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Lactate dehydrogenase is correlated with clinical stage and grade and is downregulated by si-SATB1 in ovarian cancer

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Abstract. Lactate, which is regulated by gene expression, is largely believed to favor tumor growth and survival. Elevated lactate dehydrogenase (LDH) is a negative prognostic biomarker because it is a key enzyme involved in cancer metabolism. Our previous study revealed that special AT-rich-binding protein 1 (SATB1), a genome-organizing protein, was strongly associated with high metastasis rates in ovarian cancer. However, its underlying molecular mechanisms in ovarian cancer are unclear. In the present study, we investigated whether SATB1 modulated LDH expression and examined the relationship between SATB1 and LDH in ovarian cancer. We employed transient siRNA-mediated knockdown of SATB1 in ovarian cancer and explored the effects of this knockdown on the expression levels of key glucose metabolism-related enzyme genes (G6PD, LDH, MDH1, PFK1 and TGM1) and the glucose metabolism-related protein monocarboxylate transporter 1 (MCT1). We comprehensively analyzed the cellular and molecular role of LDH in ovarian cancer to determine whether it could be a conventional clinicopathological parameter. SATB1 knockdown significantly downregulated both LDH and MCT1 levels and markedly upregulated BRCA1 and BRCA2 levels in ovarian cancer cells ($P<0.05$). Serum LDH levels in ovarian cancer patients were significantly higher than those in patients with benign ovarian tumors ($P<0.05$). LDH levels at different stages and grades differed significantly in ovarian cancer. Survival curves revealed that higher LDH expression was correlated with shorter survival ($P<0.05$). SATB1 may reprogram energy metabolism in ovarian cancer by regulating LDH and MCT1 levels to promote metastasis. Serum LDH levels presented diagnostic accuracy with high specificity and may have potential as a conventional clinicopathological parameter for ovarian cancer.

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Introduction

Among women, ovarian cancer is the seventh-most common cancer and the first-most common cause of death from gynecological cancers (1). Although some biomarkers such as CA125-a most frequently used biomarker for ovarian cancer detection encoded by the MUC16 gene and HE4-a glycoprotein, overexpressed by epithelial ovarian cancer (EOC) are used for ovarian cancer screening, diagnosis and prognosis evaluation, ovarian cancer is rarely diagnosed until it spreads and advances to later stages (III/IV) (2). Therefore, further investigation of new factors involved in ovarian cancer is required.

Recently, it has been observed that cancer cells can downregulate mitochondrial oxidative phosphorylation and increase glucose consumption and lactate release rates independently of oxygen availability (Warburg effect) (3,4), and lactate produced by anaerobic glycolysis is the primary circulating TCA substrate for cancer cells (5). This metabolic rewiring is largely believed to favor tumor growth and survival. Many molecules, such as MYC, the oncogene Kras, and the tumor suppressor TP53, regulate metabolic glycolysis and oxidative stress (6), although their underlying molecular mechanisms are unclear.

Among the enzymes involved in glycolysis, lactate dehydrogenase (LDH) is an emerging target for possible pharmacological approaches to cancer therapy (7,8). LDH has two major subunits, LDH-A and LDH-B, which can reversibly catalyze the conversion of pyruvate to lactate or lactate to pyruvate. LDH-A has a higher affinity for pyruvate and is a key enzyme in the glycolytic pathway (9-11). Evidence has revealed that lactic acid promotes tumor metastasis, and that elevated LDH is a negative prognostic biomarker since it is a key enzyme in cancer metabolism (12-15). Since glycolytic metabolism contributes to tumor growth in many cancers, efforts have been made to block tumor glycolysis by inhibiting LDH and the monocarboxylate transporters (MCTs), which regulate cancer cell lactate export. Tumor lactate export

is thought to be primarily mediated by monocarboxylate transporter 1 (MCT1), since this family member is the most commonly upregulated in human cancers (16-18). MCT1 inhibition is thought to block tumor growth by disrupting lactate transport, glycolysis and glutathione synthesis, thus inducing cell death (19-21).

SATB1 (special AT-rich-binding protein 1) is a global genome organizer that changes chromatin architecture to reprogram gene expression profiles in the genome (22-24). In our previous study, we revealed that SATB1 was important in ovarian cancer metastasis, and its high expression was associated with high metastasis rates and low survival rates in EOC (25). We demonstrated that SATB1 may be a potential novel prognostic biomarker and therapeutic target for patients with ovarian cancer. However, the significance of SATB1 expression in ovarian cancer and its regulatory mechanisms have rarely been reported.

Given that tumor cell metabolism is regulated by certain genes, and SATB1 serves as a global genome organizer, we hypothesized that SATB1 promoted ovarian cancer development by regulating the metabolic enzyme expression levels in the tumor microenvironment, such as LDH and MCT1. Similar research results have been poorly reported. The present study explored the regulatory activity of the SATB1/LDH pathway in the metabolic reprogramming of EOC cells and the potential molecular mechanism that promoted cell invasion and metastasis. Correlations between these clinical parameters and blood serum LDH levels were assessed in ovarian cancer patients. Our research may provide a theoretical basis for a new target in the energy metabolic pathway to find new anticancer therapeutics for ovarian cancer.

Materials and methods

Patients. This retrospective analysis was conducted using clinical data obtained from ovarian cancer patients who underwent surgical resection at the Department of Obstetrics and Gynecology, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine between January 2011 and March 2016. Records from 133 ovarian cancer patients and 43 normal controls were retrospectively reviewed. The clinical stage of ovarian cancer was determined based on the International Federation of Gynecology and Obstetrics (FIGO) stage. The normal controls were healthy women 35-60 years old. The present study was reviewed and approved by the Institutional Review Board and the Research Ethics Committee of Shanghai General Hospital. All participants provided informed consent to participate in the study.

Cell lines. The human ovarian serous cystadenocarcinoma cell line SKOV3 was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a humidified incubator with 5% CO_2 at 37˚C.

Transient transfection. To analyze the functional relevance and possible therapeutic potential of SATB1 inhibition, we used transient siRNA-mediated knockdown and comprehensively analyzed the cellular and molecular role of SATB1 in ovarian cancer. siRNAs were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Scrambled siRNA was used as a negative control. Prior to transfection, ($5x10^5$) cells were seeded in cell culture plates and maintained overnight under standard condition (RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin) SKOV3 cells were transfected using Lipofectamine® 3000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Sequences for siRNA transfection are listed in Table I, and the duration for transfection was 48 h.

RNA preparation and RT-qPCR. Total RNA was isolated using TRI Reagent® (Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions. A RevertAid™ H Minus First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) was used to reverse-transcribe 1 μg of total RNA with random hexamer primers. For quantitative PCR, a LightCycler® 2.0 (Roche Diagnostics GmbH, Mannheim, Germany) and the Absolute™ qPCR SYBR®-Green Capillary Mix (Thermo Fisher Scientific, Inc.) were used as previously described (26). Primers are listed in Table II. The cycling conditions consisted of a denaturation step at 95˚C for 10 min, 40 cycles at 95˚C for 15 sec, a 45-sec annealing step at 60˚C, and finally a holding temperature at 95˚C, for 15 sec, and then at 60˚C for 1 min. Gene expression was quantified based on the ΔΔCq method (27), with β-actin as the reference housekeeping gene. Primers were produced by Shanghai Sangon Biological Engineering Technology and Services Company (Shanghai, China).

Cell proliferation assay. Cell proliferation was analyzed using Cell Counting Kit-8 (CCK-8) (Beyotime Institute of Biotechnology, Nantong, China). SKOV3 cells and si-SATB1 knockout SKOV3 cells were seeded at a density of $1x10^5$ cells/well in a 96-well culture plate. Cell proliferation was detected at 24, 48 and 72 h. Subsequenlty, 100 μl of serum-free culture medium and 10 μl of CCK-8 solution were added to each well and incubated with cells at 37˚C for 1 h. The optical density at 450 nm was determined on an ELx800 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Western blotting. A dilution of $5x10^5$ SKOV3 cells were seeded in 6-well plates and transfected as aforementioned. Following 48 h of transfection, the cells were washed with PBS and lysed. Cellular protein was quantified with a Bradford assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and 50 μg of the cleared lysates were separated on a 12% SDS-PAGE gel and electrotransferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). GAPDH was used as an equal loading control. PVDF membranes were blocked in Tris-buffered saline containing 0.1% Tween-20 (TBST) with 5% non-fat dry milk for 2 h and incubated with polyclonal rabbit anti-human SATB1 antibody (1:100; cat. no. ab92307) and anti-human MCT1 antibody (1:100; cat. no. ab90582; both from Abcam, Cambridge, UK). The membranes were then washed 3 times for 5 min in PBST and incubated with goat anti-rabbit IgG H&L (HRP) (1:200; cat. no. ab205718; Abcam) in PBST for 1 h. Following 3 washes with PBST, the bands were developed using an enhanced chemiluminescence (ECL) detection

Table I. Sequences for siRNA transfection.

Gene name	Target site	DNA sequences
SATB1	620-642	GGCUAAUCCAGGUUG GAAA
(NM_001131010.2)	1339-1361	GAGGUGUCUUCCGAAAUCU
	2373-2395	CGAUGUGGCAGAAUAUAAA

SATB1, special AT-rich-binding protein 1.

Table II. Primer sequences of SATB1, MCT1, LDH-A, MDH1, MDH2, G6PD, TGM1, PFK1 and GAPDH.

Gene name	Size (bp)	Primer sequences
SATB1 (NM_001131010.2)	244	F: 5' GGCTCGTATCAACACCTATC 3' R: 5' CCTGCTCGTTTCAGTTCATC 3'
MCT1 (NM_001166496.1)	107	F: 5' GGTGGAGGTCCTATCAGCAGT 3' R: 5' CAGAAAGAAGCTGCAATCAAGC 3'
LDH-A (NM_002301.4)	191	F: 5' AGAACATGGTGATTCTAGTGTGC 3' R: 5' ACAGTCCAATAGCCCAAGAGG 3'
MDH1 (NM_001199111.1)	174	F: 5' TGCCTTCAAAGACCTGGATG 3' R: 5' TTGGCTGGATTACCCACAAC 3'
MDH2 (NM_001282403.1)	133	F: 5' CCCACGGGTTCATAGTTCAG 3' R: 5' CATCAGGGTTCGGTCAGAAG 3'
G6PD (NM_000402.4)	243	F: 5' CCTACGGCAACAGATACAAG 3' R: 5' CATACTGGAAACCCACTCTC 3'
TGM1 (NM_000359.2)	163	F: 5' GCCCACGACACAGACACATC 3' R: 5' CCACCTGCCACCCATCAAAG 3'
PFK1 (NM_000289.5)	190	F: 5' TGCCCGTGTCTTCTTTGTCC 3' R: 5' ACGCTTCACCAGGTTGTAGG 3'
GAPDH (NM_001256799.1)	110	F: 5' CACCCACTCCTCCACCTTTG 3' R: 5' CCACCACCCTGTTGCTGTAG 3'

F, forward; R, reverse; SATB1, special AT-rich-binding protein 1; MCT1, monocarboxylate transporter 1; MDH1, malate dehydrogenase 1; MDH2, malate dehydrogenase 2; G6PD, glucose-6-phosphate dehydrogenase; PFK1, phosphofructokinase-1.

system (Pierce Biotech Inc.; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The software that was used for densitometry was Image-Pro Plus (version 6.0; Media Cybernetics, Rockville, MD, USA).

LDH activity assay. LDH activity was detected using an LDH Quantification kit (A020-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, a dilution of $5x10^5$ SKOV3 cells were seeded in 6-well plates and transfected as aforementioned. Cell cultured-supernatants were harvested and then the cells were harvested and cell lysates were prepared by sonication; LDH activity was determined by calculating the pyruvic acid transferred by LDH. For tissue, one unit of LDH activity (U/gprot) meant that 1 g of tissue protein reacted with the substrates at 37˚C for 15 min to produce 1 μmol of pyruvate in the reaction system. For serum or fluid sample, one unit of LDH activity (U/l) meant that 1,000 ml of serum (or fluid) reacted with the substrates at 37˚C for 15 min to produce 1 μmol of pyruvate in the reaction system. The optical levels at 440 nm were determined on an ELX-800 microplate reader (Bio-Tek Instruments, Inc.) and experiments were performed in triplicate.

Lactate detection. A dilution of $5x10^5$ SKOV3 cells were seeded in 6-well plates and transfected as aforementioned. Cell cultured-supernatants were harvested and then the cells were harvested and cell lysates were prepared by sonication. Lactate of the cell supernatants and cell lysates was quantified using a Lactate Assay kit (A019-2; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol, which was quantified by a colorimetric change at 570 nm. The amount of lactate in the supernatant was quantified by comparison with a standard curve.

LDH activity detection in patient sera. According to the manufacturer's instructions, briefly, about 20 μl of patient sera

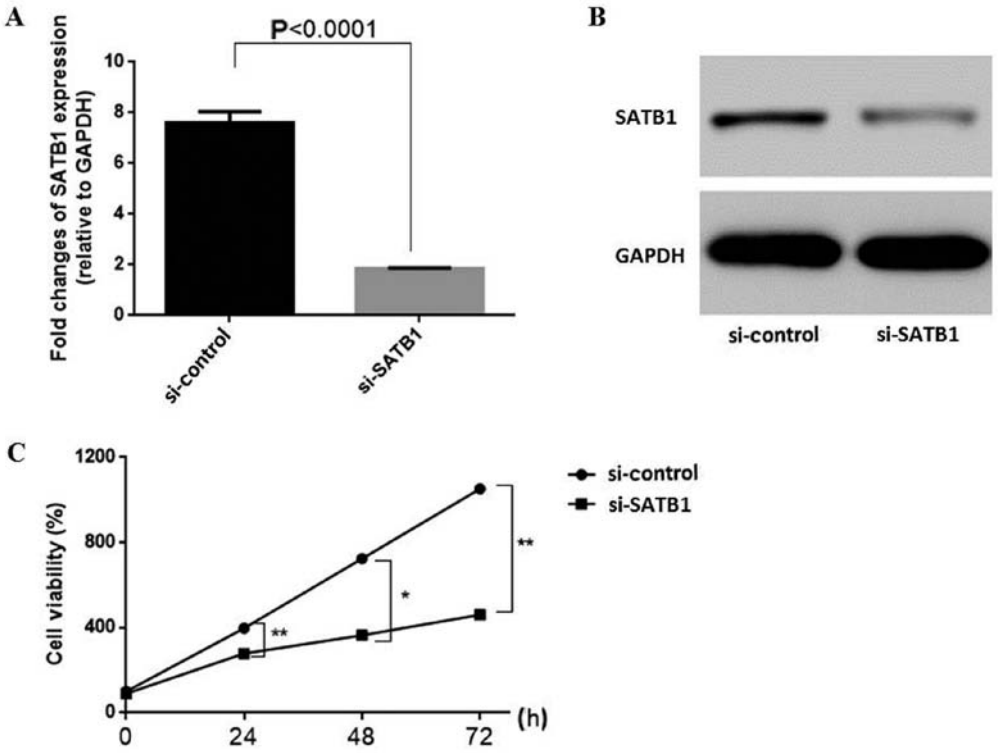

Figure 1. Inhibitory effect of SATB1-knockdown on ovarian cancer cell proliferation. (A) RT-qPCR revealed that siRNA significantly decreased SATB1 RNA expression. (B) Western blot assays revealed that siRNA significantly decreased SATB1 protein expression. (C) The CCK-8 proliferation assay revealed significantly reduced cell proliferation upon transfection of SKOV3 cells with si-SATB1. SKOV3 cells transfected with si-Control and si-SATB1 were seeded at a density of $1x10^5$ cells/well in a 96-well culture plate cultured for 24, 48 and 72 h. Subsequently, 100 μl of serum-free culture medium and 10 μl of CCK-8 solution were added to each well and incubated with cells at 37˚C for 1 h. Cell proliferation was detected, *P<0.05; **P<0.01. SATB1, special AT-rich-binding protein 1.

was dripped on VITROS™ Chemistry Products LDH Slides (Thermo Fisher Scientific, Inc.), and then enzyme complex calibrator (VITROS™ Chemistry Products Calibrator kit 3) and the automatic biochemical immunoanalyzer (VITROS™ 5600) were used to determine LDH activity. We determined LDH activity by monitoring the rate of conversion from NAD^+ to NADH, which was quantified by a colorimetric change at 340 nm.

Statistical analysis. All statistical analyses were performed using SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). T-test was used to analyze the data for two independent groups. To account for their effects on the relationship between LDH and cancer stage and grade and lymphatic metastasis, all variables were analyzed with Spearman's rank correlation coefficient analysis. Medcalc version 17.9 software (MedCalc Software bvba, Ostend, Belgium) was used for AUC comparison of LDH and CA125. P<0.05 was considered to indicate a statistically significant difference.

Results

Inhibitory effect of SATB1 knockdown on cell proliferation. To validate siRNA interference efficiency, we used RT-qPCR and western blot assays. Primers are listed in Table II. The results revealed that SATB1 expression was significantly decreased by siRNA at both the RNA (Fig. 1A) and protein levels (Fig. 1B) compared with the control group (RNA: 1.00±0.09 vs. 2.92±0.03, P<0.001). To initially explore the effects of SATB1 knockdown on ovarian cancer cell proliferation and viability, CCK-8 proliferation assays were performed. Growth curves revealed a significantly reduced cell proliferation upon transfection of SKOV3 cells with si-SATB1 (Fig. 1C) (si-SATB1 vs. si-Control, at 24 h: 321±12.73 vs. 398.56±10.02, P<0.01; at 48 h: 345±8.36 vs.785.09±12.32, P<0.05 at 72 h: 418.44±9.47 vs. 1,314.87±14.73, P<0.01). Cell proliferation was reduced by >80%, indicating that SATB1 knockdown greatly affected ovarian cancer cell proliferation.

SATB1 regulates the expression of key glucose metabolism-related molecules. Phosphofructokinase-1 (PFK1) catalyzes the important 'committed' step of glycolysis, converting glucose to pyruvate, which is a substrate for both anaerobic and aerobic glycolysis. Malate dehydrogenase 1 (MDH1) and 2 (MDH2) are key enzymes in the TCA cycle (aerobic glycolysis), while LDH, which converts pyruvic acid back to lactate is an important enzyme in anaerobic glycolysis. Glucose-6-phosphate dehydrogenase (G6PD) is important in the pentose phosphate pathway, another glucose metabolism-related pathway parallel to glycolysis. siRNA-mediated knockdown of SATB1 affected the expression levels of many of these molecules, as detected at the mRNA level by quantitative RT-PCR. The results revealed that SATB1 knockdown significantly inhibited LDH-A expression in ovarian cancer and increased the RNA expression of MDH1, MDH2, PFK1, BRCA1 and BRCA2 (Fig. 2 and Table III).

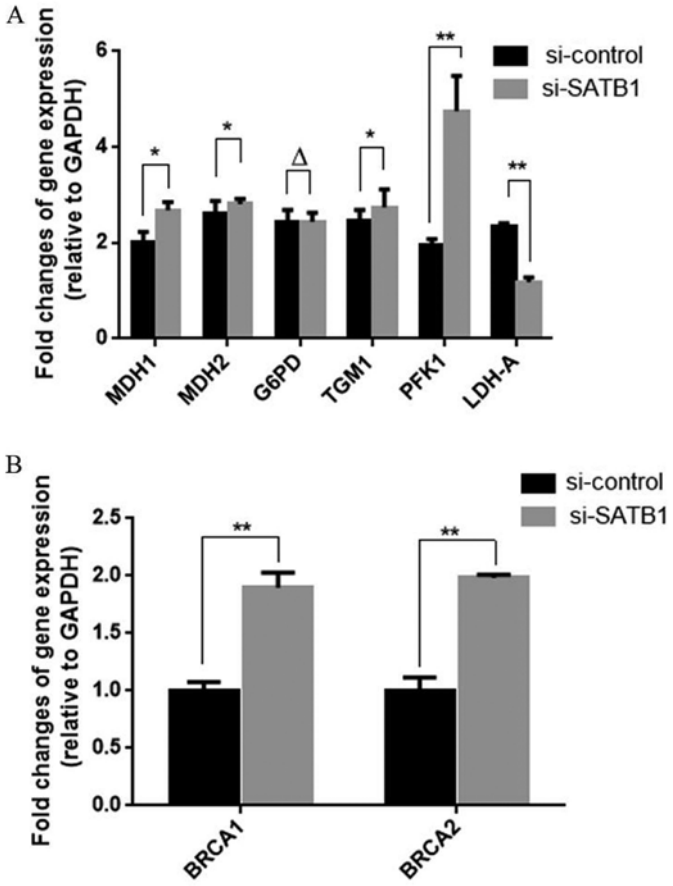

Figure 2. SATB1 regulates the expression of key glucose metabolism-related molecules and BRCA1/BRCA2. (A) RT-qPCR revealed that si-SATB1significantly decreased LDH-A RNA expression, but significantly increased the RNA expression of MDH1, MDH2 and TGM1. *P<0.05, **P<0.001, ΔP>0.05. (B) RT-qPCR revealed that si-SATB1 significantly increased the RNA expression of BRCA1 and BRCA2. **P<0.01. SATB1, special AT-rich-binding protein 1; MDH1, malate dehydrogenase 1; MDH2, malate dehydrogenase 2.

Table III. SATB1 regulates the expression of key glucose metabolism-related molecules and BRCA1/BRCA2.

	si-Control	si-SATB1	P-value (t-test)
MDH1	0.98±0.10	1.29±0.08	<0.05
MDH2	1.25±0.11	1.47±0.05	<0.05
G6PD	1.13±0.16	1.15±0.10	>0.05
TGM1	1.16±0.13	1.31±0.17	<0.05
PFK1	0.88±0.09	2.17±0.08	<0.001
LDH-A	1.12±0.13	0.14±0.11	<0.001
BRCA1	0.19±0.10	1.12±0.08	<0.01
BRCA2	0.19±0.13	1.18±0.02	<0.01

SATB1, special AT-rich-binding protein 1; MDH1, malate dehydrogenase 1; MDH2, malate dehydrogenase 2; G6PD, glucose-6-phosphate dehydrogenase; PFK1, phosphofructokinase-1.

SATB1 regulates anaerobic glycolysis by stimulating LDH and MCT1 expression and lactate production. As aforementioned, SATB1 may stimulate LDH-A expression. LDH-A converts pyruvic acid back to lactate, making it an important enzyme in anaerobic glycolysis. Therefore, we further investigated whether SATB1 regulated lactate production. MCT1 functions as a lactate/H^+ symport system to transport lactate across the cell membrane. We detected MCT1 and lactate levels in SKOV3 cells following transfection with si-SATB1 and found that si-SATB1 decreased LDH-A mRNA (Fig. 3A and Table IV) and protein expression levels (Fig. 3B), inhibiting LDH enzyme activities (Fig. 3C and Table V) and lactate production (Fig. 3D and Table V). Thus, SATB1 may be important in reprogramming ovarian cancer energy metabolism by regulating LDH and MCT1 levels.

LDH of anaerobic glycolysis is associated with poor prognosis in EOC. Lactate, which is converted from pyruvate by LDH in anaerobic glycolysis, is the primary circulating TCA substrate for cancer cells. To verify the importance of LDH in EOC, we retrospectively analyzed the LDH expression level in EOC patients. Patient baseline characteristics are displayed in Table VI. Serous adenocarcinoma was the most common subtype (65.41%), and histological grade 3 was the most frequent grade (42.86%) in our cohort. In total, 37 (27.82%), 27 (20.30%), 65 (48.87%) and 4 (3.01%) patients had stage I, II, III and IV diseases, respectively. Additionally, 22 (16.54%) patients had lymphatic metastasis. Optimal debulking was performed in 127 (95.49%) patients.

First, we compared the LDH level between normal and these EOC patients. LDH was significantly increased in EOC patients

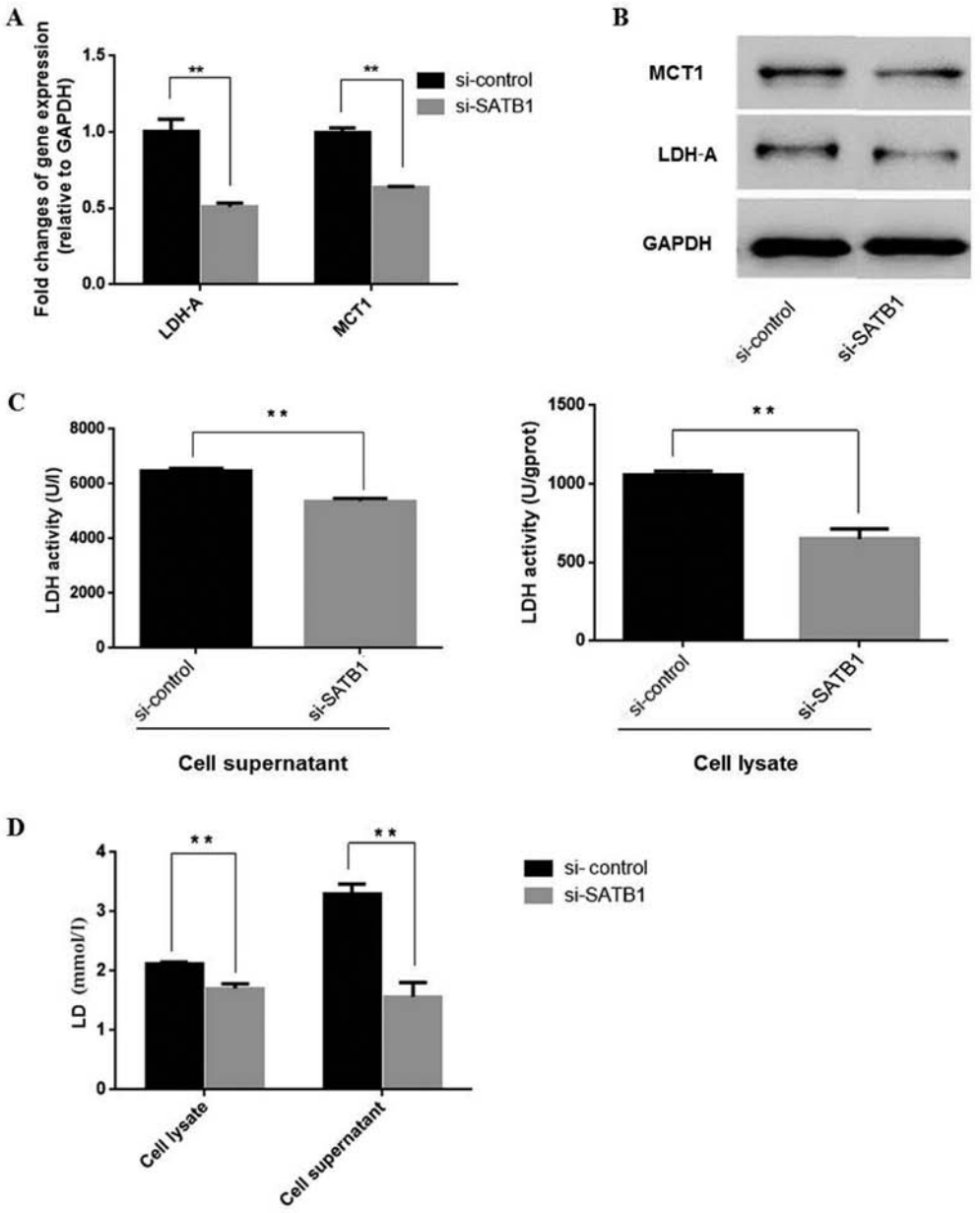

Figure 3. SATB1 plays a regular role in anaerobic glycolysis by stimulating LDH and MCT1 expression and lactate production. SKOV3 cells were treated as mentioned in the Material and methods section. Cells were harvested and prepared for RNA and protein expression analysis. (A) si-SATB1 decreased the mRNA expression level and (B) protein expression level of LDH-A and MCT1. A dilution of $5x10^5$ SKOV3 cells were seeded in 6-well plates and transfected as described in the Material and methods section. Harvested cell cultured-supernatants, and then harvested cells and cell lysates were prepared by sonication. LDH activity from cell supernatants and cell lysates was determined. (C) si-SATB1 inhibited LDH enzyme activity and (D) lactate production. **P<0.01. SATB1, special AT-rich-binding protein 1; MCT1, monocarboxylate transporter 1; LDH, lactate dehydrogenase.

Table IV. SATB1 inhibits the expression of LDH and MCT1.

	si-Control	si-SATB1	P-value (t-test)
LDH	1.12±0.17	0.14±0.10	<0.01
MCT1	1.12±0.04	0.47±0.02	<0.01

SATB1, special AT-rich-binding protein 1; LDH, lactate dehydrogenase; MCT1, monocarboxylate transporter 1.

compared with the control (398.67±45.89 vs. 589.33±12.67, P<0.001; Fig. 4A). We then drew a ROC curve to investigate the role of LDH in diagnosis of EOC (AUC=0.78, 95% CI: 0.70-0.86,

Table V. SATB1 inhibits LDH activity and LD production of ovarian cancer cells.

	si-Control	si-SATB1	P-value (t-test)
LDH (U/gprot)			
Cell lysate	1,053±29.03	648.96±67.04	<0.05
Supernatant	6,499.23±75.19	5,367.44±107.28	<0.05
LD (mmol/gprot)			
Cell lysate	2.11±0.04	1.88±0.14	<0.05
Supernatant	3.31±0.16	1.73±0.44	<0.05

SATB1, special AT-rich-binding protein 1; LDH, lactate dehydrogenase.

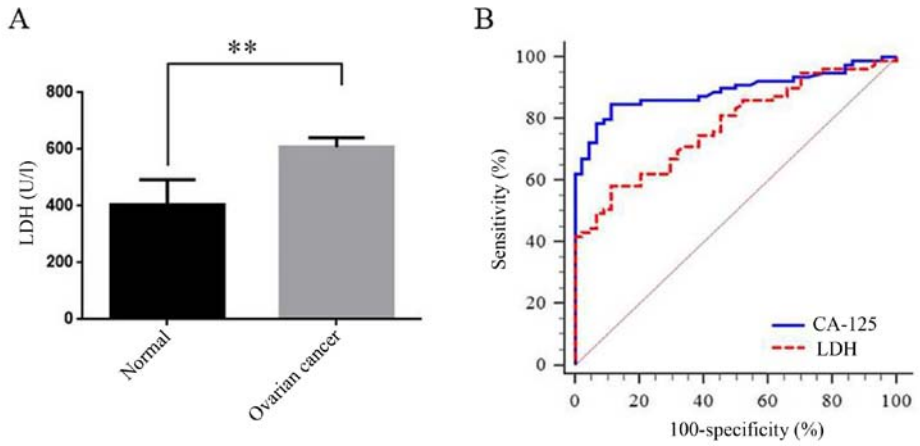

Figure 4. LDH in anaerobic glycolysis contributes to the epithelial ovarian cancer diagnosis. (A) LDH was significantly increased in epithelial ovarian cancer (**P<0.01). (B) The sensitivity of LDH for the diagnosis of epithelial ovarian cancer (vs. CA-125). LDH, lactate dehydrogenase; ovarian cancer, epithelial ovarian cancer.

Table VI. The clinical baseline characteristics of ovarian patients.

Variable	n (%)
Age (years)	Median, 53.27 (range, 20-82)
Histological type	133 (100)
Serous	87 (65.41)
Mucinous	14 (10.53)
Endometrioid	10 (7.52)
Clear cell	5 (3.76)
Undifferentiated	17 (12.78)
Differentiation	133 (100)
G1	24 (18.04)
G2	52 (39.10)
G3	57 (42.86)
Stage	133 (100)
I	37 (27.82)
II	27 (20.30)
III	65 (48.87)
IV	4 (3.01)
Lymphatic metastasis	133 (100)
Negative	111 (83.46)
Positive	22 (16.54)
Optimal debulking	133 (100)
No	6 (4.51)
Yes	127 (95.49)

Table VII. Sensitivity of LDH for the diagnosis of EOC (vs. CA125).

	Area under the curve (AUC)				Z statistic	
	n	Area	95%CI	P-value	Z score	P-value
LDH	133	0.78	0.70-0.86	<0.001	2.59	0.01
CA125	133	0.89	0.83-0.95	<0.001		

LDH, lactate dehydrogenase; EOC, epithelial ovarian cancer.

P<0.001; Fig. 4B, Table VII). It revealed that the LDH level could be a useful tool for EOC diagnosis, though the diagnostic performance of LDH was still lower than CA125 (Table VII). In addition, we also observed a statistically positive correlation between LDH and CA125, or LDH and HE4, which still indicated an important role of LDH in EOC diagnosis and prognosis (Table VIII). Last, we observed that in EOC patients, LDH was also statistically increased in high grade, high stage and non-optimal debulking (OD) cancer patients (P=0.029, 0.03 and 0.03, respectively; Fig. 5A-C). However, there was no difference between negative and positive lymphatic metastasis patients (P=0.31, Fig. 5D). Further analysis revealed that LDH level was positively correlated with stage and grade and negatively correlated with OD and survival days (Table VIII).

Discussion

SATB1 is associated with poor prognosis in ovarian cancer. Previous research has revealed that SATB1 is upregulated in human cancer tissue samples compared with matched non-cancerous adjacent tissues, and high expression of SATB1 is associated with poor patient survival (28-30). Our initial investigations also demonstrated that SATB1 regulated gene expression via the acetylation pathway, and its high expression was associated with a high metastasis rate and low survival rate in EOC. SATB1 levels have been found to be significantly associated with histological grade and poor survival and have been described in low- and high-grade ovarian cancer (25). In the present study, we employed a transient siRNA-mediated knockdown strategy to avoid possible adaptive processes upon constitutive knockdown or overexpression. We further demonstrated that SATB1 upregulated BRCA1 and BRCA2

Table VIII. Spearman's correlation analysis of the LDH level and the related biochemical measurements.

	Stage	Grade	CA125	HE4	Lymphatic metastasis	OD	Survival days
Correlation coefficient	0.27	0.32	0.37	0.35	0.11	-0.39	-0.34
P-value	0.014	0.04	0.01	0.04	0.34	<0.001	0.011
n	133	133	133	133	133	133	133

Stage: Stage I-II, stage III-IV; Grade: G1, G2+G3. LDH, lactate dehydrogenase; OD, optimal debulking.

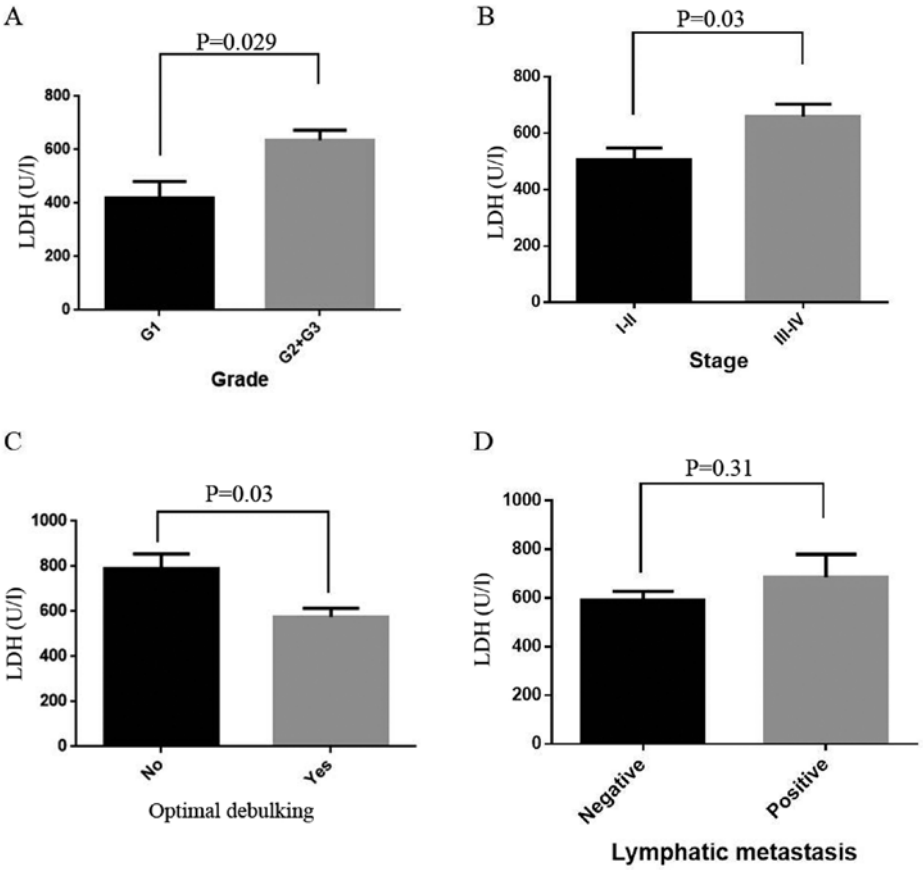

Figure 5. Correlation of LDH and the characteristics of EOC. The levels of serum LDH were detected. (A) The LDH levels were significantly different between the pathological grades of high differentiation (G1) and median-low differentiation (G2+G3) (420.60±60.65 vs. 636.20±37.38). (B) Stage of EOC was classified into early stage (I-II) and median-last stage (III-IV) (505.70±80.30 vs. 659.40±44.62), and (C) optimal debulking (792.10±63.05 vs. 577.00±37.29). (D) No significant result was observed between lymphatic metastasis and non-lymphatic metastasis (591.30±36.56 vs. 683.3±94.55). LDH, lactate dehydrogenase; EOC, epithelial ovarian cancer.

expression in ovarian cancer (data not shown). Our results demonstrated that knockdown of SATB1, a gene organizer, significantly inhibited ovarian cancer cell proliferation *in vivo*. These results were consistent with those from other research.

SATB1 reprograms ovarian cancer energy metabolism. In the present study, we revealed that SATB1, a nuclear architectural protein that organizes chromatin structure (31,32), plays an important role in reprogramming the energy metabolism of ovarian cancer cells by regulating the expression levels of LDH and MCT1. The present findings demonstrated that stable knockdown of SATB1 expression using siRNA inhibited ovarian cancer cell proliferation by downregulating both LDH and MCT1 expression. Previous studies on reducing expression also indicated that LDH is involved in tumor initiation, but its regulation mechanism in tumors has not been established. LDH converts pyruvate to lactate. Research has shown that lactate, a glycolysis end-product, is a pleiotropic tumor growth-promoting factor responsible for metabolic symbiosis in tumors. The effect of lactate primarily depends on its uptake, a process facilitated by the lactate-proton symporter MCT1. LDH and MCT1 are often overexpressed in tumor cells and are associated with high metastasis rates and poor prognosis (33). The relationship between SATB1 and these metabolism enzymes in cancer proliferation and metastasis have never been reported. In the present study, we

found that SATB1 knockdown downregulated both LDH and MCT1 levels in ovarian cancer cells. A SATB1/LDH regulation pathway may exist in EOC. SATB1 regulated LDH and then reprogramed ovarian cancer cell energy metabolism and increased anaerobic glycolysis to promote ovarian cancer metastasis.

LDH predicts poor prognosis of ovarian cancer patients. LDH, the key enzyme in the anaerobic glycolysis pathway, converts pyruvate to lactate in cancer cells and likely other highly-proliferating cells. Therefore, a high LDH level is commonly observed in cancer cells (34,35). Approximately 90% of women with advanced ovarian cancer have elevated levels of CA-125 in their blood serum, making CA-125 a popular useful tool for detecting ovarian cancer. However, CA-125 has limited specificity for ovarian cancer since elevated CA-125 levels can be found in individuals without ovarian cancer (36).

Previously, we found that LDH promoted ovarian cancer cell proliferation and metastasis, and high LDH expression was associated with poor patient survival (37). LDH may be a promising molecular predictor of EOC prognosis, providing an optional therapeutic regimen for ovarian cancer. In other words, the combination of CA-125 and LDH may be a promising factor, which warrants further study. Similarly, in the present study, we observed that LDH was significantly increased in EOC, and its expression level increased with increasing clinical stage and histological grade. Higher LDH expression was associated with a lower survival rate. A strong point of this study is that it is the first to evaluate the predictive value of LDH in ovarian cancer.

However, in the present study, we only presented the phenomenon of LDH level differences in ovarian cancer, which warrants further investigation. Therefore, further studies about the mechanism of LDH regulation of ovarian cancer need to be conducted in the future.

In conclusion, the present study was the first to assess the predictive value of LDH in ovarian cancer. We hypothesized that there may be a SATB1/LDH regulation pathway involved in metabolic reprogramming in EOC. We found that stable knockdown of SATB1 expression using siRNA inhibited ovarian cancer cell proliferation by downregulating both LDH and MCT1 expression. Additionally, we demonstrated that high LDH expression was statistically and positively correlated with stage, pathological grade, but negatively with OD. Therefore, LDH may be a clinically reliable and useful indicator to accurately predict ovarian cancer initiation and patient prognosis.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YH designed the study. JX, SL and GZ contributed to the conception of the study. JX, LZ, YZ, YS, ZZ and GZ contributed to the drafting of the study. LZ, JZ and ZZ contributed to the acquisition of the data. LZ, JZ and ZZ contributed to the analysis of the data. YZ, YS and YH contributed to the interpretation of data for the work. JX, LZ, JZ, SL and YH revised the study critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was reviewed and approved by the Institutional Review Board and the Research Ethics Committee of Shanghai General Hospital. All participants provided informed consent to participate in the study.

Patient consent for publication

All participants provided informed consent to participate in the study.

Competing interests

The authors declare that they have no competing interests.

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