

Expression of SAMHD1 and its mutation on prognosis of colon cancer

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Abstract. The expression of sterile α motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1) and its mutation play a key role in the prognosis of colon cancer. The aim of the present study was to investigate the mechanism and the role of SAMHD1 in colon cancer. Microarray data from 187 patients with colon cancer and 45 adjacent normal tissue obtained from the Gene Expression Omnibus (GEO) were analyzed. A protein-protein interaction (PPI) network was constructed to identify key genes associated with colon cancer prognosis. Cox proportional hazard regression and survival analyses were performed to identify the potential for SAMHD1 to serve as a prognostic biomarker. Immunohistochemistry (IHC) and immunofluorescence (IF) were performed to assess the expression levels and distribution of SAMHD1 in tissues and cells. Western blotting (WB) and Cell Counting Kit-8 (CCK-8) assays were used to identify the proliferation and apoptotic effects of SAMHD1 on HT-29 (Cas9-SAMHD1) cell lines. A total of 6,905 consistently differentially expressed genes were identified in the GEO database. Through the PPI network, SAMHD1 was found to be associated with Kirsten rat sarcoma virus (KRAS). SAMHD1 expression was negatively associated with KRAS. Proportional hazards regression and survival analyses demonstrated that low expression of SAMHD1 was associated with increased patient mortality. IHC and IF results demonstrated that SAMHD1 expression in patients with colon cancer was

decreased compared with controls (both $P < 0.05$). CCK-8 and WB results showed that proliferation was significantly promoted, and the expression levels of apoptosis-related proteins were significantly inhibited in the D137N and D311A groups as a result of a mutation in the deoxynucleoside triphosphohydrolase (dNTPase) site (both $P < 0.05$ vs. wild-type). Proliferation was inhibited and apoptosis-related protein expression levels were promoted in the wild-type (WT) and D137N groups following 20 $\mu\text{g/ml}$ 5-fluorouracil (5-FU) treatment (both $P < 0.05$). WB and CCK-8 results showed cell proliferation was promoted and cell apoptosis-related protein expression was inhibited in the D137N group following treatment with 20 $\mu\text{g/ml}$ 5-FU (all $P < 0.05$) compared with the WT group. In conclusion, SAMHD1 expression was low in colon cancer. The dNTPase function of SAMHD1 may inhibit colon cancer cell proliferation and may enhance apoptosis. In addition, first-line chemotherapy with 5-FU has a time-dependent effect, which may provide novel options for clinical treatment of colon cancer.

Introduction

Colon cancer is a common type of digestive system malignancy worldwide (1,2). Cancer statistics from 185 countries show that 1,104,100 novel cases and 883,200 mortalities were caused by colon cancer in 2018 (3). The incidence rate was ranked fourth and the mortality rate was ranked second among all types of cancer worldwide (3). With improved living standards and diet, the incidence of colon cancer has increased owing to high-fat diet and insufficient food fiber (4). Colon cancer is most common where the rectum and sigmoid colon connect, and it is more common in male compared with female patients (5). Prognosis and treatment of colon cancer is important. Current clinical treatment of colon cancer is based on surgery, radiotherapy and chemotherapy, but the five-year survival rate has not greatly improved (6). Mutations in Kirsten rat sarcoma virus (KRAS) is a diagnostic biomarker and therapeutic target of colon cancer (7). Therefore, determining the molecular mechanism of colon cancer prognosis is crucial to develop gene therapy.

The only dNTP hydrolase in eukaryotes, SAMHD1, is involved in multiple pathological processes (8). SAMHD1 serves

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a key role in preventing virus transcription and replication by hydrolyzing dNTP, thus protecting host cellular genome integrity (9). As an intrinsic host restriction factor, SAMHD1 exploits deoxynucleoside triphosphohydrolase (dNTPase) activity to resist human immunodeficiency virus 1 (10). Literature has shown that SAMHD1 is primarily involved in apoptosis and proliferation through hydrolyzing nucleic acids (11,12). Although some consider SAMHD1 to serve only as a dNTPase, others hypothesize that SAMHD1 can be used not only as dNTPase but also as RNase. Therefore, this remains controversial (8,13). SAMHD1 is also known as Aicardi-Goutières syndrome (AGS) gene because multipoint mutations in SAMHD1 cause familial autoimmune AGS (14-17). SAMHD1 is affected by post-translational modifications, such as phosphorylation, acetylation and methylation, which primarily affect its ability to prevent viral infection (18-22). For example, phosphomimetic mutation of T592E causes notable destabilization of the active tetrameric form of SAMHD1 and a ~3-fold decline in dNTPase activity (9). However, the T592V variant does not disrupt the crystal structure of SAMHD1, thus its dNTPase activity is not affected (9). Furthermore, SAMHD1 is acetylated on K405 by acetyltransferase arrest defective protein 1, which increases its dNTPase activity *in vitro* (21).

Previous studies have shown that SAMHD1 is associated with development of several types of cancer, including lung and colon cancer (20,23). SAMHD1 mRNA and protein expression levels have been shown to be decreased in lung adenocarcinoma tissue compared with adjacent normal controls, which suggests that the SAMHD1 promoter is highly methylated in lung adenocarcinoma, leading to decreased SAMHD1 expression (20). The decrease of SAMHD1 activity is caused by frequent mutations of SAMHD1 gene in colon cancer cells (23), which suggests that the decrease of SAMHD1 activity is associated with an increased risk for colon cancer compared with other types of cancer. A total of 164 unique mutations in SAMHD1 from a variety of cancer tissues are listed in the catalogue of cancer somatic mutations, which indicates that SAMHD1 mutation is more frequent in cancer (24). SAMHD1 mutations at residues 123, 143, 145, 201, 209, 254, 369 and 385 lead to decreased function of endogenous SAMHD1 protein and disrupt nucleotide metabolism in myeloid cells, leading to carcinogenesis (15). However, the effect of SAMHD1 on colon cancer prognosis is not clear. The present study aimed to determine expression of SAMHD1 in colon cancer and the effect of its dNTPase function on cell apoptosis and proliferation to provide potential biomarkers for prognosis of colon cancer.

Materials and methods

Dataset access and information. The Gene Expression Omnibus (GEO) database was searched (ncbi.nlm.nih.gov/geo/; GSE41258). Perl ([strawberry-perl-5.32.1.1-64bit](http://strawberry-perl-5.32.1.1-64bit.strawberryperl.com/releases.html); strawberryperl.com/releases.html) command was used to convert the genetic probe IDs in the matrix documents to the platform's genetic symbols to acquire a matrix document encompassing formal symbols. All datasets were normalized using the limma R package (R for Windows 4.1.0 Setup and <https://www.rstudio.com>). The entire genetic expression data underwent log₂ transformation.

Dataset analysis. Gene differential analysis [$|\text{LogFC}| > 1$, adjusted P-value (FDR) < 0.05] was performed by comparing tumor tissue with controls using limma R package. A volcano plot (R for Windows 4.1.0 Setup; rstudio.com) was created to present the fold-change and P-values of differentially expressed genes (DEGs) using limma R package.

Protein-protein interaction (PPI) network construction. Core genes were obtained from PPI network [Search Tool for the Retrieval of Interacting Genes/Proteins (STRING); string-db.org/] and Cytoscape software (Cytoscape_3_8_2_windows_64bit; cytoscape.org/download.html) was used to plot the net diagram. Each node denotes a protein or gene (25); edges between nodes denote molecular interactions.

Patient information. The present study was approved by the Ethics Committee of Wuxi No. 2. People's Hospital (Wuxi, China; approval no. 20180706). The patients were recruited between January and November 2020. For all patient-derived tissues, written informed consent was obtained. A total of 184 patients (sex, 98 male and 86 female; age, 19-87 years) with colon cancer and 45 adjacent normal tissues (sex, 23 male and 22 female; age, 19-85 years) were included.

Colon cancer tissue were collected to assess tumor morphology as part of routine surgery. Inclusion criteria were as follows: i) Colon cancer diagnosed by histopathological examination; ii) imaging examination confirmed that there was one or more measurable lesions; iii) routine blood examination, liver and kidney function and ECG were normal and iv) estimated survival time > 3 months. Exclusion criteria were as follows: i) Patients with other types of tumor or ii) serious underlying disease.

Immunohistochemistry (IHC). IHC was performed routinely on all primary colon cancer samples, as previously described (26,27). All samples were fixed in 10% neutral buffered formalin at room temperature for 1 h and maintained at 4°C with 30% saccharose overnight. Subsequently, all biopsy samples were embedded in optimal cutting temperature compound and sectioned in 4- μm thick slices, followed by blocking with 10% normal goat serum (cat. no. abs933; Absin) at 37°C for 60 min. The sections were incubated with SAMHD1 antibody (cat. no. ab128107; Abcam; 1:150) at 37°C for 0.5 h, followed by incubation with HRP-Polymer anti-Mouse/Rabbit IHC kit (cat. no. KIT-5020; MXB; 1:100) at 37°C for 30 min. To visualize the antigen-antibody complexes, sections were counterstained with 0.01% DAB at room temperature for 5 min and hematoxylin at room temperature for 3 min. Slides were washed for 10 min with PBS after every step. All samples were imaged using a CX31 light microscope (Olympus Corporation).

Cell culture and transfection. To eliminate background interference, HT-29 (cas9-SAMHD1) cells were constructed. NCM460 was purchased from American Tissue Culture Collection (Manassas, VA, USA), HT-29 and HT-29 (Cas9-SAMHD1) cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China) and verified by STR DNA profiling. HT-29 (Cas9-SAMHD1) cells were derived from HT-29 cells, which had previously undergone SAMHD1 gene knockout using CRISPR/Cas9 system. All cells were cultured in McCoy's

5A medium (cat. no. 12330031; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (cat. no. 16140071; Thermo Fisher Scientific, Inc.), 0.5 mmol/l L-glutamine (cat. no. 25030-024; Thermo Fisher Scientific, Inc.), 0.01 mg/ml insulin (Novo Nordisk A/S), 100 IU/ml penicillin and 100 mg/ml streptomycin (cat. no. DE17-602E; Lonza Group, Ltd.), and all cells were cultured in 37°C and 5% CO₂. 100 cm² Flasks and plates were purchased from Nunc (Thermo Fisher Scientific, Inc.) and 6-well plates were purchased from Corning Life Sciences.

HT-29 (Cas9-SAMHD1) cells were inoculated into a 6-well plate (3x10⁵) and incubated for 12 h prior to transfection in 37°C and 5% CO₂. Cells were separated into experimental and control groups with each group containing 3 µg plasmid + 5 µl Lipofectamine[®] 3000 (cat. no. L3000015, Thermo Fisher Scientific). All plasmids, including Flag-SAMHD1 (WT), Flag-SAMHD1 (Q548A), Flag-SAMHD1 (D137N) and Flag-SAMHD1 (D311A), were purchased from Shanghai GenePharma Co., Ltd. The vector for all plasmids involved in this study was PCNDA4.0. Cells were transfected at 70% confluence at room temperature. A total of 5 µl Lipofectamine 3000 was added to 125 µl DMEM/F-12 and mixed well. In addition, 3 µg DNA plasmid and 5 µl P3000[™] reagent were added to 125 µl DMEM/F-12 and mixed well. The two solutions were then mixed, maintained at room temperature for 20 min and added into the wells in a drop-wise manner. Cells were transfected in DMEM/F-12 for 6 h, then DMEM/F-12 was replaced with McCoy's 5A for 18 h. Cells were then harvested for subsequent experiments. According to preliminary results, 20 µg/ml was the most suitable concentration of 5-FU for tumor cells (data not shown).

Immunofluorescence (IF). After placing the cell slide on the 24 well plate, NCM460 normal colon epithelial and HT-29 colorectal cancer cells were inoculated in 24 well plates at a density of 1x10⁵ cells/well. Following 24 h culture at 37°C and 5% CO₂, cells were centrifuged and washed with PBS and centrifuged two times at room temperature and 1,000 x g for 5 min. 4% paraformaldehyde was fixed for 20 min and washed with PBS and centrifuged two times at room temperature for 5 min. Cells were fixed with punched with 0.1% Triton X-100 for 10 min and washed with PBS for 5 min at room temperature. Then, 0.1% BSA (cat. no. A8010, Solarbio) was used for blocking at room temperature for 1 h, followed by incubation with SAMHD1 antibody (cat. no. ab128107; Abcam; 1:100) at 4°C overnight and rinsing three times with PBS for 5 min each. Incubation with goat Anti-Mouse IgG H&L (FITC-conjugated; cat. no. ab6785, Abcam) was performed at room temperature in the dark for 1 h, followed by rinsing with PBS three times for 5 min each. The cell nuclei were stained with 10 µg/ml DAPI (cat. no. C0065; Beijing Solarbio Science & Technology Co., Ltd.) for 5 min at room temperature. Next, a drop of sealing agent was added, the film was sealed and imaging with a fluorescence microscope was performed as previously described (28,29). Images were analyzed using CellSens Standard version 1.9 software (Olympus Corporation; olympus-lifescience.com.cn/zh/software/cellsens/).

Western blotting (WB). Treated cells were harvested and lysed in RIPA buffering solution (Beyotime Institute of Biotechnology). Protein levels were determined using BCA

(Beyotime Institute of Biotechnology). Protein samples (50 µg/lane) from each group were separated using 10% SDS-PAGE, transferred onto PVDF membranes and blocked in BSA. Next, the membranes were immersed in primary antibody at 4°C overnight. The primary antibodies (all Abcam) were as follows: Bax (cat. no. ab53154; 1:1,000), Cleaved-caspase3 (cat. no. ab32042; 1:500), Caspase3 (cat. no. ab32351; 1:1,000), Bcl-2 (cat. no. ab32124; 1:1,000) and Flag (cat. no. ab205606; 1:1,000). The normal band density of GAPDH (cat. no. ab8245; Abcam; 1:1,000) at room temperature for 2 h was used as an internal reference. Then they were rinsed with TBST (0.1% Tween-20, cat. no. ST671, beyotime) three times for 10 min each. Incubation with Rabbit Anti-Mouse IgG H&L (HRP-conjugated; cat. no. ab6728, Abcam, 1:5,000) or goat Anti-Rabbit IgG H&L (HRP, ab6721; Abcam, 1:5,000) was performed at room temperature for 1 h, followed by rinsing with TBST three times for 10 min each. Protein detection was performed using BeyoECL Star (cat. no. P0018AM, beyotime). The bands were quantified using the Image Lab System version 6.1 (Bio-Rad, bio-rad.com/SearchResults?search_api_fulltext=Image+Lab). These assays were performed at least three times, as previously described (30,31).

Cell counting kit-8 (CCK-8) assay. To detect cell proliferation, CCK-8 assays (cat. no. C0037; Beyotime Institute of Biotechnology) were performed. HT-29 (Cas9-SAMHD1) cells at the logarithmic growth stage were collected and digested to prepare HT-29 (Cas9-SAMHD1) cell suspension at room temperature for 1 min by trypsin digestion (cat. no. 25200072, Gibco). Next, 1x10⁴ cells/well were inoculated into 96-well plates. Following 24 h incubation at 37°C and 5% CO₂, cell viability in each group was detected prior to transfection. The 200 ng Flag-SAMHD1 (WT), Flag-SAMHD1 (Q548A), Flag-SAMHD1 (D137N) and Flag-SAMHD1 (D311A) plasmids were then transfected into HT-29 (Cas9-SAMHD1) cells. Cell viability was detected 24 and 48 h after transfection. A total of 10 µl CCK-8 solution was added to 90 µl culture medium. The medium was replaced in each well for testing, and cells were incubated at 37°C for 1 h. A microplate reader was used to measure optical density in each well at a wavelength of 450 nm, as previously described (32).

Following 24 h transfection at 37°C and 5% CO₂, cell viability in the WT and D137N groups was detected. Cell viability was detected for 24 and 48 h following 20 µg/ml 5-FU treatment in 37°C and 5% CO₂, as aforementioned.

Statistical analysis. SPSS 19.0 (IBM Corp.) was used to analyze the data. Differences between multiple groups were analyzed using one-way ANOVA followed by Tukey's honestly significant difference post hoc test. Data are expressed as mean ± SEM. An independent samples t-test was used to assess differences between two groups; χ^2 test was conducted to determine differences in proportion. Progression-free survival was determined using the Kaplan-Meier method and survival differences were analyzed using log-rank test. Cox proportional hazards model was used for multivariate analysis of prognostic factors. Two-sided P<0.05 was considered to indicate a statistically significant difference. All assays were performed at least three times.

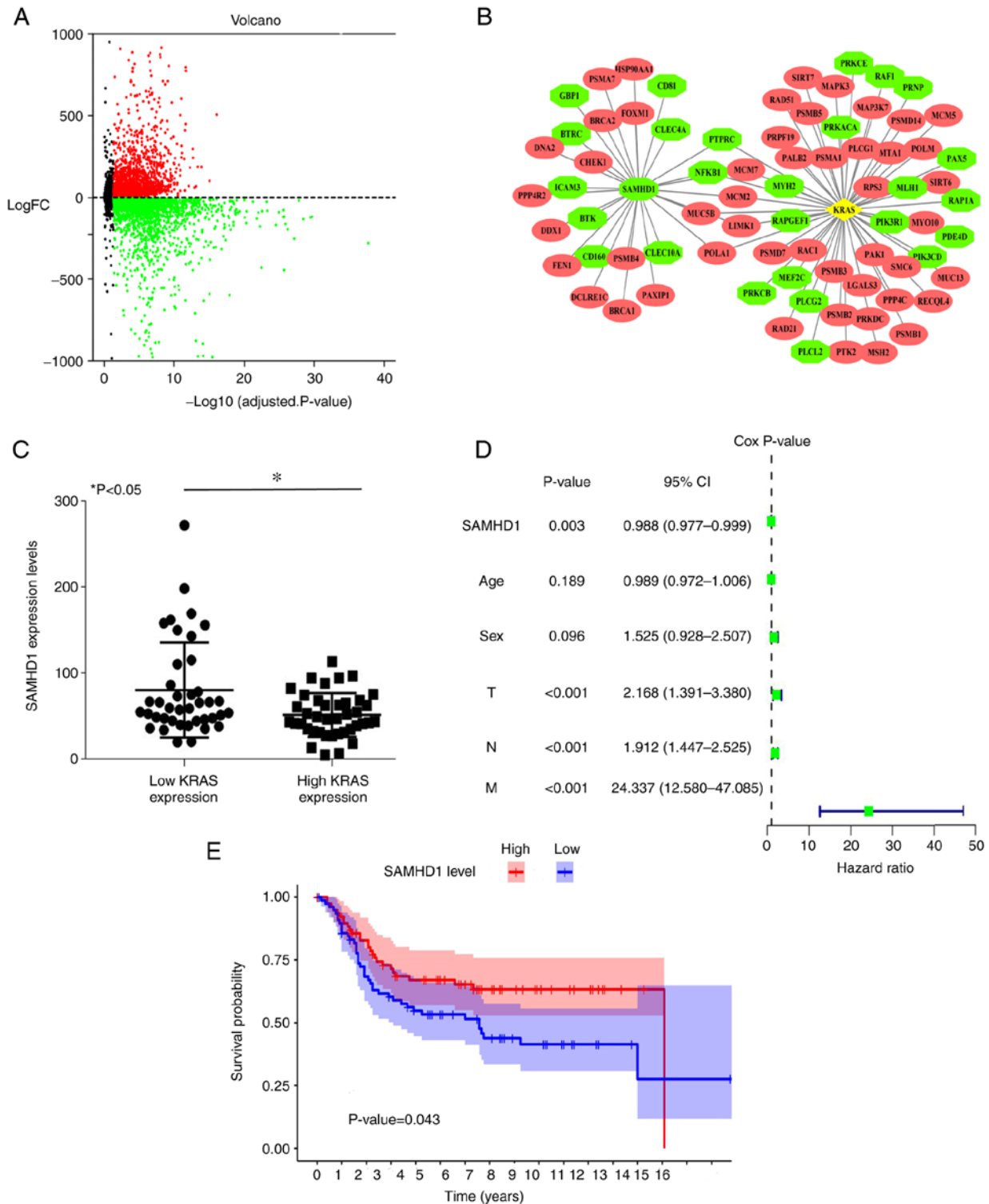


Figure 1. Identification of DEGs in colon cancer. (A) DEGs were identified using microarray volcano plots. (B) Using a protein-protein interaction network, SAMHD1 was found to be associated with KRAS. (C) SAMHD1 expression was negatively associated with KRAS ($P=0.0035$). (D) Cox proportional hazards model was used to analyze the effect of univariate data (SAMHD1 expression, age, sex and TNM) on mortality of patients with colon cancer. (E) Survival analysis showed that the survival time of patients with low SAMHD1 was significantly shorter compared with that of patients with high SAMHD1 expression. DEGs, differentially expressed genes; FC, fold change; KRAS, Kirsten rat sarcoma virus; SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1.

Results

Identification of DEGs in colon cancer. GSE41258 genetic expression matrix and corresponding annotated files were acquired from GEO database. The dataset, which included

187 patients with colon cancer and 45 adjacent normal tissue, was analyzed using the GPL596 Affymetrix Human Genome U133 Plus 2.0 Array. A total of 6,905 DEGs were identified from the GSE41258 dataset using the R package. DEGs were identified using microarray volcano plots (Fig. 1A). STRING

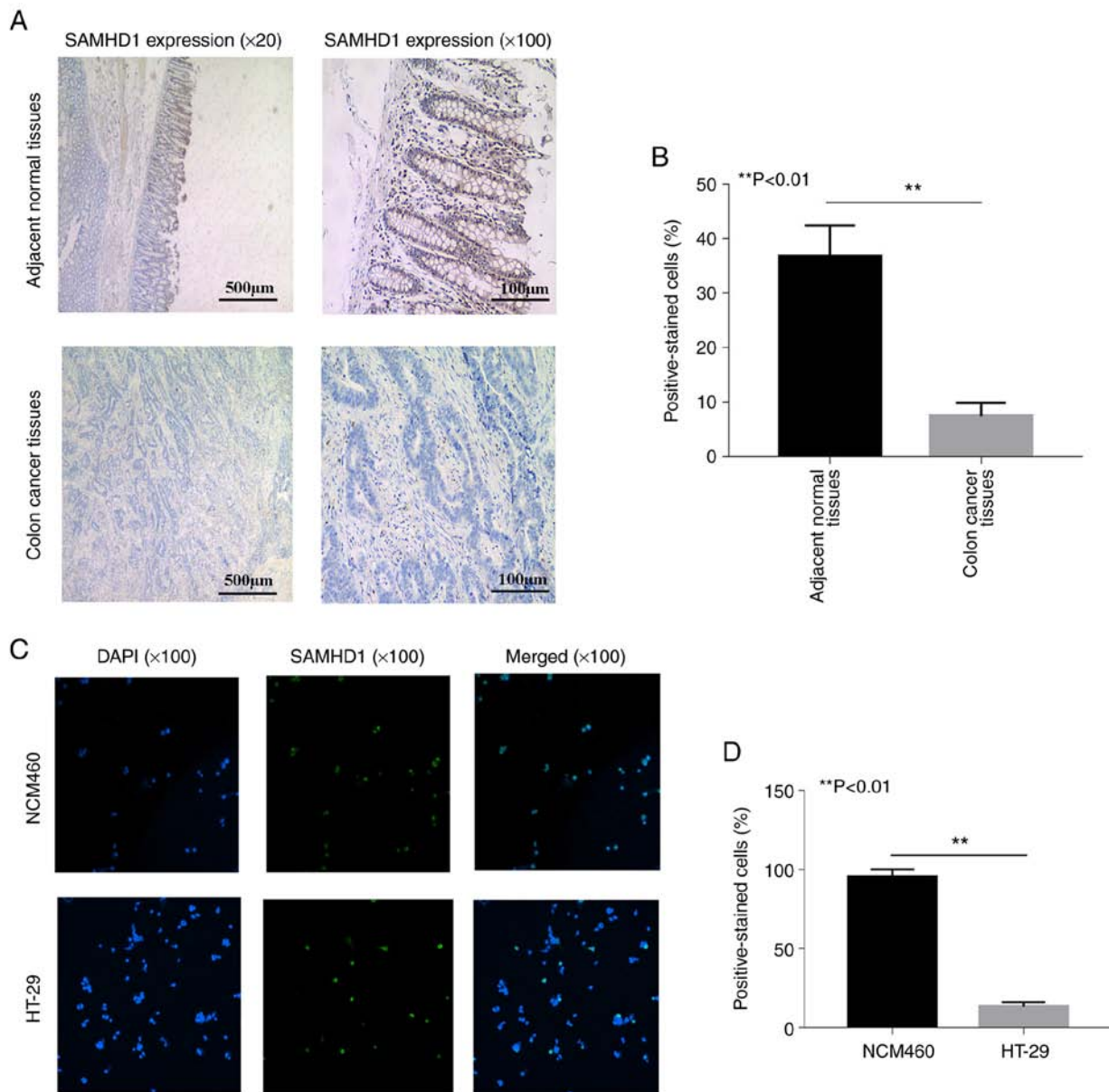


Figure 2. Low expression of SAMHD1 in colon cancer. (A) Representative IHC staining images and (B) quantitative analysis of colon cancer tissue revealed lower expression of SAMHD1 in colon cancer tissues compared with adjacent normal tissues. (C) Representative immunofluorescence staining images and (D) quantitative analysis revealed significantly lower SAMHD1 expression in HT-29 colorectal cancer cells compared with expression in NCM460 normal colon epithelial cells, which was consistent with IHC. **P<0.01. IHC, immunohistochemistry; SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1.

database and Cytoscape were used to construct and visualize a PPI network of DEGs. The location of DEG was visualized by PPI analysis. The KRAS mutation is common in colon cancer (occurring in 30-50% of cases) and has been recognized as a key molecular marker for predicting response to anti-epidermal growth factor receptor monoclonal antibodies, cetuximab and panitumumab in metastatic colon cancer (33). Using the PPI network, SAMHD1 was found to be associated with KRAS and the expression of SAMHD1 is upregulated in colon cancer. The red is up and green is downregulation. (Fig. 1B). A total of 187 patients with colon cancer from the GEO database were divided into high and low KRAS expression groups. SAMHD1 expression was negatively associated with KRAS (Fig. 1C). Cox proportional hazards model was used to analyze the effect of multivariate data (SAMHD1 expression, age, sex and TNM) on

patient mortality. Cox proportional hazards regression analysis and survival analysis found that low SAMHD1 expression was a factor that led to patient mortality (Fig. 1D). The survival time of patients with low SAMHD1 expression was significantly shorter compared with that of patients with high SAMHD1 expression (Fig. 1E). According to bioinformatics, low expression of SAMHD1 was associated with poor prognosis.

Low expression of SAMHD1 in colon cancer. IHC analysis of colon cancer tissue revealed lower expression of SAMHD1 compared with expression in adjacent normal tissue (Fig. 2A and B). The nucleus of healthy tissue exhibited diffuse brown staining, indicating the presence of SAMHD1. However, low SAMHD1 expression was detected in patients with colon cancer. To verify these results, expression levels of

Table I. SAMHD1 expression in colon cancer.

Clinicopathological characteristic	SAMHD1 negative (n=148)	SAMHD1 positive (n=36)	χ^2	P-value
Sex				
Male	76	22	0.353	0.1930
Female	72	14		
Age, years				
≤ 65	76	20	0.205	0.6510
> 65	72	16		
T				
1+2	37	21	14.905	<0.0001
3+4	111	15		
N				
0	38	13	7.677	0.0220
1	44	16		
2	66	7		
M				
0	59	21	4.019	0.0450
1	89	15		

SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1.

SAMHD1 were measured in HT-29 colorectal cancer cells. IF revealed lower SAMHD1 expression in HT-29 compared with NCM460 healthy colonic epithelial cells (Fig. 2C and D), which was consistent with IHC results.

To determine the association between SAMHD1 expression and clinical features, patients were divided into two groups: Positive ($\geq 5\%$) and negative ($< 5\%$) SAMHD1 expression (Table I). No significant differences in age and sex were observed between the two groups. There was a significant association between SAMHD1 expression and N, T and M stage. These data indicated that SAMHD1 may be a biological marker for diagnosis and prognosis of patients with colon cancer.

D137N mutation of SAMHD1 inhibits apoptosis and promotes proliferation of HT-29 (Cas9-SAMHD1). Post-translational modification and associated sites of SAMHD1 are presented in Table II. WB showed SAMHD1 expression was significantly lower in the HT-29 (Cas9-SAMHD1) group compared with HT-29 (Fig. 3A and B), which indicated the HT-29 (Cas9-SAMHD1) cell model was successfully constructed. Then, three classical mutation sites of SAMHD1 were selected: Q548A, D137N and D311A (11,12). Q548A is RNase deletion, D137N is dNTPase deletion, D311A is RNase deletion and dNTPase deletion (11,12). WB analysis showed that there was no significant difference in Caspase3, Cleaved-caspase3, Bax and Bcl-2 expression levels between the Q548A and WT groups (Fig. 3C and D). Compared with the WT group, expression levels of Caspase3, Cleaved-caspase3 and Bax significantly decreased, whereas Bcl-2 expression significantly increased in the D137N and D311A groups, which indicated that D137N and D311A groups significantly inhibited the expression of apoptosis-related

proteins. CCK-8 results showed that the difference in proliferation between the Q548A and WT groups was not statistically significant (Fig. 3E). Compared with WT group, proliferation increased in the D137N and D311A groups.

5-FU treatment promotes apoptosis and inhibits proliferation of HT-29 (Cas9-SAMHD1). WB analysis showed that in the WT and D137N groups, Caspase3, Cleaved-caspase3 and Bax expression significantly increased and Bcl-2 significantly decreased over time following 20 $\mu\text{g/ml}$ 5-FU treatment (Fig. 4A and B), potentially promoting cell apoptosis. However, compared with the WT group, Caspase3, Cleaved-caspase3 and Bax expression levels were significantly lower and the Bcl-2 level was significantly higher in the D137N group, suggesting a potentially inhibiting effect on the expression of apoptosis-related proteins. CCK-8 results showed that, following 20 $\mu\text{g/ml}$ 5-FU treatment, proliferation in the WT and D137N groups was significantly decreased over time (Fig. 4C). However, compared with the WT group, viability in the D137N group was significantly higher at each time point.

Discussion

The human SAMHD1 gene was first cloned from human dendritic cell cDNA in 2000 (34). Its protein expression is induced by IFN- γ (34,35). In addition to being associated with occurrence and development of lung and colon cancer, low-level exogenous SAMHD1 expression leads to a notable decrease in development, proliferation and colony formation of HUT78 human T lymphocyte leukemia cells by promoting apoptosis, and SAMHD1 may be a therapeutic target against cancer involving T lymphocytes (20,23,36). The number of

Table II. Modifications in SAMHD1 in point mutation.

Mutation	Flanking sequence	Modification	Records, n	
			LTP	HTP
S6	MQRADsEQPSkRP	Phosphorylation	0	4
K11	ADsEQPSkRPRCDDs	Ubiquitylation	0	4
S18	kRPRCDDsPRtPsNt	Phosphorylation	3	62
T21	RCDDsPRtPsNtPsA	Phosphorylation	4	106
S23	DDsPRtPsNtPsAEA	Phosphorylation	0	15
N24	DsPRtPsntPsAEAD	Phosphorylation	1	18
T25	sPRtPsNtPsAEADW	Phosphorylation	3	45
S27	RtPsNtPsAEADWsP	Phosphorylation	0	8
E29	PsNtPsAeADWsPGL	Phosphorylation	0	10
S33	PsAEADWsPGLLELHP	Phosphorylation	4	50
V63	RGGFEePvLLkNIRE	Ubiquitylation	0	1
K66	FEePvLLkNIRENEI	Ubiquitylation	0	10
S93	FENLGVsSsLGERkkL	Phosphorylation	0	1
K98	VsSsLGERkkLLsyIQ	Ubiquitylation	0	1
K99	SsLGERkkLLsyIQR	Ubiquitylation	0	3
S102	GERkkLLsyIQRLVQ	Phosphorylation	0	2
Y103	ERkkLLsyIQRLVQI	Phosphorylation	0	1
T138	LLVRIIDtPQFQRLR	Phosphorylation	0	1
Y146	PQFQRLRyIkQLGGG	Phosphorylation	0	1
K148	FQRLRyIkQLGGGYy	Ubiquitylation	0	1
Y155	kQLGGGYyVFPGASH	Phosphorylation	0	1
E255	NGIKPVMeQYGLIPE	Acetylation	0	1
K269	EEDICFIkEQIVGPL	Ubiquitylation	0	1
S278	QIVGPLEsPVEDSLW	Phosphorylation	1	24
E281	GPLEsPVeDSLWPYk	Ubiquitylation	0	1
S283	LEsPVEDsLWPYkGR	Phosphorylation	0	1
K288	EDSLWPYkGRPENkS	Ubiquitylation	0	1
K294	YkGRPENkSFLYEIV	Acetylation	0	1
K294	YkGRPENkSFLYEIV	Ubiquitylation	0	1
K304	LYEIVSNkRNGIDVD	Ubiquitylation	0	5
K312	RNGIDVDkWDyFARD	Ubiquitylation	0	3
Y315	IDVDkWDyFARDCHH	Phosphorylation	0	3
K332	IQNNFDYkRFIKFAR	Sumoylation	0	2
E346	RVCEVDNeLRICARD	Ubiquitylation	0	1
Y360	DKEVGNLyDMFHTRN	Phosphorylation	0	1
T384	KVGNIIIDtMItdAFL	Phosphorylation	0	2
T387	NIIDtMItdAFLKAD	Phosphorylation	0	2
K405	EITGAGGkKYRISTA	Phosphorylation	0	1
Y419	AIDDMEAyTKLTDNI	Phosphorylation	0	1
Y432	NIFLEILySTDPKLL	Phosphorylation	0	1
K446	KDAREILkQIEYRNL	Ubiquitylation	0	9
K455	IEYRNLfkYVGETQP	Ubiquitylation	0	4
K467	TQPTGQIkIkREDYE	Ubiquitylation	0	1
K469	PTGQIkIkREDYESL	Sumoylation	0	3
K478	EDYESLPkEVAAsAKP	Ubiquitylation	0	1
S482	SLPkEVAAsAKPkVLL	Phosphorylation	0	1
K484	PkEVAAsAkPkVLLDV	Ubiquitylation	0	1
K486	EVAAsAKPkVLLDVKL	Phosphorylation	0	2
Y507	VDVINMDyGMQEKNP	Phosphorylation	0	1
S519	KNPIDHVsfYCKTAP	Phosphorylation	0	1

Table II. Continued.

Mutation	Flanking sequence	Modification	Records, n	
			LTP	HTP
K534	NRAIRITkNQVSQLL	Ubiquitylation	0	1
K544	VSQLLPEkFAEQLIR	Ubiquitylation	0	4
Y553	AEQLIRVyCKKVDRk	Phosphorylation	0	1
K560	yCKKVDRkSLYAARQ	Phosphorylation	0	1
T579	WCADRNFtKPQDGDV	Phosphorylation	0	1
T592	DVIAPLIkPQkkEWN	Phosphorylation	15	64
K595	APLIkPQkkEWNDsT	Sumoylation	1	2
K596	PLIkPQkkEWNDsTS	Ubiquitylation	0	1
S601	QkkEWNDsTSVQNpT	Phosphorylation	0	1
T608	sTSVQNpTRLREASK	Phosphorylation	0	1
S616	RLREASKsRVQLFkD	Phosphorylation	0	2
K622	KsRVQLFkDDPM	Sumoylation	0	2

HTP, high throughput paper (records in which this modification site was assigned using only proteomic discovery mass spectrometry); LTP, low throughput paper (records in which modification site was determined using methods other than discovery mass spectrometry); SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1.

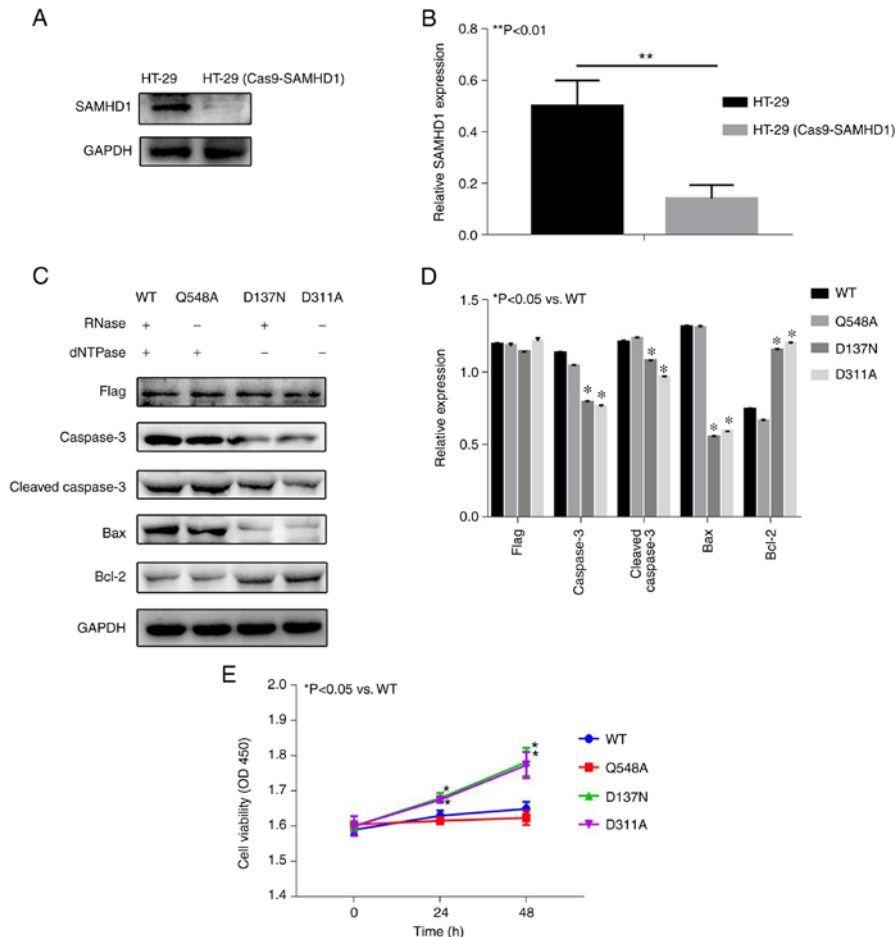


Figure 3. D137N mutation of SAMHD1 inhibits apoptosis and promotes proliferation of HT-29 (Cas9-SAMHD1). (A) Successful construction of HT-29 (Cas9-SAMHD1) model. (B) Semi-quantitative analysis of WB images from (A); GAPDH was used as a positive control. $^{**}P < 0.01$. (C) Representative WB images and (D) semi-quantitative analysis of apoptosis-related protein expression in WT, Q548A, D137N and D311A groups. One-way ANOVA followed by Tukey's honestly significant difference post hoc test was used to evaluate the difference between groups. $^{*}P < 0.05$ vs. WT. (E) Cell Counting Kit-8 assay was used to detect cell viability in the WT, Q548A, D137N and D311A groups at 0, 24 and 48 h. $^{*}P < 0.05$ vs. WT. OD, optical density; SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1; WB, western blotting; WT, wild-type.

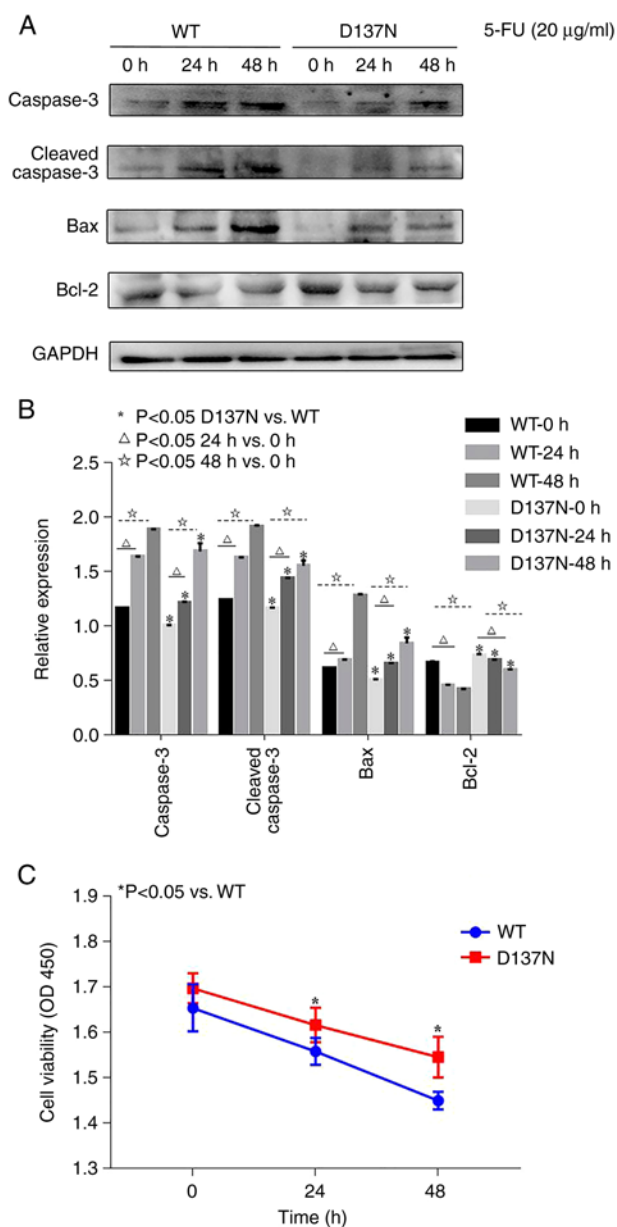


Figure 4. 5-FU treatment promotes apoptosis and inhibits proliferation of HT-29 (Cas9-SAMHD1). (A) Apoptosis-related protein expression was detected by WB analysis following treatment with 20 $\mu\text{g/ml}$ 5-FU for 48 h. (B) Semi-quantitative analysis of the WB images in (A); GAPDH was used as a positive control. One-way ANOVA followed by Tukey's honestly significant difference post hoc test was used to evaluate the difference between groups. * $P < 0.05$ D137N vs. WT; $\Delta P < 0.05$ 24 h vs. 0 h; $\star P < 0.05$ 48 h vs. 0 h. (C) Cell Counting Kit-8 assay was used to determine cell viability following 20 $\mu\text{g/ml}$ 5-FU treatment for 0, 24 and 48 h. * $P < 0.05$ vs. WT. 5-FU, fluorouracil; OD, optical density; SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1; WB, western blotting; WT, wild-type.

SAMHD1 mutations and expression levels vary between types of solid cancer, such as lung adenocarcinoma and colon cancer, and affect the efficiency of clinical first-line chemotherapy drug ara-C in leukemia (20,23,37). Multipoint gene mutations of SAMHD1 were detected in leukemia cells, resulting in decreased mRNA and protein expression levels of SAMHD1 (38,39). The sensitivity of THP-1 human myeloid leukemia monocytes, which do not exhibit dNTPase activity of SAMHD1, to anti-metabolites, such as fludarabine, decitabine, cytarabine and clofarabine, has improved (40). SAMHD1

protects cancer cells from anti-nucleoside metabolites, such as ara-C (37,40-42). Certain SAMHD1 mutation results in loss of dNTPase activity, leading to abnormal dNTP accumulation, which causes rapid cancer cell proliferation (43-46) and immune system dysfunction (44). In addition, SAMHD1 may protect cancer cells from DNA replication inhibitors, such as antimetabolite antineoplastic agents (43,47). As a result, SAMHD1 is considered a potential biomarker for the stratification of patients with AML based on response to ara-C and a potential therapeutic agent against ara-C-refractory AML (37). It is hypothesized that a similar phenomenon may occur in colon cancer. Previous studies have found that SAMHD1 mutations increase whole mutation rates in colon cancer cells, and SAMHD1 may be a prognostic marker of colorectal cancer (23,48). D137N (dNTPase deletion) and D311A mutation (RNase and dNTPase deletion) prevent the antiviral activity of SAMHD1, whereas the Q548A mutation (RNase deletion) does not significantly inhibit its antiviral activity (11). The present study analyzed the association between SAMHD1 and KRAS and found that SAMHD1 was expressed at low levels in colon cancer. The effects of three classic SAMHD1 mutations (Q548A, D137N and D311A) on apoptosis and proliferation of colon cancer cells were subsequently assessed. The effect treatment with first-line drug 5-FU against colon cancer cells with D137N mutation is time-dependent.

The incidence and mortality rate of colon cancer in 2018 ranked fourth (3) in all types of cancer worldwide, which showed that the present research has high clinical value. In addition, a review of The Cancer Genome Atlas found that, among 15 types of cancer with frequent SAMHD1 mutations, mutation rate of SAMHD1 in colon cancer ranked third and frequent mutations in SAMHD1 gene that result in its down-regulation have also been found in colon cancer (23). Here, SAMHD1 was differentially expressed between the adjacent normal tissue group and the colorectal cancer group. Therefore, the present study investigated expression of SAMHD1 in colon cancer. In the present study, 6,905 DEGs were identified using R software firstly. The classical colorectal cancer gene KRAS was selected and found different SAMHD1 expression levels in KRAS-L and KRAS-H in colorectal cancer. SAMHD1 and KRAS were found to be negatively associated. Cox proportional hazards regression and survival analyses demonstrated that low expression of SAMHD1 was associated with increased patient mortality. This is consistent with the conclusions of previous studies in AML (49). Therefore, it was hypothesized that SAMHD1 may serve a key role in colorectal cancer. The expression of SAMHD1 in colorectal cancer was assessed in tissue and cells; its mRNA expression was lower in tumor compared with that in adjacent normal tissue. Similarly, compared with NCM460 normal colon epithelial cells, SAMHD1 expression in HT-29 colorectal cancer cells was decreased, which was consistent with the results of bioinformatic analysis. The effect of mutations in the dNTPase functional site of SAMHD1 on proliferation and apoptosis of colon cancer was investigated. Therefore, three previously reported mutation sites of SAMHD1 were selected: Q548A, D137N and D311A (11,12). The Q548A mutation had no significant effect on proliferation and apoptosis of HT-29 cells compared with WT group, whereas D137N and D311A notably promoted proliferation and inhibited apoptosis of

HT-29 cells. WT and D137N groups were treated with 20 $\mu\text{g/ml}$ 5-FU for 24 and 48 h. The results showed cell proliferation was inhibited and apoptosis-related protein expressions were promoted in WT and D137N groups over time following 20 $\mu\text{g/ml}$ 5-FU treatment. However, compared with the WT group, cell proliferation in the D137N group was higher and apoptosis-associated protein expression levels were lower following 20 $\mu\text{g/ml}$ 5-FU treatment.

In conclusion, SAMHD1 was expressed at low levels and was associated with prognosis in colon cancer. The dNTPase function of SAMHD1 may inhibit proliferation and promote apoptosis in colon cancer cells. In addition, first-line chemotherapy with 5-FU had a time-dependent effect on colon cancer cells, which may provide novel options for clinical treatment of colon cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PS conceived and designed the experiments. ZZ and PL performed the experiments, analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript. ZZ and PS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the ethics committee of Wuxi No. 2. People's Hospital (Wuxi, China; approval no. 20180706). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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