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LSD1-mediated epigenetic modification contributes to proliferation and metastasis of colon cancer

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Background: Emerging evidence has demonstrated that lysine-specific demethylase 1 (LSD1) has an important role in many pathological processes of cancer cells, such as carcinogenesis, proliferation and metastasis. In this study, we characterised the role and molecular mechanisms of LSD1 in proliferation and metastasis of colon cancer.

Methods: We evaluated the correlation of LSD1, CDH-1 and CDH-2 with invasiveness of colon cancer cells, and investigated the roles of LSD1 in proliferation, invasion and apoptosis of colon cancer cells. We further investigated the mechanisms of LSD1-mediated metastasis of colon cancer.

Results: Lysine-specific demethylase 1 was upregulated in colon cancer tissues, and the high LSD1 expression was significantly associated with tumour-node-metastasis (TNM) stages and distant metastasis. Functionally, inhibition of LSD1 impaired proliferation and invasiveness, and induced apoptosis of colon cancer cells *in vitro*. The LSD1 physically interacted with the promoter of *CDH-1* and decreased dimethyl histone H3 lysine 4 (H3K4) at this region, downregulated CDH-1 expression, and consequently contributed to colon cancer metastasis.

Conclusion: Lysine-specific demethylase 1 downregulates the expression of CDH-1 by epigenetic modification, and consequently promotes metastasis of colon cancer cells. The LSD1 antagonists might be a useful strategy to suppress metastasis of colon cancer.

Colon cancer is the third most common malignancy worldwide (Center *et al*, 2009; Cui *et al*, 2011) and the second leading cause of cancer deaths in the United States (American Cancer Society, 2012), recurrence and metastasis are the leading cause of death among colon cancer patients. Epithelial-mesenchymal transition (EMT) is now considered to be the initial and necessary step in the metastatic cascade. During EMT, epithelial cells acquire fibroblast-like characteristics, including loss of cell polarity, reduced intercellular adhesion, increased motility and invasive capacity (Boyer *et al*, 2000). Transcription factors, such as snai1, slug, zeb1, zeb2 and twist, can promote EMT by suppressing CDH-1 (E-cadherin) expression (Kang and Massagué, 2004; Waldmann *et al*, 2008; Kato *et al*, 2010; Shah and Kakar, 2011) and consequently contribute to cancer metastasis.

Lysine-specific demethylase 1 (LSD1), the first discovered histone demethylase, is required in Snail/Slug-mediated transcriptional repression during EMT, in the absence of LSD1, Snail and Slug fail to repress CDH-1 transcription (Lin *et al*, 2010; Baron *et al*, 2011; Ferrari-Amorotti *et al*, 2013). The Snail/Gfi-1 (SNAG) domain of Snail/Slug assembles a histone H3-like structure and serves as a molecular 'hook' to interact with the LSD1–CoREST complex, and brings this complex to its targeted gene promoters through the binding of the E-box through the zinc-finger motifs (Lin *et al*, 2010; Ferrari-Amorotti *et al*, 2013). The LSD1/CoREST complex functions as a reversible nanoscale binding clamp, recruits and anchors a variety of substrate peptides with high sequence similarity to the H3-histone tail (Hwang *et al*, 2011; Baron and Vellore, 2012a, b).

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As a lysine-specific demethylase belonging to the flavin-dependent amine oxidase family, LSD1 specifically catalysed the demethylation of mono- and di-methylated histone H3 lysine 4 (H3K4) and H3 lysine 9 (H3K9) through a redox process. Overexpression of LSD1 promotes proliferation, migration and invasion of various cancer cells (Cho *et al*, 2011; Lv *et al*, 2012). And knocking down of LSD1 with small-interfering RNAs (siRNAs) resulted in suppression of proliferation and metastasis of various cancer cells (Hayami *et al*, 2011; Lv *et al*, 2012; Pollock *et al*, 2012; Willmann *et al*, 2012; Jin *et al*, 2013; Zhao *et al*, 2013).

Our previous studies (Ding *et al*, 2013) have shown that LSD1 had significantly higher expression, in contrast to the significantly lower expression of CDH-1, in colon cancer at high tumour-node-metastasis (TNM) stages (Edge *et al*, 2010) and with distant metastasis. Positive expression of LSD1 and negative expression of CDH-1 may predict a worse prognosis of colon cancer. We speculated that LSD1 may promote metastasis of colon cancer by decreasing the level of dimethylated histone H3 lysine4 (H3K4m2) at the *CDH-1* promoter and repressing *CDH-1* transcription, which requires confirmation by further *in vitro* experiments. Therefore, we attempted to investigate the expression of LSD1, CDH-1 and CDH-2 (N-cadherin) in several colon cancer cell lines, and to analyse their relationship with proliferation and invasion abilities of colon cancer, we also aimed to determine the mechanism of colon cancer metastasis regulated by LSD1.

MATERIAL AND METHODS

Immunohistochemical staining. The archival formalin-fixed and paraffin wax-embedded tissue blocks of 108 colon cancer and 30 normal colon mucosa removed by surgery from 2006 to 2008 were retrieved from the Department of Pathology, Xiangya Hospital, Central South University. Primary antibodies were directed towards LSD1 (rabbit monoclonal, 1 : 100; R&D Systems, Minneapolis, MN, USA). Serial sections of 5 μm were cut from the tissue blocks, deparaffinised in xylene, and hydrated in a graded series of alcohol. Staining was then performed using the EnVision + anti-rabbit system (Dako Corporation, Carpinteria, CA, USA). Negative control staining was carried out by substituting non-immune rabbit and phosphate-buffered saline (PBS) for the primary antibodies. This study was conducted with the approval of the Ethics Committee of Xiangya Hospital, China.

Cell lines and cell culture. Colon cancer cell lines used in this study were purchased from American Type Culture Collection (Sigma-Aldrich Corp., St Louis, MO, USA). These cells include Lovo, SW620, HT-29, HCT-8 and HCT-116. HEK293 cell was obtained from Cancer Research Institute of Central South University, China. HT-29 cells and HCT-8 cells were cultured with RPMI-1640 medium (Sigma-Aldrich Corp.), Lovo cells were cultured with F-12K medium (Sigma-Aldrich Corp.), SW620 cells were cultured with L-15 medium (Sigma-Aldrich Corp.), HCT-116 cells were cultured with McCoy's5a medium (Sigma-Aldrich Corp.) and HEK293 cells were cultured with Modified Eagle's minimal essential medium (Sigma-Aldrich Corp.). All media were supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Biowest, Nuaillé, France). All of the cell lines were grown in 5% CO_2 at 37 °C in incubators with 100% humidity.

Real-time reverse transcription–PCR. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). Gene expression of LSD1 was monitored by real-time PCR using 'Assays on Demand' (Applied Biosystems, Alameda, CA, USA). Expression values were normalised to the geometric mean of GAPDH. The primers that used to amplify the cDNA were as follows: F: 5′-CCTGAAGAACC ATCGGGTGT-3′, R: 5′-CCTTCTGGGTCTGTTGTGGT-3′ for LSD1; F: 5′-TCATGAGTGTCCCCCGGTAT-3′, R: 5′-TCTTGAA GCGATTGCCCC AT-3′ for *CDH-1*; F: 5′-CCTTTCAAACA CAGCCACGG-3′, R: 5′-TGTTTGGGT CGGTCTGGA TG-3′ for *CDH-2*; and F: 5′-CTCATGACCACAGTCCATGC-3′, R: 5′-TT CAGCTCTGGGATGACCTT-3′ for GAPDH.

Western blot analysis. Protein lysates were extracted from cells and blotted as described previously (Kahl *et al*, 2006). The membranes were incubated for 1–2 h using the following antibodies and dilutions: LSD1, 1 : 1000; CDH-1, 1 : 2000; CDH-2, 1 : 2000.

Small interference RNA and transfection. Cells were seeded with 5×10^4 cells in 24-well plates, then incubated for 2–4 days in standard medium in the presence of 10–20 nmol l^{-1} siRNA directed against LSD1. The siRNA LSD1 sequences used in this study were as follows: siLSD#1 (sense: 5′-GCCACCCAGA GAUAUUACUTT-3′, anti-sense: 5′-AGUAAUAUCUCUGGGUG GCTT-3′); siLSD#2 (sense: 5′-CCGGAUGACUUCUCAAGAATT -3′, anti-sense: 5′-UUCUUGAGAAGUCAUCCGGTT-3′); siLSD#3 (sense: 5′-CCACGAGUCAAAC CUUUAUTT-3′, anti-sense: 5′-AUAAAGGUUUGACUCGUGGTT-3′); or control siRNA (sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, anti-sense: 5′-ACGUGACACGUUCGGAGAATT-3′). Cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Invasion assay. Invasion assays were performed as previously described (Wu *et al*, 2009; Lin *et al*, 2010). Briefly, invasion chambers were coated with BD Matrigel matrix according to the manufacturer's protocol (BD Biosciences, San Jose, CA, USA). Cancer cells were seeded on top of the Matrigel in the upper chamber, and the bottom chamber was filled with culture medium containing chemoattractant. Cells that invade through the Matrigel-coated membrane after 24 h were fixed with paraformaldehyde, followed by staining with crystal violet. All experiments were conducted at least three times in triplicate.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide growth inhibition assay. Cells were seeded at a density of 5×10^4 per well and cultured in standard medium, replaced daily. Treatment with siRNA directed against LSD1, and tranylcypromine (Sigma-Aldrich) was accomplished as indicated. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the manufacturer's protocol (Bioassay Systems, Hayward, CA, USA).

Apoptosis assay. Apoptosis assay was performed as previously described (Gorczyca *et al*, 1998). According to the manufacturer's instructions, 2×10^5 cells were collected and washed twice in PBS. Cells were then resuspended in binding buffer and stained with AnnexinV-FITC and propidium iodide (Promab, Hunan, China). Stained cells were analysed with a flow cytometer (BD FACSAria; BD Biosciences). The measurements were performed independently for at least three times with similar results.

Chromatin immunoprecipitation and histone demethylation assay. Chromatin immunoprecipitation (ChIP) analysis was performed using Protein A and Protein G Dynabeads (Invitrogen) as previously reported (McGarvey *et al*, 2006). Cells were exposed to 1% formaldehyde to crosslink proteins, and 1.0×10^7 cells were used for each ChIP assay. The antibodies against H3 and LSD1 were from R&D Systems, and antibodies against H3K4m2 were from Invitrogen. Quantitative ChIP was performed using qPCR on the ABI PRISM 7900 real-time PCR detection system (Applied Biosystems). Primer sequences for qPCR of *CDH-1* promoter for ChIP were as follows: F: 5′-AGTCCCACAACAGCATAGGG-3′, R: 5′-TTCTGAACTCAGGCGATCCT-3′. Sheared genomic DNA

was used as a positive control (input) and for the normalisation of DNA immunoprecipitated by LSD1.

Statistical analysis. Statistical analysis was performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). Statistical analysis was performed with Student's *t*-test and ANOVA. Values of $P<0.05$ were considered to be statistically significant.

RESULTS

LSD1 expression is upregulated in colon cancer tissues. We first examined the expression levels of LSD1 in 108 colon cancer tissues and 30 normal colon tissues by immunohistochemistry (IHC) obtained in Xiangya Hospital, Central South University. High LSD1 expression was detected in the nuclei of malignant cells, while low staining was observed in any of non-neoplastic tissues (Figure 1). Specifically, the expression of LSD1 was observed in 67% (72 out of 108) of colon cancer tissues and 37% (11 out of 30) of normal colon mucosa, indicating a significant elevation of LSD1 expression in tumours compared with normal control tissues ($P=0.005$, Table 1). High levels of LSD1 expression (score: '++' to '+++') were detected in 52 (48%) tumour tissues and 5 (17%) benign colon tissues, respectively. As shown in Table 1, there was no significant correlation of LSD1 expression with gender, age and Broders' classification ($P>0.05$). However, the expression of LSD1 was significantly more in colon cancer with high TNM stages and distant metastasis ($P<0.05$). Therefore, we hypothesised that LSD1 may have an important role in metastasis of colon cancer.

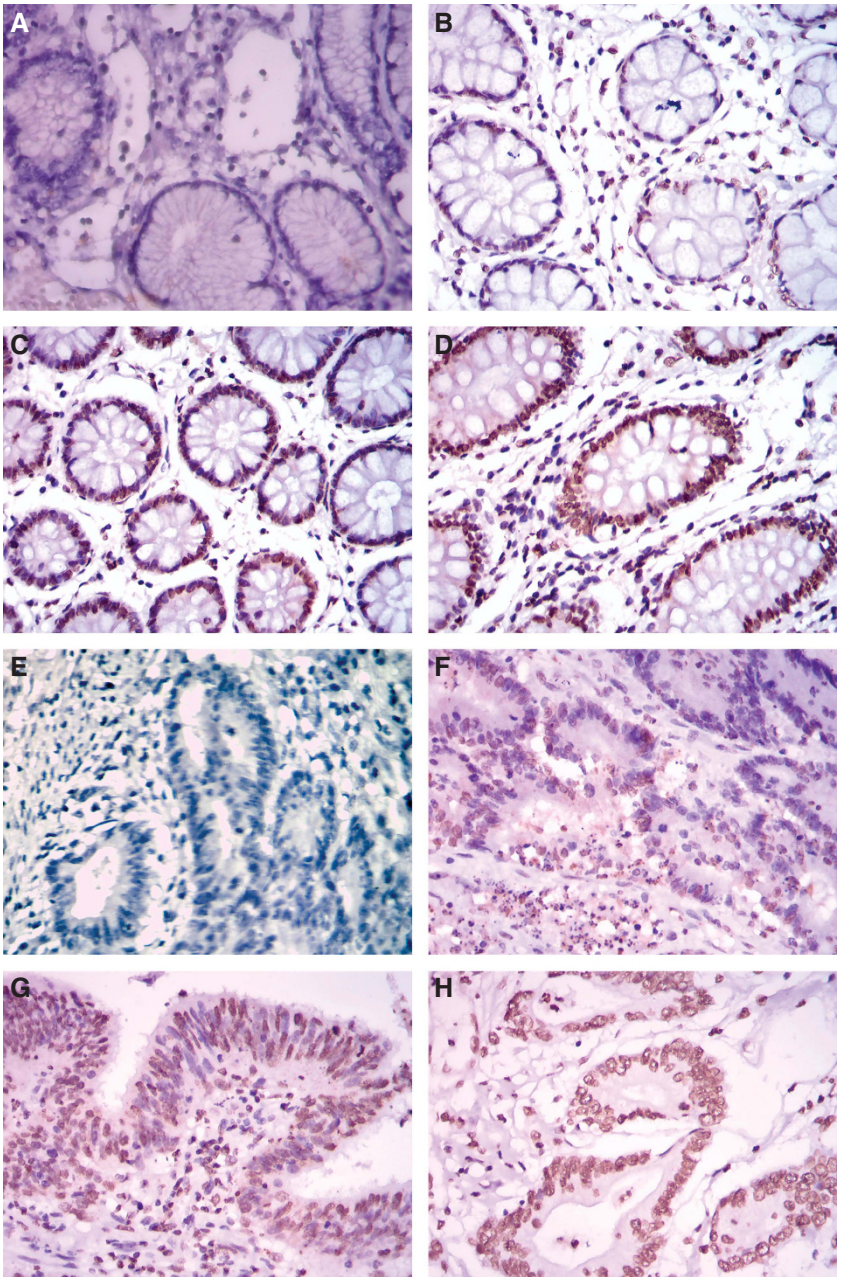


Figure 1. (**A–D**) Representative LSD1 staining in normal control specimens. (**A**) Negative, (**B**) weak, (**C**) moderate and (**D**) strong. (**E–H**) Representative LSD1 staining in colon cancer specimens. (**E**) Negative, (**F**) weak and (**G**) moderate and (**H**) strong. LSD1 was positively expressed in 37% (11 out of 30) of control specimens and 67% (72 out of 108) of colon cancer specimens, respectively.

Table 1. Statistical analysis of LSD1 expression levels in colon cancer and control specimens

		LSD1 (+)			
Item	**Case (*n*)**	***n***	**%**	χ^2	***P*-value**
Origin of specimens					
Colon cancer	108	72	67		
Normal control tissues	30	11	37	7.61	0.005
Gender					
Male	65	42	72		
Female	43	30	81	0.31	0.578
Age					
<50 years	37	26	84		
⩾50 years	71	46	72	0.33	0.566
TNM stage					
Stage I	19	7	37		
Stage II	34	23	68		
Stage III	47	34	72		
Stage IV	8	8	100	12.3	0.006
Broders' classification					
Grade I	18	8	44		
Grade II	58	41	71		
Grade III	26	18	69		
Grade IV	6	5	83	5.25	0.154
Distant metastasis					
Yes	8	8	100		
No	100	64	64	4.32	0.038

Abbreviations: LSD1 = lysine-specific demethylase 1; TNM = tumour-node-metastasis.

Correlation of LSD1, CDH-1 and CDH-2 with invasiveness of colon cancer cell lines. To evaluate the correlation of LSD1, CDH-1 and CDH-2 with invasiveness of colon cancer, we detected their expression in colon cancer cells by RT–PCR and western blot. The results showed that the mRNA and protein levels of LSD1 and CDH-2 in SW620 cell line were significantly higher than those in the Lovo, HT-29, HCT-8, HCT-116 and HEK293 (control) cell lines. However, the levels of CDH-1 in SW620 cell line were evidently lower than the other five cell lines ($P<0.05$, Figure 2A–C).

Using Transwell Invasion Assay, we detected the invasiveness of six cell lines. The ability to cross matrigel indicated the invasiveness of cancer cells. Interestingly, the amount of invading cells crossing the matrigel basement membrane was evidently higher in SW620 cell line than that in the other five cell lines ($P<0.05$, Figure 2D and E). Linear correlation was used to analyse the correlation between expression of LSD1, CDH-1, CDH-2 in six cell lines and invasiveness of the six cell lines due to normal distribution data ($Z=0.727$, $P=0.665$), and the results showed that the protein and mRNA levels of LSD1 and CDH-2 correlated positively with the invasiveness of colon cancer cells, whereas that of CDH-1 exhibited a negative correlation with the invasiveness of colon cancer cells ($P<0.05$, Tables 2 and 3; Figure 2F–K).

Inhibition of LSD1 impairs proliferation and invasiveness, and induces apoptosis of colon cancer cells *in vitro*. We performed a knockdown experiment using siRNAs targeting LSD1, and tranylcypromine, a chemical inhibitor for LSD1 (Karytinos *et al*, 2009; Culhane *et al*, 2010; Schenk *et al*, 2012), to further investigate the roles of LSD1 in proliferation and invasion of colon cancer cells. Considering SW620 cell line was more aggressive than the other cell lines, it was used to perform the following experiment. First, three independent siRNAs targeting LSD1 (siLSD#1, #2 and #3) were transfected to SW620 cells to detect gene-silencing efficiency. As shown in Figure 3, the knockdown effect of siLSD#2 were better than that of the other two siRNAs at both mRNA and protein levels. Then, the MTT Cell Proliferation Assay was used for evaluation of optimal drug concentration in response to tranylcypromine. The cellular proliferation declined along with tranylcypromine treatment in a concentration-dependent manner. And it was notably lower in the 2.5 mM-, 5 mM- and 10 mM-treated groups than in the 0.5 mM- or 1 mM-treated groups ($P<0.05$). However, there was no significant difference between the 0.5 mM- and 1 mM-treated groups, or the 2.5 mM-, 5 mM- and 10 mM-treated groups ($P>0.05$, Figure 3D).

Using the siLSD#2 and tranylcypromine (2.5 mM), we performed Transwell Invasion Assay, Cell Proliferation Assay and Apoptosis Assay in SW620 cell lines, and found a significant suppression of invasion and growth, and induced cell apoptosis by siRNA and tranylcypromine ($P<0.05$, Figure 3E–I). No suppressive effect was found when we used control siRNAs (Figure 3J–L). Interestingly, upregulation of CDH-1 and downregulation of CDH-2 were observed after treated with siLSD#2 and tranylcypromine for 72 h ($P<0.05$, Figure 3J–L). However, the LSD1 level of SW620 cells remained almost the same after treated with tranylcypromine, which might demonstrate that tranylcypromine cannot downregulate the expression level, but inhibit the enzymatic activity of LSD1.

LSD1 regulates EMT via demethylation of *CDH-1* gene. Given that inhibition of LSD1 accompanied by upregulation of CDH-1 and downregulation of invasiveness of colon cancer, we speculated that LSD1 can promote metastasis of colon cancer by downregulating CDH-1 expression. To assess whether the promoter of *CDH-1* is directly regulated by LSD1, which consequently would lead to an enrichment of activating histone marks, ChIP analysis was performed using anti-LSD1 and anti-H3K4m2 antibodies in SW620 cells, LSD1-silenced SW620 cells and tranylcypromine-treated SW620 cells (transfected with siLSD#2 or treated with tranylcypromine for 48 h). The results confirmed that LSD1 is present at the proximal promoter of *CDH-1* in cells of all the three groups, and quantitative analysis revealed that the enrichment of LSD1 at the proximal promoter of *CDH-1* was significantly higher in SW620 and tranylcypromine-treated SW620 cells than in LSD1-silenced SW620 cells (Figure 4A–C), which was in accordance with invasiveness of SW620 cells and LSD1-silenced SW620 cells, but was inconsistent with invasiveness of tranylcypromine-treated SW620 cells. We further investigate whether tranylcypromine influenced the enzymatic activity of LSD1.

Both di- and tri-methylated H3K4 are associated with active transcription (Kouzarides, 2007; Li *et al*, 2007). Using an antibody specific for dimethyl H3K4 (H3K4m2), we detected relatively high levels of H3K4m2 at the promoter of the *CDH-1* gene in LSD1-silenced SW620 cells and tranylcypromine-treated SW620 cells, and a significant decrease in this active mark specifically at the promoter region in SW620 cells (Figure 4D and E). Therefore, we conclude that the expression of LSD1 leads to a specific decrease in H3K4m2 at *CDH-1* promoters, downregulates the CDH-1 expression, and consequently contributes to colon cancer metastasis; Tranylcypromine cannot downregulate the protein level, but suppress the enzymatic activity of LSD1 at the proximal promoter of *CDH-1*. Downregulation of protein level or inhibition of enzymatic activity of LSD1 can prevent metastasis of colon cancer

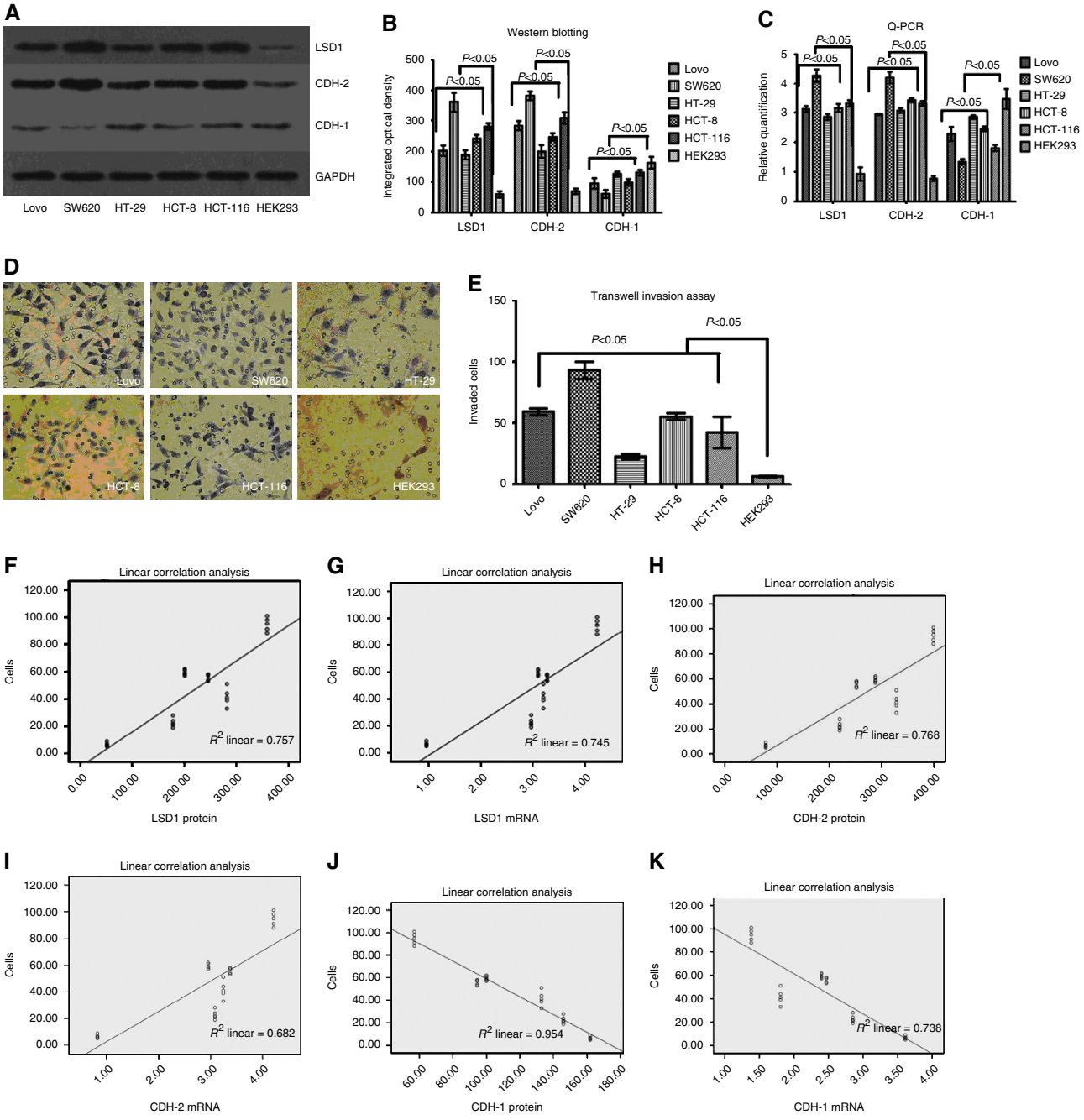


Figure 2. LSD1 and CDH-2 expression correlated positively with the invasiveness of colon cancer cells, whereas the CDH-1 exhibited a negative correlation with the invasiveness of colon cancer cells. (**A–C**) LSD1 and CDH-2 had significantly higher expression, in contrast to the significantly lower expression of CDH-1 in SW620 cells than the other cells. (**D** and **E**) SW620 cells exhibited significantly higher invasive ability. Bars represent mean ± standard deviation of three independent experiments. (**F–K**) The protein and mRNA levels of LSD1 and CDH-2 correlated positively with the invasiveness of colon cancer cells, whereas that of CDH-1 exhibited a negative correlation with the invasiveness of colon cancer cells.

Table 2. Correlation analysis (Linear correlation) between the protein levels of LSD1, CDH-1 and CDH-2 and invasiveness of six cell lines

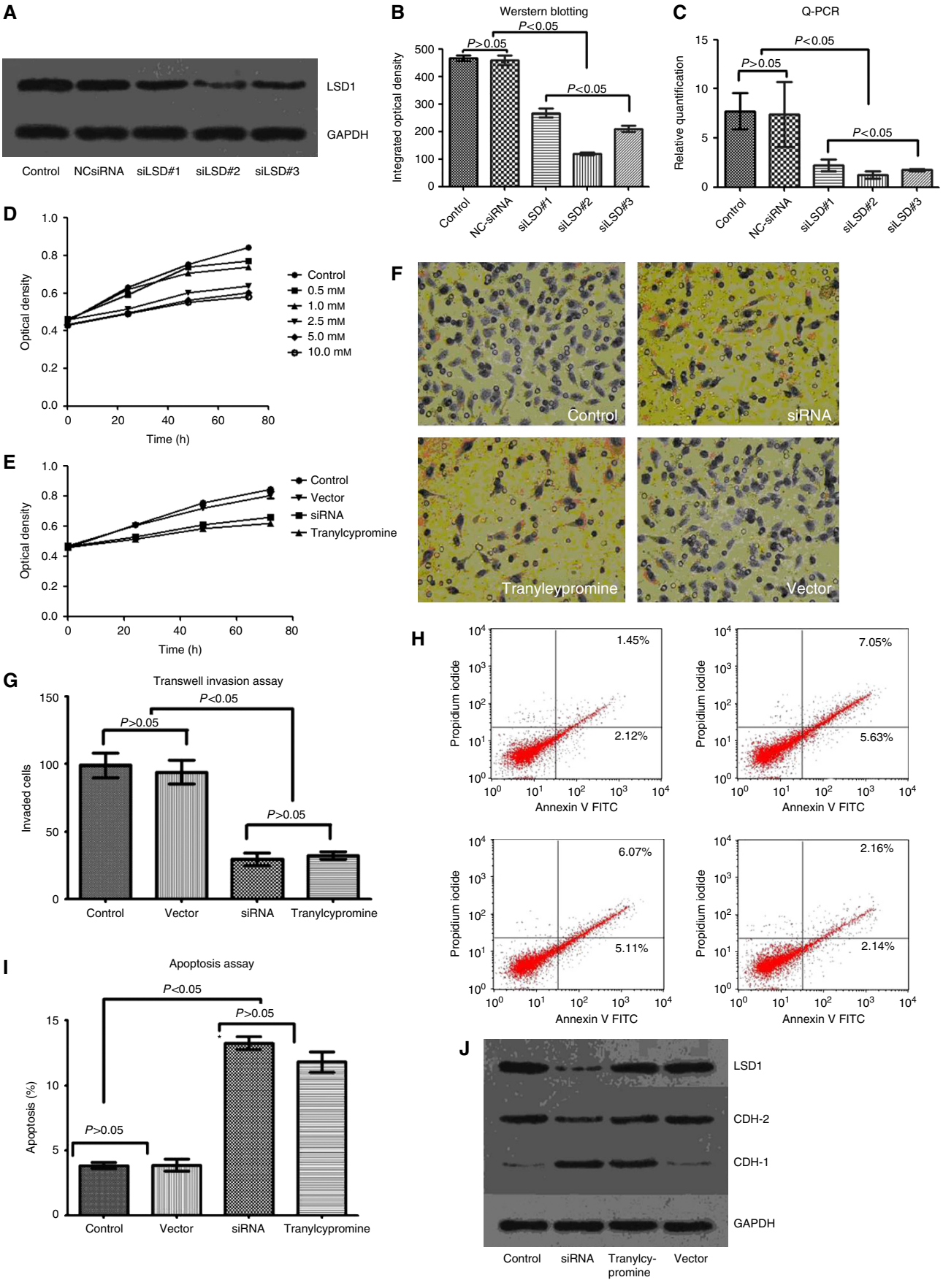
Item	β	F	P
LSD1	0.87	87.28	<0.05
CDH-2	0.88	92.48	<0.05
CDH-1	−0.98	87.28	<0.05

Abbreviation: LSD1 = lysine-specific demethylase 1.

Table 3. Correlation analysis (Linear correlation) between the mRNA levels of LSD1, CDH-1 and CDH-2 and invasiveness of six cell lines

Item	β	F	P
LSD1	0.86	81.74	<0.05
CDH-2	0.83	60.06	<0.05
CDH-1	−0.86	79.05	<0.05

Abbreviation: LSD1 = lysine-specific demethylase 1.


A
LSD1
GAPDH
Control NCsiRNA siLSD#1 siLSD#2 siLSD#3
B
Werstern blotting
P<0.05
P>0.05
P<0.05
Integrated optical density
Control NC-siRNA siLSD#1 siLSD#2 siLSD#3
C
Q-PCR
P<0.05
P>0.05
P<0.05
Relative quantification
Control NC-siRNA siLSD#1 siLSD#2 siLSD#3
D
Optical density
Time (h)
Control
0.5 mM
1.0 mM
2.5 mM
5.0 mM
10.0 mM
E
Optical density
Time (h)
Control
Vector
siRNA
Tranylcypromine
F
Control
siRNA
Tranyleypromine
Vector
G
Transwell invasion assay
P<0.05
P>0.05
P>0.05
Invaded cells
Control Vector siRNA Tranylcypromine
H
Propidium iodide
Annexin V FITC
1.45%
2.12%
7.05%
5.63%
6.07%
5.11%
2.16%
2.14%
I
Apoptosis assay
P<0.05
P>0.05
P>0.05
Apoptosis (%)
Control Vector siRNA Tranylcypromine
J
LSD1
CDH-2
CDH-1
GAPDH
Control siRNA Tranylcy-promine Vector

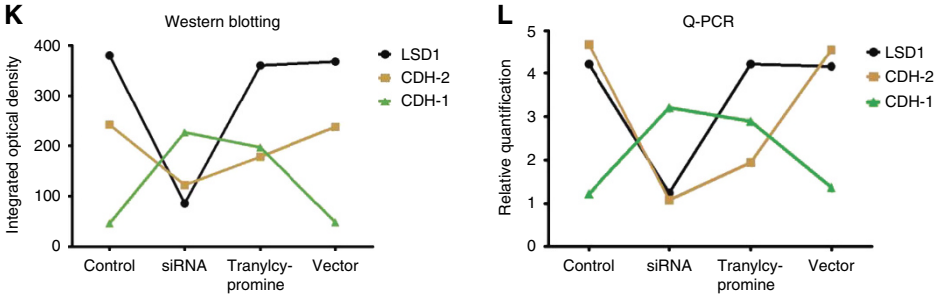


Figure 3. Inhibition of LSD1 impairs proliferation and invasiveness of colon cancer cells. (**A–C**) The knockdown effect of siLSD#2 was more efficient than the other two siRNAs at protein and mRNA levels. (**D**) Cellular proliferation declined along with tranylcypromine treatment in a concentration-dependent manner, and it was notably lower in the 2.5 mM-, 5 mM- and 10 mM-treated groups than in the 0.5 mM- or 1 mM-treated groups. (**E**) Cell Proliferation Assay showed that siLSD#2 and tranylcypromine effectively suppressed proliferation of colon cancer cells. (**F** and **G**) Transwell Invasion Assay revealed that siLSD#2 and tranylcypromine effectively inhibited invasiveness of colon cancer cells. (**H** and **I**) Apoptosis Assay showed that suppression of LSD1 with siRNA and tranylcypromine induced apoptosis of colon cancer cell. (**J–L**) Downregulation of LSD1 and CDH-2 and upregulation of CDH-1 were observed after treated with siLSD#2. Bars represent mean ± standard deviation of three independent experiments.

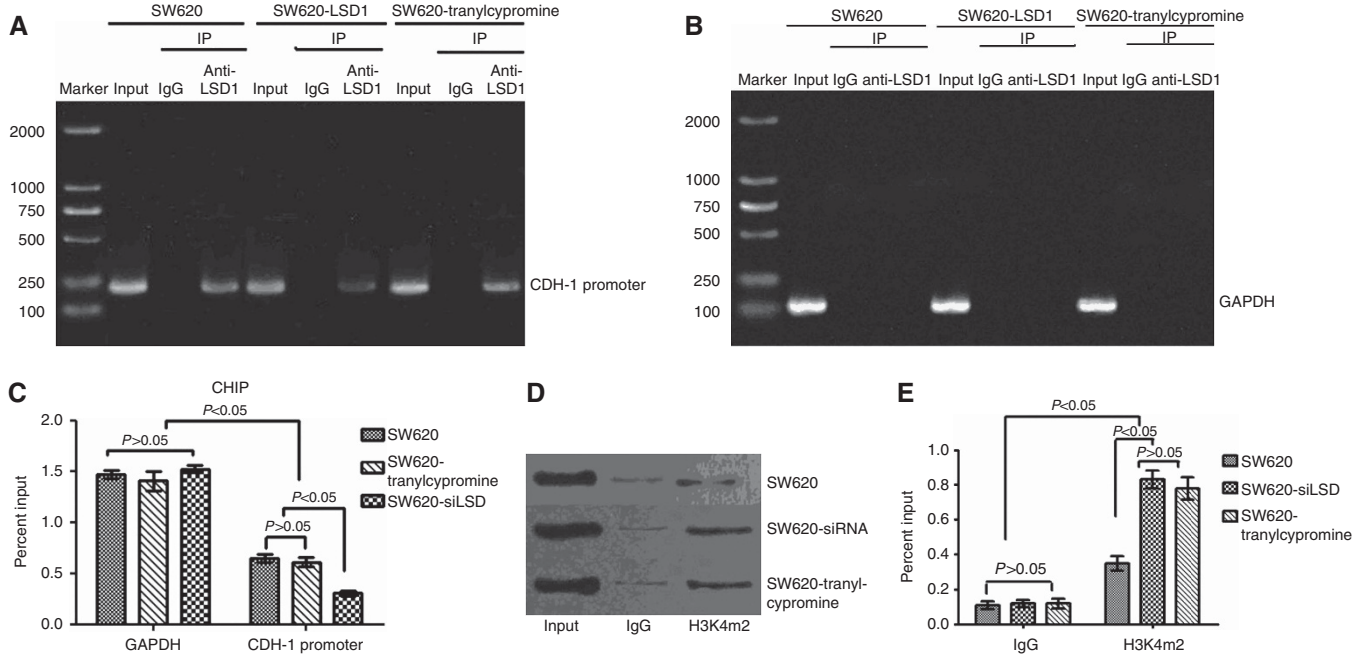


Figure 4. LSD1 reduces H3K4 dimethylation at the promoter of *CDH-1*. (**A–C**) The enrichment of LSD1 at the proximal promoter of *CDH-1* (immunoprecipitated with anti-LSD1) was significantly lower in LSD1-silenced SW620 cells than in SW620 cells and tranylcypromine-treated SW620 cells. (**D** and **E**) The level of H3K4m2 at the promoter of the *CDH-1* gene in LSD1-silenced SW620 cells and tranylcypromine- treated SW620 cells was significantly higher than that in SW620 cells. Values represent mean ± standard deviation of three independent experiments.

effectively. Since LSD1 cannot remove trimethylation of H3K4 (H3K4m3) (Shi *et al*, 2004), we did not perform ChIP assay for the abundance of H3K4m3 at the *CDH-1* promoter.

DISCUSSION

Metastasis is the major cause of mortality among colon cancer patients. Therefore, it is important for us to understand mechanisms that help colon cancer cells to acquire invasive/metastatic potential (Singh *et al*, 2011). This study has demonstrated that LSD1 was significantly upregulated in colon cancer, by IHC, western blot and real-time PCR. With high levels of LSD1 and CDH-2, and low levels of CDH-1, SW620 cells showed significantly higher invasive potential than the other colon cancer cells, indicating that LSD1 and CDH-2 correlated positively with the invasiveness of colon cancer cell lines, whereas the CDH-1 exhibited a negative correlation with the invasiveness of colon cancer cell lines.

Lysine-specific demethylase 1 is composed of three domains, including an N-terminal SWIRM domain, a conserved motif shared by many chromatin regulatory complexes, an amine oxidase domain (AO domain) and a C-terminal Tower domain (Forneris *et al*, 2005; Shi and Whetstine, 2007; Yang *et al*, 2007). The LSD1 cooperates with CoREST, CtBP24 corepressor complex, demethylates histone H3-K4 and H3-K9 through this interaction (Lee *et al*, 2005; Gatta and Mantovani, 2008), and regulates the expression of its target gene by this epigenetic modification. Lysine-specific

demethylase 1 is found to participate in development and differentiation (Lan *et al*, 2008; Adamo *et al*, 2011; Whyte *et al*, 2012), regulation of chromatin remodelling, cell apoptosis and methylation of DNA and histone (Ouyang and Gill, 2009; Wang *et al*, 2009; Amente *et al*, 2010; Deleris *et al*, 2010; Hayami *et al*, 2011; He *et al*, 2011; Yeh *et al*, 2011). More importantly, LSD1 is involved in many pathological processes of cancer, such as carcinogenesis, proliferation, metastasis and apoptosis (Huang *et al*, 2007; Scoumanne and Chen, 2007; Schulte *et al*, 2009; Hayami *et al*, 2011; Bennani-Baiti *et al*, 2012).

The LSD1/CoREST complex dynamics works as a nanoscale clamp opening/closing on several hundred nanosecond time scales reversibly (Baron and Vellore, 2012a, b), making LSD1/CoREST a potential docking site for multiple protein partners sharing high N-terminus sequence similarity with H3 histone tail (Baron *et al*, 2011; Hwang *et al*, 2011; Baron and Vellore, 2012a, b; Laurent *et al*, 2012). The H3 histone tail binding pocket is considered to be a potential allosteric site regulating the opening/closing motion of the clamp (Hwang *et al*, 2011; Baron and Vellore, 2012a, b). Binding of CoREST can not only protect LSD1 from proteasomal degradation, but also regulate the structure of the AO domain of LSD1 to control the interaction of LSD1 with its substrate (Lin *et al*, 2010). In fact, LSD1/CoREST complex does not have the function of cadherin recognition, which is mainly based on the binding of the E-box through the zinc-finger motifs of transcription factor (e.g., Snail and slug) (Lin *et al*, 2010; Ferrari-Amorotti *et al*, 2013).

Our experiment revealed that inhibition of LSD1 impaired proliferation and invasiveness, and induced apoptosis of colon cancer cells *in vitro*. The LSD1 is required for cell proliferation in both p53-dependent and -independent manners, deficiency in LSD1 can lead to a partial cell-cycle arrest in G(2)/M and sensitises cells to growth suppression induced by DNA damage or murine double minute 2 (MDM2) inhibition (Scoumanne and Chen, 2007). Through enhancement of cell-cycle progression, LSD1 could promote growth of cancer cells, whereas inhibition of LSD1 could suppress the G1-to-S progression (Hayami *et al*, 2011), and even induced cells apoptosis (Wang *et al*, 2009; Wen *et al*, 2012).

Cancer invasion and metastasis are landmark events that transform a locally growing tumour into a systemic, metastatic and live-threatening disease (Christofori, 2003). As an important regulator of cell shape, growth and polarity, CDH-1 has a crucial role in epithelial cell–cell adhesion and in the maintenance of tissue architecture (Angst *et al*, 2001; Greenspon *et al*, 2011). Indeed, CDH-1 serves as a widely acting suppressor of invasion and growth of epithelial cancers (Hazan *et al*, 2004). The loss of CDH-1-mediated cell–cell adhesion is a prerequisite for tumour cell invasion and metastasis formation (Friedl and Alexander, 2011; Yip and Seow, 2012). Transcriptional factor Snail can repress the expression of CDH-1 by epigenetic mechanisms dependent on the interaction of its N-terminal SNAG domain with LSD1 (Ferrari-Amorotti *et al*, 2013). Di- and tri-methylation of H3K4 (H3K4m2/m3) is associated with actively transcribed genes (Santos-Rosa *et al*, 2002; Miao and Natarajan, 2005; Morillon *et al*, 2005). In the process of EMT, LSD1 removes dimethylation of lysine 4 on histone H3 (H3K4m2) at the *CDH-1* promoter, and downregulates the CDH-1 expression (Lin *et al*, 2010; Huang *et al*, 2011), which is also proved by our experiment.

Since LSD1 has been demonstrated to be overexpressed in colon cancer with higher TNM stages and distant metastasis, inhibition of LSD1 impaired proliferation and invasiveness, and induced apoptosis of colon cancer cells *in vitro*, the use of LSD1 inhibitors may provide an important potential therapy of cancer. Due to the high structural and mechanistic similarities between LSD1 and amine oxidases, monoamine oxidase (MAO) covalent inhibitors such as pargyline, tranylcypromine and polyamine analogues have been shown to inhibit LSD1 enzymatic activity (Huang *et al*, 2007, 2009). In our experiment, tranylcypromine inhibited LSD1 enzymatic activity and suppressed the growth and invasiveness of colon cancer cells effectively. Inhibitors of LSD1 could also induce the reexpression of the aberrantly silenced gene and result in significant inhibition of the growth of colon cancer xenograft model *in vivo* (Santos-Rosa *et al*, 2002).

However, most of the MAO inhibitors do not selectively target LSD1 and therefore, limits their use as therapeutics owing to potential side effects (Willmann *et al*, 2012). Unlike the non-selective MAO inhibitors that form a covalent bond between FAD and the compounds, the selective and reversible inhibitors such as Namoline (Willmann *et al*, 2012) and CBB compounds (Wang *et al*, 2011) can specifically interact with LSD1 and inhibit its activity without forming a covalent bond, and impair proliferation of cancer cells *in vitro* and *in vivo* (Wang *et al*, 2011; Willmann *et al*, 2012). Unfortunately, these reversible LSD1 inhibitors are either modest in activity or polycationic in nature. Therefore, more effective, high-affinity, non-covalent and fully reversible LSD1 inhibitors still have yet to be found.

In conclusion, LSD1 was expressed significantly higher in colon cancer with higher TNM stages and distant metastasis. Inhibition of LSD1 impairs proliferation and invasiveness, and induces apoptosis of colon cancer cells *in vitro*. By removing dimethylation of lysine 4 on histone H3 (H3K4m2) at the *CDH-1* promoter, LSD1 downregulates the CDH-1 expression, and contributes to metastasis of colon cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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