, J. Chen, Y. Kuang, J. Fei, C. Jiang, P. Wang, D. Pei, S. Ding, X. Xie, Lithium, an anti-psychotic drug, greatly enhances the generation of induced pluripotent stem cells, Cell Res. 21 (2011) 1424–1435.

[14] N. Sakane, H.S. Kwon, S. Pagans, K. Kaehlcke, Y. Mizusawa, M. Kamada, K.G. Lassen, J. Chan, W.C. Greene, M. Schnoelzer, M. Ott, Activation of HIV transcription by the viral Tat protein requires a demethylation step mediated by lysine-specific demethylase 1 (LSD1/KDM1), PLoS Pathog. 7 (2011) e1002184.

[15] S. Hayami, J.D. Kelly, H.S. Cho, M. Yoshimatsu, M. Unoki, T. Tsunoda, H.I. Field, D.E. Neal, H. Yamaue, B.A. Ponder, Y. Nakamura, R. Hamamoto, Overexpression of LSD1 contributes to human carcinogenesis through chromatin regulation in various cancers, Int. J. Cancer 128 (2011) 574–586.

[16] S. Lim, A. Janzer, A. Becker, A. Zimmer, R. Schule, R. Buettner, J. Kirfel, Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology, Carcinogenesis 31 (2010) 512–520.

[17] L. Gao, J. Alumkal, Epigenetic regulation of androgen receptor signaling in prostate cancer, Epigenetics 5 (2010) 100–104.

[18] P. Kahl, L. Gullotti, L.C. Heukamp, S. Wolf, N. Friedrichs, R. Vorreuther, G. Solleder, P.J. Bastian, J. Ellinger, E. Metzger, R. Schule, R. Buettner, Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence, Cancer Res. 66 (2006) 11341–11347.

[19] Y. Wang, H. Zhang, Y. Chen, Y. Sun, F. Yang, W. Yu, J. Liang, L. Sun, X. Yang, L. Shi, R. Li, Y. Li, Y. Zhang, Q. Li, X. Yi, Y. Shang, LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer, Cell 138 (2009) 660–672.

[20] T. Lv, D. Yuan, X. Miao, Y. Lv, P. Zhan, X. Shen, Y. Song, Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer, Plos One 7 (2012) e35065.

[21] X. Hu, X. Li, K. Valverde, X. Fu, C. Noguchi, Y. Qiu, S. Huang, LSD1-mediated epigenetic modification is required for TAL1 function and hematopoiesis, Proc. Natl. Acad. Sci. USA 106 (2009) 10141–10146.

[22] W. Remmele, U. Hildebrand, H.A. Hienz, P.J. Klein, M. Vierbuchen, L.J. Behnken, B. Heicke, E. Scheidt, Comparative histological, histochemical, immunohistochemical and biochemical studies on oestrogen receptors, lectin receptors, and Barr bodies in human breast cancer, Virchows Arch. A Pathol. Anat. Histopathol. 409 (1986) 127–147.

[23] J.T. Lynch, W.J. Harris, T.C. Somervaille, LSD1 inhibition: a therapeutic strategy in cancer?, Expert Opin Ther. Targets 16 (2012) 1239–1249.

[24] S. Lv, W. Bu, H. Jiao, B. Liu, L. Zhu, H. Zhao, J. Liao, J. Li, X. Xu, LSD1 is required for chromosome segregation during mitosis, Eur. J. Cell Biol. 89 (2010) 557–563.

[25] J. Huang, R. Sengupta, A.B. Espejo, M.G. Lee, J.A. Dorsey, M. Richter, S. Opravil, R. Shiekhattar, M.T. Bedford, T. Jenuwein, S.L. Berger, P53 is regulated by the lysine demethylase LSD1, Nature 449 (2007) 105–108.

[26] J.H. Schulte, S. Lim, A. Schramm, N. Friedrichs, J. Koster, R. Versteeg, I. Ora, K. Pajtler, L. Klein-Hitpass, S. Kuhfittig-Kulle, E. Metzger, R. Schule, A. Eggert, R. Buettner, J. Kirfel, Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy, Cancer Res. 69 (2009) 2065–2071.

[27] A. Spannhoff, A.T. Hauser, R. Heinke, W. Sippl, M. Jung, The emerging therapeutic potential of histone methyltransferase and demethylase inhibitors, ChemMedChem 4 (2009) 1568–1582.