Histone acetyltransferase 1 promotes ovarian cancer progression by regulating cell proliferation and the cell cycle

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Abstract. Ovarian cancer (OC) is the most common cause of gynecological cancer-related death. Histone acetyltransferase 1 (HAT1) has generated interest as a potential target for therapy due to it being involved in a variety of diseases, including cancer. However, to the best of our knowledge, the role of HAT1 in OC has not yet been investigated. In the present study, HAT1 was upregulated in OC and the high expression of HAT1 was associated with unfavorable prognosis. The transcription factor forkhead box protein A1 (FOXA1) transcriptionally regulated HAT1 expression. Furthermore, HAT1 knockdown in OC cells significantly suppressed cell proliferation and colony formation. In addition, the inhibition of HAT1 promoted cell cycle arrest, and reduced cyclin-dependent kinase (CDK)2, CDK4 and cyclin E levels in OC cells. Taken together, the present data suggested that HAT1 served an oncogenic role in OC; therefore, HAT1 may represents a new potential therapeutic target in OC treatment.

Introduction

Ovarian cancer (OC) has the greatest fatality rate among all global gynecological malignancies, with epithelial OC being the most frequently occurring type (1,2). Due to a lack of accurate early screening techniques for OC, diagnosis is delayed and ~50% of patients die within 5 years of diagnosis (3,4). Despite recent therapeutic breakthroughs, OC still has poor results due to the unknown gene regulation network that underpins its etiology (5). Notably, there is an abundance of clinical prognostic indicators for OC, such as CA125; however, the identification of meaningful biomarkers for early detection and improving treatment outcomes in OC is required.

Histone acetyltransferases (HATs) and deacetylases are capable of controlling DNA transcription by regulating histone acetylation and deacetylation. Overexpression or inappropriate recruitment of HATs have been associated with the development and progression of malignant tumors (6). HAT1 can only acetylate newly generated histone H4, and was the first HAT discovered and remains one of the insufficiently studied members of the HAT family (7). HAT1 is upregulated in numerous types of solid tumors, including lung, breast, esophageal and liver cancer, and functions as an oncogene to promote carcinogenesis (8-11). It has also been revealed that HAT1 operates as a transcription factor (TF), modulating cancer cell metabolism and treatment resistance (12,13).

To date, to the best of our knowledge, the role and function of HAT1 in OC have not been investigated. The present study aimed to elucidate the HAT1 expression levels in specimens from patients with OC and healthy human samples. Then the molecular mechanism of upstream regulator of HAT1 will be studied, as well as the function of HAT1 in regulating OC cell proliferation and colony formation activities. Furthermore, the role of HAT1 in regulation of cell cycle pathway in OC cells will be analyzed, and whether knockdown of HAT1 could decrease cell cycle-related protein expression levels in OC cells. Thus, the present results may contribute to a better understanding of the potential role of HAT1 in OC, which could provide new biomarkers for novel therapeutic strategies in OC.

Materials and methods

Cell culture and reagents. Human OC cell lines OVCAR3, HEY, A2780 and SKOV3, and the immortalized ovarian epithelial cell line IOSE386 were grown in RPMI-1640 medium with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin and 100 μg/ml streptomycin (Thermo Fisher Scientific, Inc.). The cells were cultured in a 37˚C incubator with 5% CO_2, and cells were detached using 0.25% trypsin and passaged every 2 days. Small interfering RNA (siRNA) transfection was used to silence HAT1 expression in 3x10^6 HEY and SKOV3 cells at 150 nM concentration,

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and a siRNA transfection reagent (cat. no. sc-29528, Santa Cruz Biotechnology, Inc.) was used to transfect cells at room temperature for 48 h according to the manufacturer's guidelines. siRNAs including scrambled siRNA (siSCR) and siHAT1 were purchased from the Dharmacon (Revvity, Inc.). The ON-TARGETplus Human HAT1 siRNA-SMART pool contained four target sequences of HAT1 together in the tube: 5'-GCUACAGACUGGAUAUUAAUU-3', 5'-CAGAUGAACCAAAUAGAAAUU-3', 5'-CAACACAGCAAUUGAACUAUU-3' and 5'-AGGAACUAGUGGAAGAUUAUU-3'; the siRNA also contained four target sequences of siSCR: 5'-UGGUUUACAUGUCGACUAA-3', 5'-UGGUUUACAUGUUGUGUGA-3', 5'-UGGUUUACAUGUUUUCUGA-3' and 5'-UGGUUUACAUGUUUUCCUA-3'. The FOXA1 overexpression plasmid was synthesized by YouBio Biotechnology. A total of 1 μg pcDNA3-FOXA1 plasmid and 1 μg pcDNA3-Flag control plasmid was transfected into $3x10^6$ HEY cells for 48 h using the FuGENE® HD transfection reagent at room temperature according to the manufacturer's instructions (Roche Diagnostics).

Western blot analysis. Lysates from whole cells (IOSE386, OVCAR3, HEY, A2780 and SKOV3) were extracted using RIPA buffer (Thermo Fisher Scientific, Inc.) with a protease inhibitor cocktail. Cell suspensions were homogenized and centrifuged at 16,000 x g for 20 min at 4˚C to extract the supernatant. Equal amounts of protein (15 μg) were separated by SDS-PAGE (Upper layer 4% stacking gel and lower layer 8% separating gel) and transferred to a PVDF membrane, which was blocked with 5% non-fat milk for 2 h at room temperature. Subsequently, the membrane was treated with the indicated primary antibodies at 4˚C overnight and then horseradish peroxidase-conjugated secondary antibodies (1:2,000; Cat.31430, Cat.31460; Thermo Fisher Scientific, USA) for another 2 h at room temperature. Signals were detected using an enhanced chemiluminescence reagent from Pierce (Thermo Fisher Scientific, USA) and analyzed with a ImageQuant LAS 4000 and ImageQuant TL 8.1 software (GE Healthcare). The following primary antibodies were used in the present study: Anti-HAT1 (1:1,000; cat no. 11432-1-AP; Proteintech Group, Inc.), anti-CDK2 (1:1,000; cat no. 10122-1-AP; Proteintech Group, Inc.), anti-FOXA1 (1:1,000; cat no. 20411-1-AP; Proteintech Group, Inc.), anti-CDK4 (1:1,000; cat no. 12790; Cell Signaling Technology, Inc.), anti-cyclin E (1:1,000; cat no. 20808; Cell Signaling Technology, Inc.) and anti-GAPDH (1:5,000; cat no. 60004-1-Ig; Proteintech Group, Inc.).

RNA isolation and reverse transcription-quantitative PCR (qPCR). TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was adopted for total mRNA isolation from cell lines (IOSE386, OVCAR3, HEY, A2780 and SKOV3). mRNA was reverse transcribed with PrimeScript™ RT Master Mix (Takara Bio, Inc.) as follows: Primer annealing at 25˚C, 5 min; Reverse transcription: 42˚C, 30-60 min; Enzyme inactivation: 85˚C 5 min; and Hold: 4˚C. qPCR was performed on QuantStudio 5 qPCR equipment (Applied Biosystems; Thermo Fisher Scientific, Inc.) with Universal SYBR Green Master reagent, and the cycling conditions were: Initial denaturation: 95˚C, 3 min; PCR cycles (40 cycles): Denaturation: 95˚C, 15 sec and Annealing/extension: 60˚C, 30 sec (with fluorescence acquisition); and Melting curve analysis: 60-95˚C. The relative gene expression levels were normalized to those of the internal control, GAPDH, using the $2^{-\Delta\Delta Cq}$ method (14). The primer sequences were as follows: HAT1, forward, 5'-AAGCCATTCGGAACCTTACTTC-3', reverse 5'-AGTGCCATCTTTCATCATCCAC-3'; and GAPDH, forward 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse 5'-GGCTGTTGTCATACTTCTCATGG-3'.

Colony formation assay. 500 Cells (HEY and SKOV3 cells) were trypsinized, assessed and cultivated for 2 weeks in medium containing 10% FBS. Subsequently, the cells were washed in PBS, fixed with 4% paraformaldehyde for 15 min and stained with 1% crystal violet for 30 min at 37˚C. The colonies visible at the plates were counted with ImageJ software v1.41 (National Institutes of Health) and images were captured.

Cell viability assay. 2000 HEY and SKOV3 cells were trypsinized, measured and seeded into 96-well plates with medium containing 10% FBS. Each well received 10 μl Cell Counting Kit-8 (CCK-8) solution (Dojindo Laboratories, Inc.) and the cells were cultured in an incubator at 37˚C for 1 h. At various time points (24, 48, 72, 96, 120 h), the absorbance was measured at 450 nm with a microplate reader and analyzed (Bio-Rad Laboratories, Inc.).

Cell cycle assay. A total of $5x10^6$ HEY and SKOV3 cells were collected, washed with PBS and were subsequently fixed with 70% cold ethanol at 4˚C overnight. The cells were then incubated with 500 μl PI/RNase A staining solution (Nanjing KeyGen Biotech Co., Ltd.) for 1 h at room temperature in the dark, according to the manufacturer's instructions, and were examined using a BD FACSCanto flow cytometer (BD Biosciences) and analyzed using FlowJo software version 4.5 (Tree Star, Inc.).

EdU staining. The EdU reagent was used to measure cell proliferation (Guangzhou RiboBio Co., Ltd.). A total of $2x10^4$ HEY cells were cultured in 96-well plates. Cells were then treated with 50 μM EdU solution for an additional 2 h in 37˚C. After washing with PBS, the cells were fixed at room temperature with 4% paraformaldehyde for 30 min and then permeabilized with 0.5% Triton X-100 for 10 min. Cells were exposed to 200 μl Apollo staining solution in the EdU kit for 30 min at room temperature in the dark. Finally, the DNA was stained with 200 μl 1 X Hoechst 33342 for 30 min at room temperature in the dark. Cells were then examined under a fluorescence microscope with excitation wavelength at 567 nm.

Cell survival assay. HEY and SKOV3 cells were seeded into 96-well plates ($5x10^3$/well) and treated with 0.0, 0.5, 1.0, 5, 10, 20, 30 and 50 μM) of HAT1 inhibitor JG-2016 or DMSO as indicated (Cat.HY-154944; MedChemExpress) for 72 h at room temperature. OD values were determined using a luminometer at an absorbance of 450 nm with the CCK-8 Kit as aforementioned (Dojindo Laboratories, Inc.), and cell viability levels were calculated based on OD values.

Luciferase reporter assay. The potential transcriptional factor FOXA1 binding sites of the HAT1 promoter region were analyzed through the UCSC Genome Browser (genome.ucsc.edu/) and TF database JASPAR (https://jaspar.elixir.no/). The pmiRGLO vectors (Promega Corporation) containing HAT1 wild-type (WT) and HAT1 mutant (MUT) sequences were constructed and Sanger sequenced. Subsequently, the HEY cells were co-transfected with pmiRGLO WT/MUT vectors and an empty or FOXA1 vector using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following 48 h of transfection, the Luciferase Assay Kit (Promega Corporation) was used to assess the activities of firefly and *Renilla* luciferase, and the firefly luciferase activity levels were adjusted to *Renilla* luciferase.

Bioinformatics analysis. The HAT1 protein expression data from healthy human tissue and OC tissues was derived from antibody-based protein profiling using immunohistochemistry from the Human Protein Atlas database (https://www.proteinatlas.org/). The mRNA and protein expression levels of HAT1 in healthy human tissue (n=133) and OC tissues (n=374) were analyzed using The Cancer Genome Atlas (TCGA data (https://www.cancer.gov/ccg/research/genome-sequencing/tcga) through the TNMplot analysis platform (tnmplot.com/analysis/), the University of Alabama at Birmingham Cancer data analysis Portal (UALCAN) and Clinical Proteomic Tumor Analysis Consortium (ualcan.path.uab.edu/index.html; gdc.cancer.gov/about-gdc/contributed-genomic-data-cancer-research/clinical-proteomic-tumor-analysis-consortium-cptac). Survival analysis was performed using the Kaplan-Meier method and log-rank testing to analyze the prognostic value of the HAT1 gene (kmplot.com/analysis/; https://www.gsea-msigdb.org/gsea/index.jsp); the patient samples were split into two groups according to various quantile expressions of HAT1 (Cutoff value used in analysis: 1383), and the hazard ratios with 95% confidence intervals and log-rank P-values were calculated; the Kaplan-Meier survival plots with the number at risk, hazard ratios and log-rank P-values were obtained using the Kaplan-Meier plotter website. The LinkedOmics database was used to perform HAT1 gene and pathway analysis using both over-representation analysis and gene set enrichment analysis (linkedomics.org; cutoff value, 2000). The binding regions of FOXA1 in HAT1 promoter region were analyzed by Cistrome DB (cistrome.org/db/#/) with the public chromatin immunoprecipitation-sequencing data downloaded from the Gene Expression Omnibus (GEO) datasets GSM 1538430/803461/1858655. The binding peaks of FOXA1 in HAT1 promoter indicated that FOXA1 transcriptionally regulated HAT1 expression. The cBioPortal database (cbioportal.org/) was used to analyze the correlation between FOXA1 and HAT1 through Pearson's correlation coefficient. Transcriptional factor FOXA1 was enriched at the HAT1 promoter region as suggested by Cistrome DB. The association between HAT1 and regulatory proteins of DNA replication and pyrimidine metabolism pathways in OC tissues were analyzed through cBioPortal database (https://www.cbioportal.org/).

Statistical analysis. Experiments were repeated at least three times and data are presented as the mean ± SD, with results analyzed using GraphPad 8.0 software (Dotmatics). The differences between two groups were analyzed via unpaired Student's t-test. HAT1 expression levels between the OC cell lines and the normal IOSE386 cell line were analyzed with one-way ANOVA and Dunnett's post hoc test. The significance of differences in HAT1 expression in the bioinformatics analysis was determined using Welch's t-test, or one-way ANOVA with Dunnett's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of HAT1 are upregulated in OC tissues and higher HAT1 levels indicate poor prognosis. HAT1 was the first HAT) discovered; however, its biological functions together with the cell mechanisms involved in cancer progression are poorly characterized. To identify the potential role of HAT1 in OC, the immunohistochemistry staining results of HAT1 in OC tissue were analyzed in the Human Protein Atlas database, and HAT1 staining was found to be higher in OC compared with in healthy human specimens (Fig. 1A). Through TCGA) database analysis, the OC tissues were found to have higher expression levels of HAT1 compared with healthy human tissues (Fig. 1B). In addition, HAT1 protein levels were increased in OC samples (Fig. 1C) and were associated with tumor grades (Fig. 1D). The association of HAT1 with overall survival was analyzed using the Kaplan-Meier plotter website and the results showed that higher HAT1 levels indicated shorter survival rates (Fig. 1E). These findings suggested that HAT1 may act as a tumor promoter in OC.

HAT1 levels are increased in OC cell lines. To determine the role of HAT1 in OC development, the mRNA expression levels of HAT1 were assessed in different OC cell lines: A2780, HEY, OVCAR3 and SKOV3. The results showed that HAT1 expression levels were generally upregulated in OC cell lines compared with normal IOSE386 cell line, especially in HEY and SKOV3 cells, which were used in subsequent study (Fig. 2A). Similarly, the protein levels of HAT1 were increased in OC cells (Fig. 2B). Thus, HAT1 may induce ovarian tumorigenesis.

FOXA1 transcriptionally regulates HAT1 expression in OC. TFs are important for the modulation of various signaling pathways associated with cell homeostasis and disease conditions (15). To identify the upstream regulator of HAT1 in OC, the TF database was used to predict the potential TFs of HAT1 and it was revealed that the TF FOXA1 may regulate HAT1. Among cancer-related TFs, FOXA1 is an important molecule that regulates multiple aspects of cancer cells, including cell proliferation and cancer metastasis. The potential FOXA1 binding sites of the HAT1 promoter region were then analyzed through the UCSC Genome Browser and JASPAR databases (Fig. 3A). Subsequently, WT and MUT HAT1 luciferase reporter plasmids were constructed (Fig. 3A). Overexpression of FOXA1 could promote the activities of HAT1 WT plasmids, as determined through the luciferase reporter assay; however, this was not observed with HAT1 MUT plasmids (Fig. 3B and C). Compared with the vector control, overexpression of FOXA1 significantly increased HAT1 expression levels (Fig. 3D). In addition, the cBioPortal database was adopted

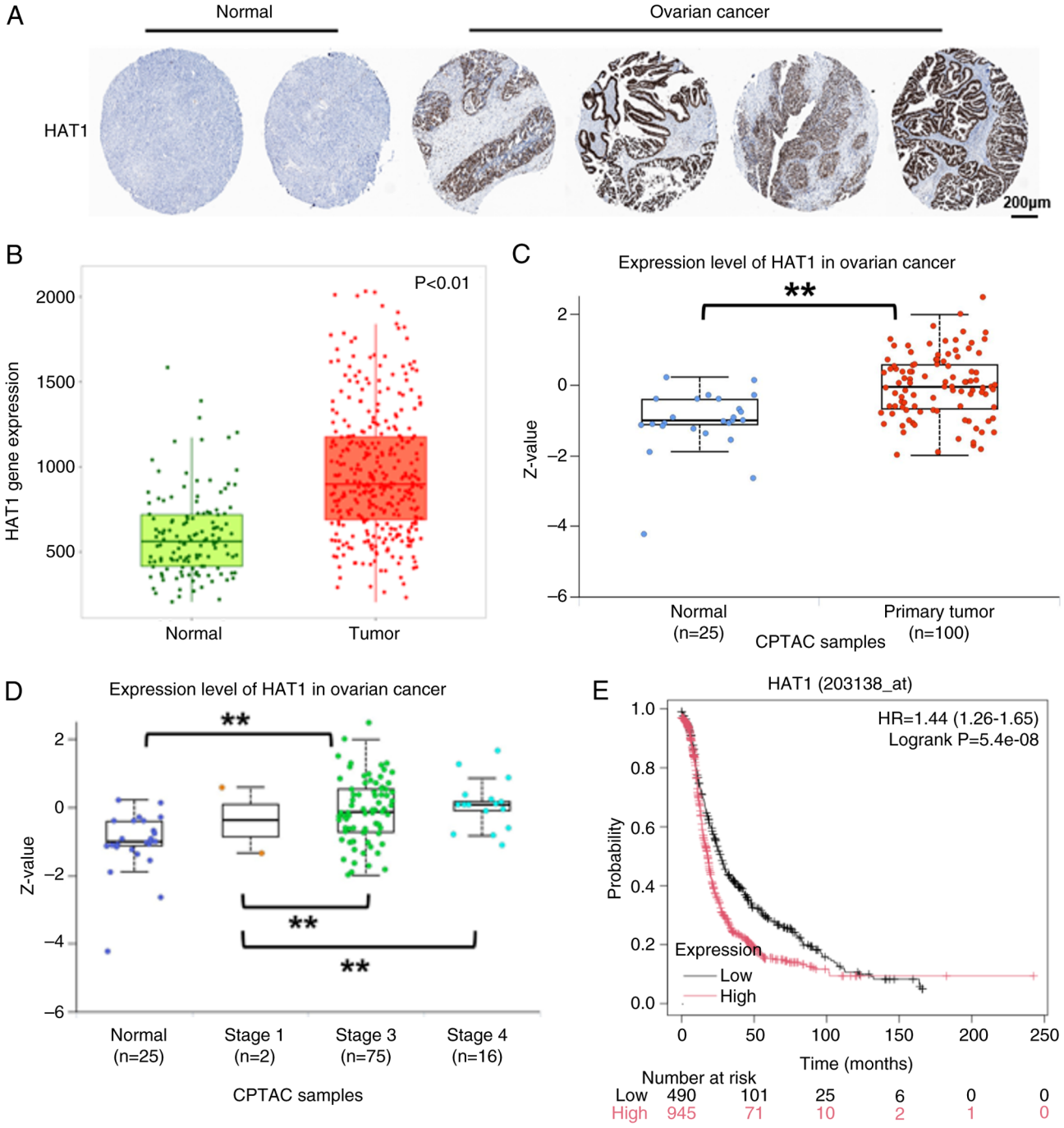

Figure 1. HAT1 levels are upregulated in human ovarian cancer tissue and higher HAT1 levels indicate a poor prognosis. (A) Representative images depicting the representative immunostaining of HAT1 in ovarian cancer tissues and healthy human tissues in The Human Protein Altas database. Scale bar=200 μm. (B) Analysis of the TNMplot database showed that HAT1 was highly expressed in ovarian cancer tissues compared with healthy controls. (C) Protein levels of HAT1 in ovarian cancer and healthy human samples from healthy controls were analyzed in the CPTAC database. (D) HAT1 protein levels were upregulated with tumor grade in ovarian cancer tissues. (E) Higher HAT1 levels were associated with an unfavorable prognosis in patients with ovarian cancer. Data are presented as the mean ± SD. **P<0.01. HAT1, histone acetyltransferase 1; CPTAC, Clinical Proteomic Tumor Analysis Consortium; HR, hazard ratio.

to analyze the correlation between FOXA1 and HAT1, and a positive association was observed between FOXA1 and HAT1 levels in OC tissues from TCGA data in the cBioPortal database (Fig. 3E). FOXA1 expression levels were also upregulated in OC tissues as suggested in TCGA database (Fig. 3F). Subsequently, the chromatin immunoprecipitation-sequencing data conveyed that FOXA1 was enriched in the promoter region of HAT1 (Fig. 3G). In conclusion, these data showed that FOXA1 functioned as a TF of HAT1.

HAT1 is involved in DNA replication and pyrimidine metabolism pathways. To explore the regulatory pathways associated with HAT1 expression levels, OC data from TCGA database were downloaded and analyzed, before being separated into HAT1 high- and low-expression groups section. The LinkedOmics database suggested that differentially expressed genes of HAT1 were enriched in several biological processes, such as 'DNA replication', 'pyrimidine metabolism' and 'RNA transport' (Fig. 4A). The gene set enrichment analysis results

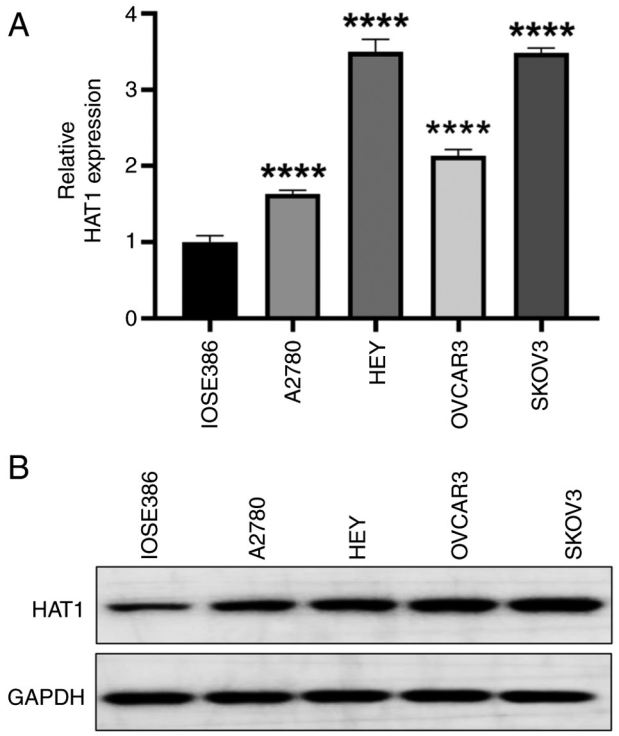

Figure 2. HAT1 expression levels are upregulated in human ovarian cancer cell lines. (A) Relative expression levels of HAT1 were detected by reverse transcription-quantitative PCR in A2780, HEY, OVCAR3, SKOV3 and normal IOSE386 cell lines. (B) Western blot analysis of HAT1 expression in A2780, HEY, OVCAR3, SKOV3 and IOSE386 cell lines. ****P<0.0001 vs. IOSE386. HAT1, histone acetyltransferase 1.

showed that the HAT1 high-expression group was positively associated with the 'DNA replication' and 'pyrimidine metabolism' pathways (Fig. 4B). Subsequently, the association between HAT1 and regulatory proteins of DNA replication and pyrimidine metabolism pathways in OC tissues were analyzed, and the expression levels of HAT1 were revealed to be positively correlated with proliferating cell nuclear antigen (PCNA), replication protein A1 and DNA polymerase α catalytic subunit from the cell cycle pathway (Fig. 4C). Furthermore, HAT1 levels were positively correlated with thymidine kinase 1, ribonucleoside-diphosphate reductase subunit M (RRM)1 and RRM2, which are involved in the pyrimidine metabolism pathway (Fig. 4D).

HAT1 induces OC cell viability and colony formation. To confirm the tumor-promoting role of HAT1, HEY and SKOV3 OC cell lines were used to knockdown HAT1 expression (Fig. 5A). As determined by CCK-8 assay, HAT1 knockdown markedly suppressed cell proliferation (Fig. 5B). In the present study, cells were also treated with the HAT1 inhibitor JG-2016, and the results showed that JG-2016 significantly inhibited cell proliferation (Fig. 5C). In addition, inhibition of HAT1 reduced colony formation activities in HEY and SKOV3 cell lines (Fig. 5D). Thus, the present results indicated that HAT1 contributed to tumorigenic growth.

HAT1 expression is associated with CDK2, CDK4 and cyclin E levels. Human HEY OC cells were transfected with negative control and HAT1 siRNAs. These cells were then stained with an EdU probe, which functions as a cell proliferation marker, and the results showed that HAT1 knockdown significantly suppressed EdU activities (Fig. 6A). The cell cycle regulatory pathway integrates into other hallmarks of cancer, including metabolism remodeling and immune escape, and promotes tumorigenesis (16). In the present study, inhibition of HAT1 suppressed cell cycle progression and reduced the percentage of cells at S phase (Fig. 6B). Cell cycle-related proteins levels were then analyzed and it was revealed that suppression of HAT1 decreased CDK2, CDK4 and cyclin E levels (Fig. 6C). Furthermore, positive associations were shown between HAT1 and CDK2, CDK4 and cyclin E levels in OC tissues from TCGA data obtained from the cBioPortal database (Fig. 6D). Thus, HAT1 was suggested to regulate the cell cycle pathway in OC.

Discussion

OC has become one of the most frequently diagnosed cancers in female patients worldwide, with an expected 313,000 cases and 207,000 mortalities in 2020, ranking as the third highest cause of death among gynecological cancers (17). The high mortality rate is partly due to the heterogeneity of the tumor combined with the absence of symptoms at the early stages of OC, which limits early diagnosis, resulting in most patients presenting with advanced stages of OC when identified (18). Histone modifications, as major chromatin regulators, play an important role in the etiology of numerous diseases, especially cancer (19). Acetylation, specifically lysine acetylation, is a dynamic epigenetic process with a notable role in cell cycle, cell survival or to cellular processes, which capable of targeting both histone and non-histone proteins (16,20). Epigenetic modification via acetylation regulates gene transcriptional processes and protein stability, activity and localization (21). HAT1 was the first identified HAT; however, its biological significance remains to be elucidated. Several studies have highlighted the importance of HAT1 in regulating cellular processes that are altered in a variety of diseases, including cancer (13,22,23). HAT1 protein knockdown or knockout in various pre-clinical models has conveyed that HAT1 is likely a new potential therapeutic target in OC treatment (13,22,23). In the present study, the mRNA and protein expression levels of HAT1 were found to be elevated in OC tissues, and elevated HAT1 levels were associated with shorter survival rate.

FOXA1 belongs to the FOXA TF family and has a fork-head (or winged helix) DNA-binding domain of ~100 amino acids. FOXA1 functions as a TF and attaches to the chromo-some to induce nucleosome remodeling, which allows other TFs to bind to the chromosome and perform tissue-specific transcriptional programs (24-28). FOXA1 can be pro- or antitumorigenic in various human malignancies (29). Up to 80% of estrogen receptor-positive breast cancer cases are FOXA1-positive, and FOXA1 expression is linked with improved prognosis (29). Furthermore, FOXA1 upregulation has been shown to be inversely linked with interferon signa-ture and activated tumor-specific $CD8^+$T cells in patients with prostate cancer, and to increase cancer immunoresistance and resistance to chemotherapy in nude mice and patients with prostate cancer (30). FOXA1 acts as a tumor suppressor during its development of endometrial cancer (31). In the present

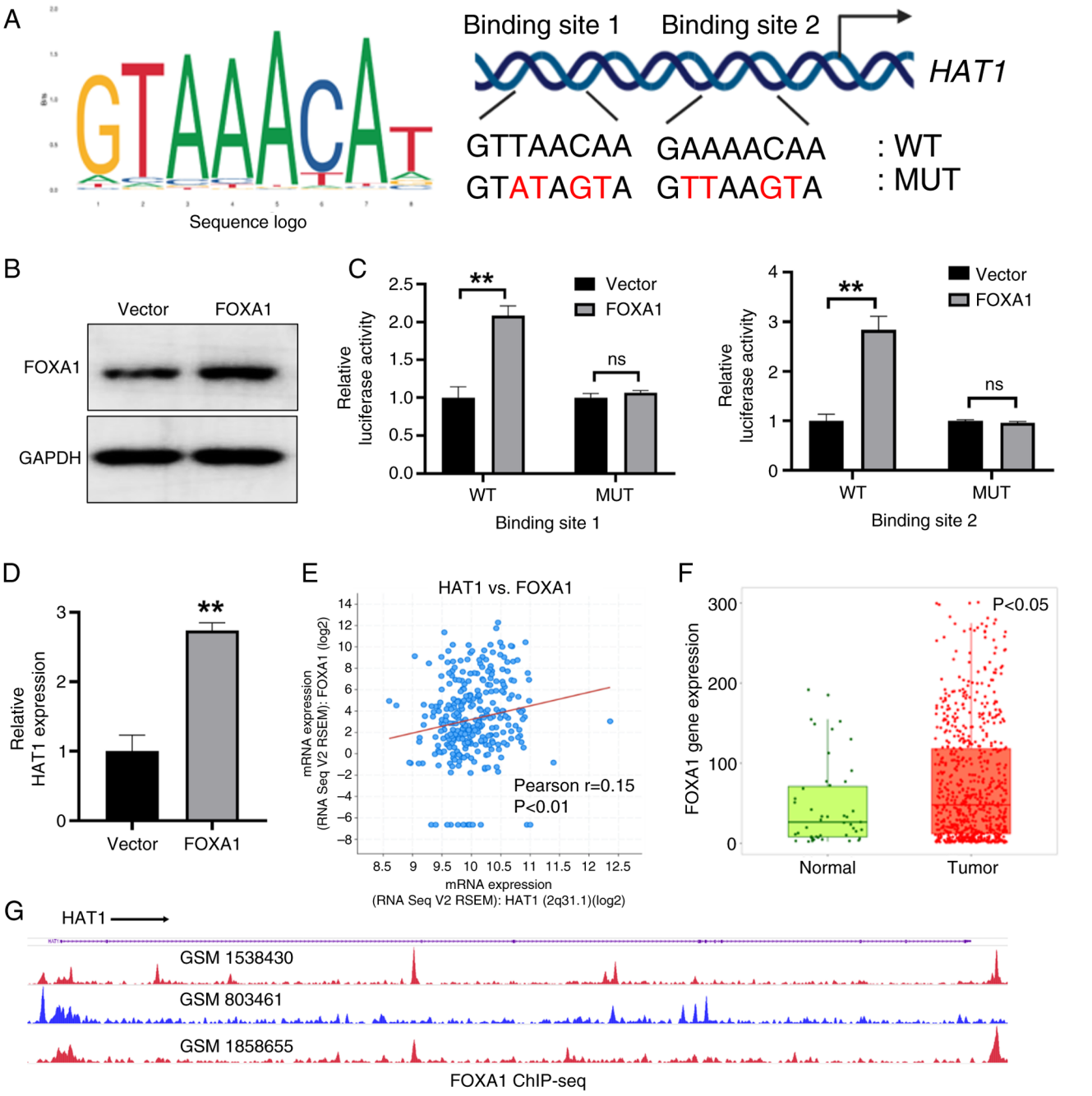

Figure 3. FOXA1 transcriptionally regulates HAT1 expression levels. (A) FOXA1 binding sites in the HAT1 promoter region were predicted using the JASPAR database. MUT constructs were generated at the binding sequence regions as indicated. (B) The expression levels of FOXA1 in HEY cells transfected with an empty or FOXA1 vector. (C) HEY cells were transfected with pmirGLO reporter vectors containing either WT or MUT plasmids alongside an empty or FOXA1 vector. Luciferase activities were determined 24 h after transfection. (D) Overexpression of FOXA1 induced HAT1 expression levels. (E) cBioPortal database was adopted to analyze the correlation between FOXA1 and HAT1. Pearson's rank correlation between HAT1 and FOXA1 was analyzed in ovarian cancer tissues. (F) FOXA1 levels in ovarian cancer tissues from TCGA were determined using TNMplot database. (G) FOXA1 was enriched at the HAT1 promoter region as suggested by Cistrome DB. Data are presented as the mean ± SD of three replicates. **P<0.01 vs. vector or as indicated; ns, not significant. ChIP-seq, chromatin immunoprecipitation sequencing; FOXA1, forkhead box protein A1; HAT1, histone acetyltransferase 1; WT, wild-type; MUT, mutant.

study, the TF FOXA1 was shown to regulate HAT1 levels, and there was a positive association between FOXA1 and HAT1 in OC.

The functional importance of HAT1 has been investigated in a number of cell models to improve understanding of its biological function. Previous investigation has suggested that HAT1 is briefly localized on chromatin, adjacent to DNA replication sites, where it plays a regulatory role in replication fork stalling (32). According to additional research, the loss of HAT1 enhances genomic instability, making cells vulnerable to DNA double-strand breaks (33). The significance of HAT1 in replication and chromatin assembly has previously been shown (34).

In the present study, the expression levels of HAT1 were positively correlated with PCNA in the DNA replication pathway. PCNA is the eukaryotic DNA sliding clamp that interacts with cell cycle proteins, including cyclins and CDKs, involved in DNA replication (35). A previous study has shown

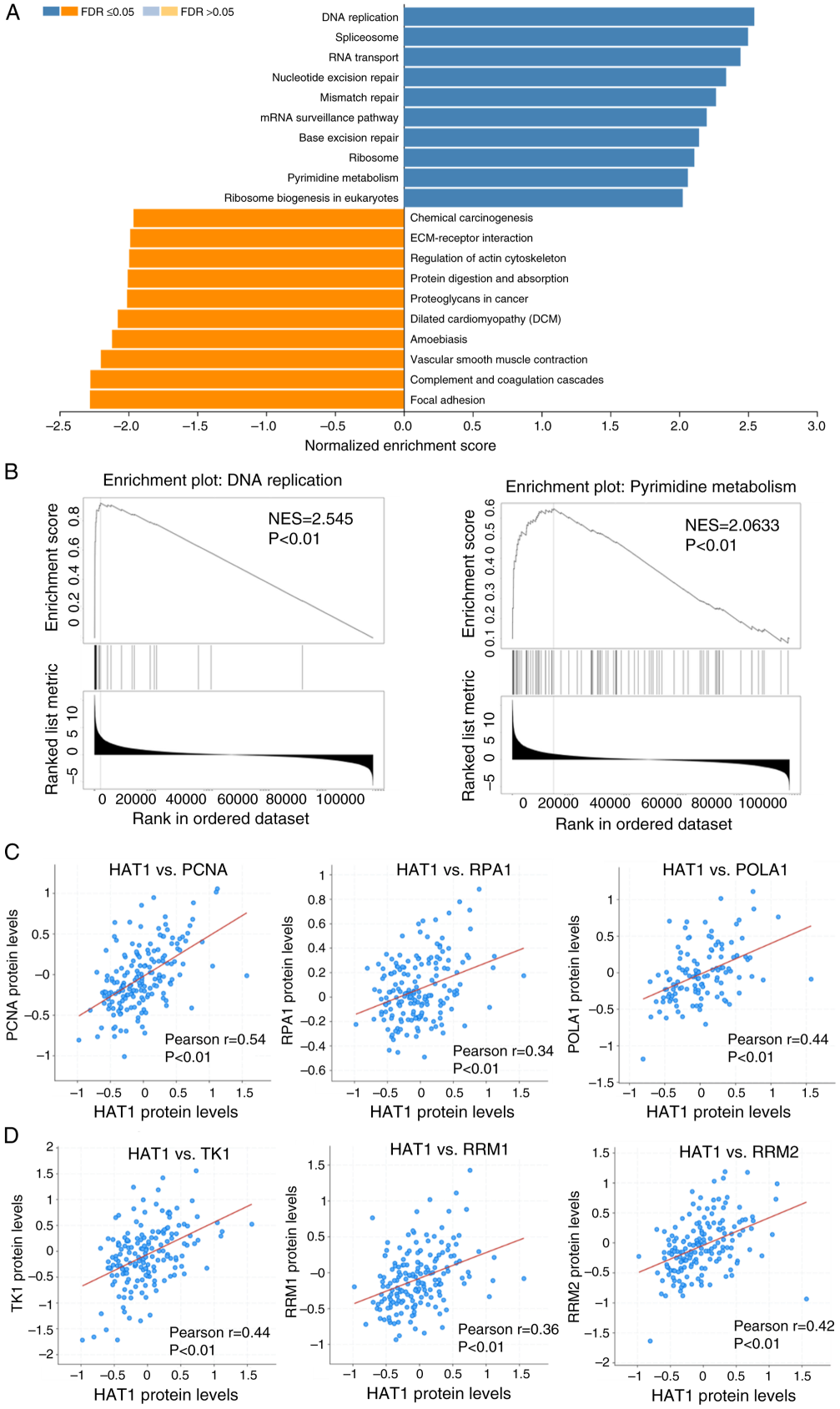

Figure 4. Relationship between HAT1 and DNA replication and pyrimidine metabolism pathways. (A) LinkedOmics database suggested that differentially expressed genes of HAT1 were enriched in several biological processes, such as 'DNA replication', 'pyrimidine metabolism' and 'RNA transport'. (B) Gene set enrichment analysis revealed that genes altered by HAT1 were positively associated with 'DNA replication' as well as 'pyrimidine metabolism' pathways. (C) cBioPortal database was used to analyze the correlations between HAT1 and regulatory proteins of the DNA replication pathway. Pearson's rank correlation between HAT1 and DNA replication-related proteins (PCNA, RPA1 and POLA1) was analyzed in ovarian cancer tissues. (D) Pearson's rank correlation between HAT1 and pyrimidine metabolism-related proteins (TK1, RRM1 and RRM2) was analyzed in ovarian cancer tissues as suggested in the cBioPortal database. HAT1, histone acetyltransferase 1; PCNA, proliferating cell nuclear antigen; RPA1, replication protein A1; POLA1, DNA polymerase α catalytic subunit; TK1, thymidine kinase 1; RRM, ribonucleoside-diphosphate reductase subunit M; NES, normalized enrichment score; FDR, false discovery rate.

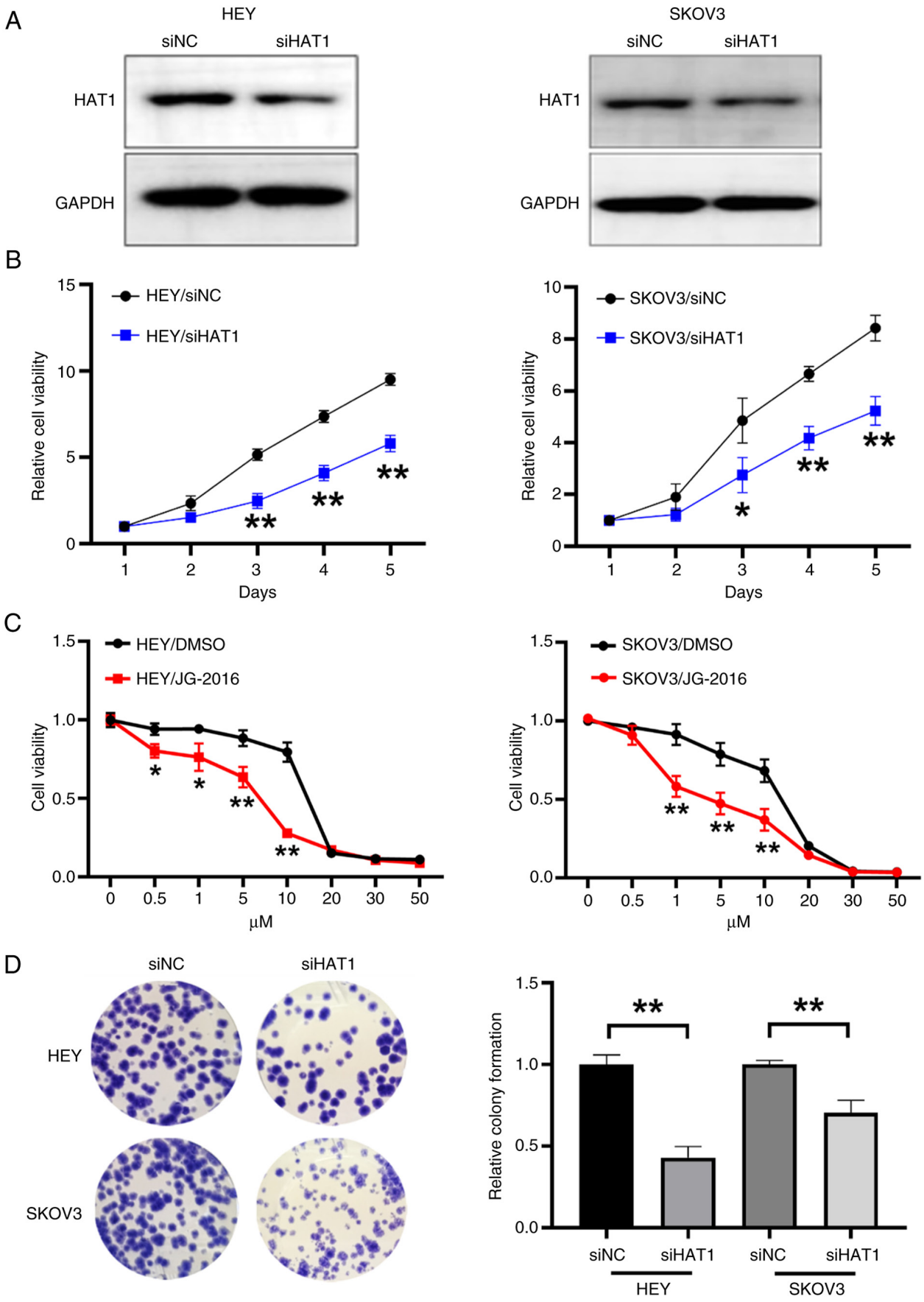

Figure 5. Inhibition of HAT1 suppresses cell viability and colony formation *in vitro*. (A) Western blot analysis of HAT1 expression in HEY and SKOV3 cells transfected with HAT1 siRNA or NC siRNA. (B) Cell Counting Kit-8 assays were performed to determine cell viability after HAT1 was knocked down in HEY and SKOV3 cells. (C) HEY and SKOV3 cells were treated with HAT1 inhibitor JG-2016 for 72 h, which significantly inhibited cell viability. (D) Knockdown of HAT1 decreased the colony formation capacity of HEY and SKOV3 cells. Data are presented as the mean ± SD of three replicates. *P<0.05 and **P<0.01 vs. siNC in figure 5B and DMSO group in figure 5C. HAT1, histone acetyltransferase 1; si, small interfering; NC, negative control.

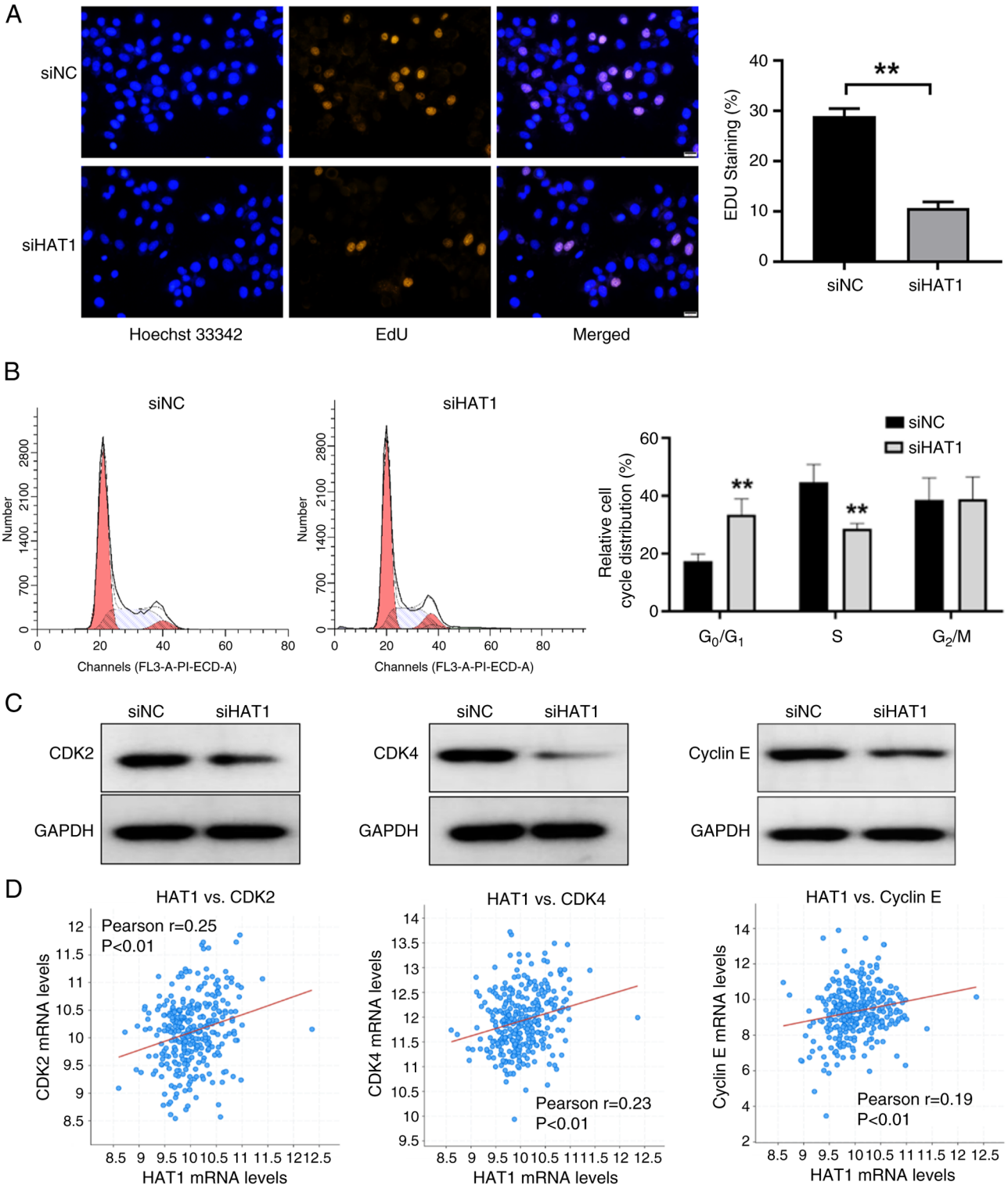

Figure 6. HAT1 knockdown inhibits cell proliferation. (A) EdU assays of HEY cells were performed showing that suppression of HAT1 attenuated cell proliferation activities. Scale bar, 20 μm. (B) Cell cycle analysis was carried out on the HAT1 knockdown and siNC cell lines, and the distribution of G_0/G_1, S and G_2/M percentages were analyzed. (C) Western blot analysis of cell cycle-related protein expression after knockdown of HAT1. (D) Pearson's rank correlation between HAT1 and CDK2, CDK4 and cyclin E was analyzed in ovarian cancer tissues. Data are presented as the mean ± SD of three replicates. **P<0.01 vs. siNC. HAT1, histone acetyltransferase 1; si, small interfering; NC, negative control; CDK, cyclin-dependent kinase.

that PCNA is a key factor in DNA replication during the cell cycle and is directly associated with prostate tissue proliferation (36). PCNA inhibition induces S-phase arrest of human prostatic epithelial cancer cell lines (37). PCNA also interacts with the cyclin-CDK complex in several eukaryotic cells (38). It has been noted that CDK2 could directly interact with PCNA in numerous cells, supporting the linkage between cell cycle checkpoints and PCNA (39). Thus, PCNA is considered an accessory protein in cellular processes, contributing to cell division, DNA replication and various cell cycle controls. In

the present study, HAT1 was suggested to regulate the cell cycle pathway in OC. The cell cycle is a series of interconnected events that allow the cell to continue growing and proliferating. Cell cycle arrest serves as a survival mechanism that enables cancer cells to compensate for their own DNA that has been damaged. CDKs are key components of the cell cycle machinery that, when activated, enable the cell to move from one phase to the next (40). Cyclins positively regulate CDKs, whereas naturally generated CDK inhibitors (CDKIs) adversely regulate them. Cancer is characterized by cell cycle dysregulation, with cells upregulating cyclins or lacking CDKIs continuing to grow abnormally (41). The cell cycle also protects the cell against DNA damage. The present research conveyed that suppression of HAT1 decreased CDK2, CDK4 and cyclin E levels; thus, HAT1 may be a potential therapeutic target in OC.

Notably, findings have revealed the importance of HAT1 in a variety of physiological processes, encouraging additional research to determine its biological function (12,42). HAT1 has been linked to a variety of cellular functions, including proliferation, DNA replication and cellular metabolism (12,42). The JG-2016 compound has shown relative specificity toward HAT1 compared with other acetyltransferases, suppressing the proliferation of human cancer cell lines and interfering with tumor growth (43). The present study is, to the best of our knowledge, the first report of a small-molecule inhibitor of the HAT1 enzyme complex and represents a step toward targeting the HAT1 pathway for cancer therapy. In the present study, the inhibition of HAT1 was discovered to promote cell cycle arrest, and reduce CDK2, CDK4 and cyclin E levels in OC cells. Thus, the present work highlighted the importance of FOXA1 as an upstream regulator mediating HAT1 expression levels and HAT1 as a potential oncogene in OC. Therefore, specifically inhibiting HAT1 expression may provide a unique strategy for OC treatment.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XH conceptualized and designed the study, performed experiments and wrote the manuscript. JLi, YZ, LZ, LY and XO developed methodology, analyzed data and performed experiments. LL and JLiu performed experiments, analyzed data and confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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