A novel lncRNA 495810 promotes the aerobic glycolysis in colorectal cancer by stabilizing pyruvate kinase isozyme M2

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Abstract. Aberrant expression of long non-coding RNA (lncRNA) plays an important role in malignant progression of colon cancer and has become a new therapeutic target. In the present study, it was found that the expression of a novel lncRNA 495810 was significantly upregulated in colon cancer and correlated with poor prognosis in patients with colorectal cancer. The highly expressed lncRNA 495810 promoted the proliferation and inhibited apoptosis of CRC cells. Furthermore, the results of gene enrichment analysis indicated that 495810-targeted genes were enriched in the glycolysis pathway and overexpression of 495810 enhanced aerobic glycolysis in colon cancer cells. More importantly, the expression of lncRNA 495810 was positively correlated with the glycolytic rate-limiting enzyme pyruvate kinase isozyme M2 (PKM2). Notably, the data suggested that lncRNA 495810 physically interacted with PKM2 protein and enhanced PKM2 protein stability via the ubiquitin-proteasome pathway. The present findings suggested that lncRNA 495810, a glycolysis-related oncogenic lncRNA, is a potential biomarker for predicting prognosis and a therapeutic target for colon cancer.

Introduction

According to the 2021 global cancer statistics, colorectal cancer (CRC) is a common gastrointestinal malignancy with the third highest incidence and the second highest mortality (1,2). The failure of early diagnosis and the lack of effective therapeutic targets contribute to the poor prognosis of CRC (3,4). Therefore, finding new targets for the diagnosis and therapy of CRC is necessary and urgent.

The long non-coding RNA (lncRNA), a group of novel regulatory RNA, is a non-coding transcript >200 nucleotides in length (5). It has been shown that lncRNA is aberrantly expressed in numerous tumors and plays critical roles in various tumor processes (6-8), including tumor cell death (9), metabolic remodeling (10), migration and infiltration (11), reflecting their important roles in carcinogenesis. For instance, lncRNA GAS5 is downregulated in colon cancer and restrains the proliferation of colon cancer by inhibiting the activation of the Wnt/β-catenin signaling pathway (12); lncRNA NALT1 is upregulated and induces invasion and metastasis of gastric cancer through the NOTCH signaling pathway (10); lncRNA MALAT1 regulates proliferation of SKOV3 cells and is overexpressed in ovarian cancer tissues (9). Accordingly, lncRNAs are considered novel diagnostic and therapeutic targets for anticancer research.

Metabolic reprogramming, particularly aerobic glycolysis, is one of the malignant features of tumors (13). Cancer cells prefer to produce energy by glycolysis rather than via oxidative phosphorylation, even in the presence of oxygen. This phenomenon is defined as the 'Warburg effect', also known as aerobic glycolysis (14,15). Recently, an increasing number of studies suggested that lncRNAs may play a role in tumorigenesis by reprogramming glucose metabolism (16-18). Upregulation of lncRNA PVT1 expression in tumors has been reported to promote aerobic glycolysis in gallbladder cancer cells by competitively binding to endogenous miR-143 to regulate HK2 expression (19). LncRNA MACC1-AS1 promotes the stabilization of MACC1 mRNA, which in turn activates the expression of its downstream target genes Glut1, HK2 and LDHA, promotes aerobic glycolysis in tumor cells and leads to malignant growth of gastric cancer cells (20).

In a previous study by the authors, a novel lncRNA 495810 (named according to its ENST ID: ENST00000495810) expressed in CRC cells was discovered (21). However, its clinical significance and molecular mechanism in CRC have not been reported. In the current study, data demonstrated that lncRNA 495810 is significantly upregulated in colon cancer and is associated with poor prognosis in patients with CRC. LncRNA 495810 functions as an oncogene and promotes aerobic glycolysis in colon cancer. Mechanistically, lncRNA 495810 positively correlates with pyruvate kinase isozyme M2

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(PKM2) and regulates its proteasomal degradation to promote the level of aerobic glycolysis in CRC cells.

Materials and methods

Chemicals. DMEM/F12 (HAM) 1:1 medium and fetal bovine serum (FBS) were from Biological Industries; 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and cycloheximide (CHX) were obtained from Beijing Solarbio Science & Technology Co., Ltd. The RNAiso Plus was purchased from Takara Bio, Inc. All-in-one First Strand cDNA Synthesis Kit II and 2X SYBR Green qPCR Master Mix were from Sevenbio (http://www.7bio.com.cn/index.php). Turbofect was obtained from Thermo Fisher Scientific, Inc. Annexin V-FITC apoptosis detection kit was purchased from Nanjing KeyGen Biotech Co., Ltd. Antibodies for PKM2 (cat. no. 15822-1-AP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; cat. no. 10494-1-AP) were obtained from Proteintech Group, Inc. ATP assay kit (cat. no. S0026B) was obtained from Beyotime Institute of Biotechnology. Glucose assay kit (cat. no. F006-1-1) and pyruvate acid test kit (cat. no. A081-1-1) were purchased from Nanjing Jiancheng Bioenigeering Institute. HiPerFect Transfection Reagent were purchased from Qiagen GmbH. MG132 was obtained from MedChemExpress.

Cell lines and cell culture. The CRC cell lines HT-29, DLD1, HCT116, SW620, SW480 and human colon epithelial cell line (FHC) were preserved in the authors' laboratory.. The cells were cultured in DMEM/F12 medium containing 10% FBS and placed in an incubator at 37˚C with 5% CO_2.

Reverse transcription-quantitative PCR (RT-qPCR) assay. Total RNA was extracted from cells with TRIzol® reagent (Takara Bio, Inc.) and 500 ng of RNA was reverse-transcribed. The reverse transcription was performed according to the manufacturer's instructions. LncRNA and mRNA levels were quantified using RT-qPCR. The PCR reaction in the following conditions: one cycle at 94˚C for 30 sec, followed by 40 cycles at 94˚C for 5 sec, 60˚C for 15 sec and 72˚C for 10 sec. The quantitative analysis was performed using the $2^{-\Delta\Delta Cq}$ method (22). GAPDH was used as a reference gene. All primers used in the experiment are shown in Table SI.

Bioinformatics. Kaplan-Meier survival analysis for CRC patients with high or low gene expression level was performed through Gene Expression Profiling Interactive Analysis 2 (GEPIA 2; http://gepia2.cancer-pku.cn/#index). The coding potential of lncRNA was estimated by the coding-potential assessment tool (CPAT; http://lilab.research.bcm.edu/). LncRNA PhyloCSF value analysis was performed by the UCSC genome browser (http://genome.ucsc.edu/) with the PhyloCSF data hub. The metascape database (www.metascape.org) was used for gene enrichment analysis and the protein-protein interaction (PPI) network. The expression data of PKM2 and the correlation analysis of two genes in CRC tissues were performed through the GEPIA 2 (http://gepia2.cancer-pku.cn/#correlation). The interaction between lncRNA 495810 and PKM2 was predicted via RNA-Protein Interaction Prediction (RPISeq) (http://pridb.gdcb.iastate.edu/RPISeq/index.html). A potential PKM2-binding region of lncRNA 495810 (401-452) was predicted using catRAPI omics (http://s.tartaglialab.com/page/catrapid_omics_group).

Nuclear and cytoplasmic RNA fractionation analysis. The collected cells were lysed with hypotonic buffer (pH 7.4 25 mM Tris-HCl, 1 mM $MgCl_2$, 5 mM KCl and 1% NP-40), centrifuged (4˚C, 5,000 g, 5 min), and the supernatant was received to obtain cytosolic fractions. Then, the aforementioned pellet was lysed with nuclear resuspension buffer (pH 7.9 20 mM HEPES, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and 1 mM PMSF) and centrifuged (4˚C, 13,000 g, 10 min) to obtain nuclear fractions. Nuclear and cytoplasmic RNA were extracted with TRIzol reagent. Finally, the expression levels of GAPDH, U6, and lncRNA 495810 in the nuclei and cytoplasm of CRC cells were detected using RT-qPCR assays.

siRNA and plasmid transfection. To assay siRNA transfection, the cells were inoculated into a six-well plate, and 10 nM siRNA was transfected with HiPerFect Transfection Reagent for 48 h according to the manufacturer's protocol. For the overexpression assay, lncRNA 495810 overexpression plasmid and the control plasmid (pCDH; both from Miiaoling Biotech Science) were transfected into colon cancer cells using Turbofect reagent according to the manufacturer's instructions. After transfection for 48 h, the cells were collected for subsequent analysis or assay. The siRNA sequences (Shanghai GenePharma Co., Ltd) were as follows: lncRNA 495810-homo-112 (sense, 5'-CCA UACCAUCAAUGGUCAUTT-3' and antisense, 5'-AUGACC AUUGAUGGUAUGGTT-3').

Cell viability assay. The HT-29, SW620 and HCT116 cells (~$5x10^3$) were seeded into 96-well plates and were incubated overnight to adhere to the wall. Cells were transfected with si-495810 or 495810 for 48 h. Then 10 μl of MTT (0.5 mg/ml) was added to each well for 4 h. After the formazan crystals formed, they were dissolved in DMSO and the OD value at 570 nm was evaluated. The cell survival rate was calculated with the following formula: Cell survival rate (%)=(OD570 treated/OD570 control) x100.

Cell apoptosis analysis. Cells were resuspended with 500 μl binding buffer at the density of $1x10^5$ cells/ml, and then 5 μl connexin V-FITC and 5 μl propidium iodide were added. The cells were incubated at room temperature in the dark for 15 min. After that, the samples were detected and analyzed by flow cytometry (CytoFLEX; Beckman Coulter, Inc.).

Transwell assays. The SW620 and HCT116 cells (~$2x10^4$) were seeded into 24-well Transwell plates (cat. no. 3422; Corning, Inc.) and 200 μl serum-free DMEM medium (Gibco; Thermo Fisher Scientific, Inc.) in the upper compartment and 660 μl DMEM medium supplemented with 15% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to the lower compartment. The plate was incubated at 37˚C for 48 h. The migrated cells on the lower surface were detected.

Metabolic assays. Cells were seeded into the six-well plate, and medium and cells were collected after transfection with

si-495810 or 495810 for 48 h. The medium was used to measure the contents of glucose and pyruvate acid, and the production of ATP was determined after cell lysis. The methods of measuring glucose, ATP and pyruvate acid were according to the manufacturer's instructions. Briefly, the medium was added to the reaction solution in the glucose and pyruvate acid kit, respectively, and its OD was measured at 505 nm to calculate its content. The cell lysate was added to the ATP assay solution, its fluorescence intensity was measured, and its content was calculated.

In vitro transcription and RNA pull-down. The templates for *in vitro* transcription were obtained from PCR. The primers containing the T7 promoter of 495810 are listed in Table SI. *In vitro* transcription was conducted using a TranscriptAid T7 High Yield Transcription kit (Thermo Fisher Scientific, Inc.) following the manufacturer's protocol and the target RNA was labeled with biotin using Biotin RNA Labeling Mix 10X.

Cell lysates were prepared using IP lysis buffer. Next, the labeled RNA was incubated with streptavidin magnetic beads for 1 h at room temperature with agitation. Then, the bead-RNA complex and RNA-protein binding reaction master were incubated for 2 h at 4˚C with rotation. Finally, the eluted interacting proteins were obtained.

Mass spectrometry. Mass spectrometry was performed at Bioprofile. Mass spectrometry experiments were performed on a Q Exactive HF-X mass spectrometer that was coupled to Easy nLC (Thermo Fisher Scientific, Inc.). The full MS scans were acquired at a resolution of 60,000 at m/z 200, and 15,000 at m/z 200 for MS/MS scan. The maximum injection time was set to for 50 ms for MS and 50 ms for MS/MS. Normalized collision energy was 28 and the isolation window was set to 1.6 Th. Dynamic exclusion duration was 60 sec.

Western blot assay. Western blotting was performed as previously described (21). Total protein was extracted by WBIP (Solarbio Science & Technology Co., Ltd.) and its concentration was determined by BCA assay. The same amount (60 μg) of protein was separated by 10% SDS-PAGE and transferred to the PVDF membrane. The membrane was blocked for 40 min at room temperature with 5% skim milk and incubated with primary antibodies against anti-PKM2 or GAPDH overnight at 4˚C. The corresponding secondary antibodies (anti-Rabbit, ZB-2306/anti-Mouse, ZB-2305) were incubated for 2 h at room temperature the following day. The expression changes of target proteins were observed. Relative protein levels were analyzed using ImageJ software (National Institutes of Health). Expression of GAPDH was used as the internal control.

CHX chase assay. To observe the protein degradation process, HT-29, HCT116 and SW620 cells (~1x10^5) were treated with CHX (20 μg/ml) for 0, 2, 4, 6 and 8 h, respectively. Total protein was extracted at indicated time points and analyzed by western blotting.

Statistical analysis. The data are presented as the mean ± SEM, data error bar indicating standard deviation. Single-variable comparisons were performed by independent samples t-test. Continuous variables (N≥3 groups) were analyzed by Single factor analysis of variance (ANOVA), followed by Tukey's post-hoc test. Cox proportional hazards regression models were applied to determine the independent factors that influence survival. Experimental data were executed using GraphPad Prism 9 software (GraphPad Software, Inc.). The data of TCGA was performed in the GEPIA 2. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LncRNA 495810 is upregulated in CRC. To examine the clinical significance of lncRNA 495810 in colon cancer, the endogenous expression of lncRNA 495810 in normal colonic epithelial FHC and different CRC cell lines was first examined. It was found that lncRNA 495810 was highly expressed in most detected CRC cells compared with FHC (Fig. 1A). Then the expression of lncRNA 495810 was estimated in 68 paired samples of adjacent tissues and CRCs. The results revealed that lncRNA 495810 expression level was significantly increased in colon cancer tissues (Fig. 1B). Subsequently, the pathological and clinical value of lncRNA 495810 were assessed. The results revealed that high expression of lncRNA 495810 was associated with poor survival by survival analysis (Fig. 1C). Through COX regression analysis, it was identified that the expression level of lncRNA 495810 was a prognostic factor for CRC (Tables I and SII). The overall and disease-free survival curve for CRC patients with high or low lncRNA 495810 expression level further proved that high lncRNA 495810 expression levels indicated a poor prognosis in patients (Fig. 1D and E), and it was correlated with advanced clinical grade and stage (Fig. 1F).

To determine whether 495810 is a lncRNA, the characteristics of lncRNA 495810 were detected. The chromosomal coordinate ranges of lncRNA 495810 were chr7:26195712-26201301, and its size was 484 bp in length (Table SIII). Then, the CPAT displayed that 495810 had no coding ability (Fig. S1A). In addition, the PhyloCSF value of 495810, which was calculated to verify the conservation of the sequence, was minus (Fig. S1B). Nuclear and cytoplasmic fractions in CRC cells were also isolated and RT-qPCR was performed. The results demonstrated that 495810 was mainly localized in the nucleus (Fig. 1G). Collectively, the data indicated that 495810 is a lncRNA and exhibits oncogene potential.

High expression of lncRNA 495810 promotes CRC growth. The promotional effect of lncRNA 495810 on colorectal carcinogenesis was then characterized by functional assays. HCT116/SW620 cells with relatively lower or higher lncRNA 495810 expression were selected for lncRNA 495810 overexpression and knockdown. The HT-29 cells with moderate expression were used in both knockdown and overexpression experiments, which were used as parallel experimental groups (Figs. 1A, S1C and D). The results demonstrated that lncRNA 495810 knockdown significantly inhibited cell proliferation in SW620 and HT-29 cells, whereas overexpression of lncRNA 495810 increased CRC cell proliferation of HCT116 and HT-29 cells (Fig. 2A and B). Moreover, lncRNA 495810 knockdown significantly increased, whereas overexpression of lncRNA 495810 inhibited apoptotic levels (Fig. 2C and D).

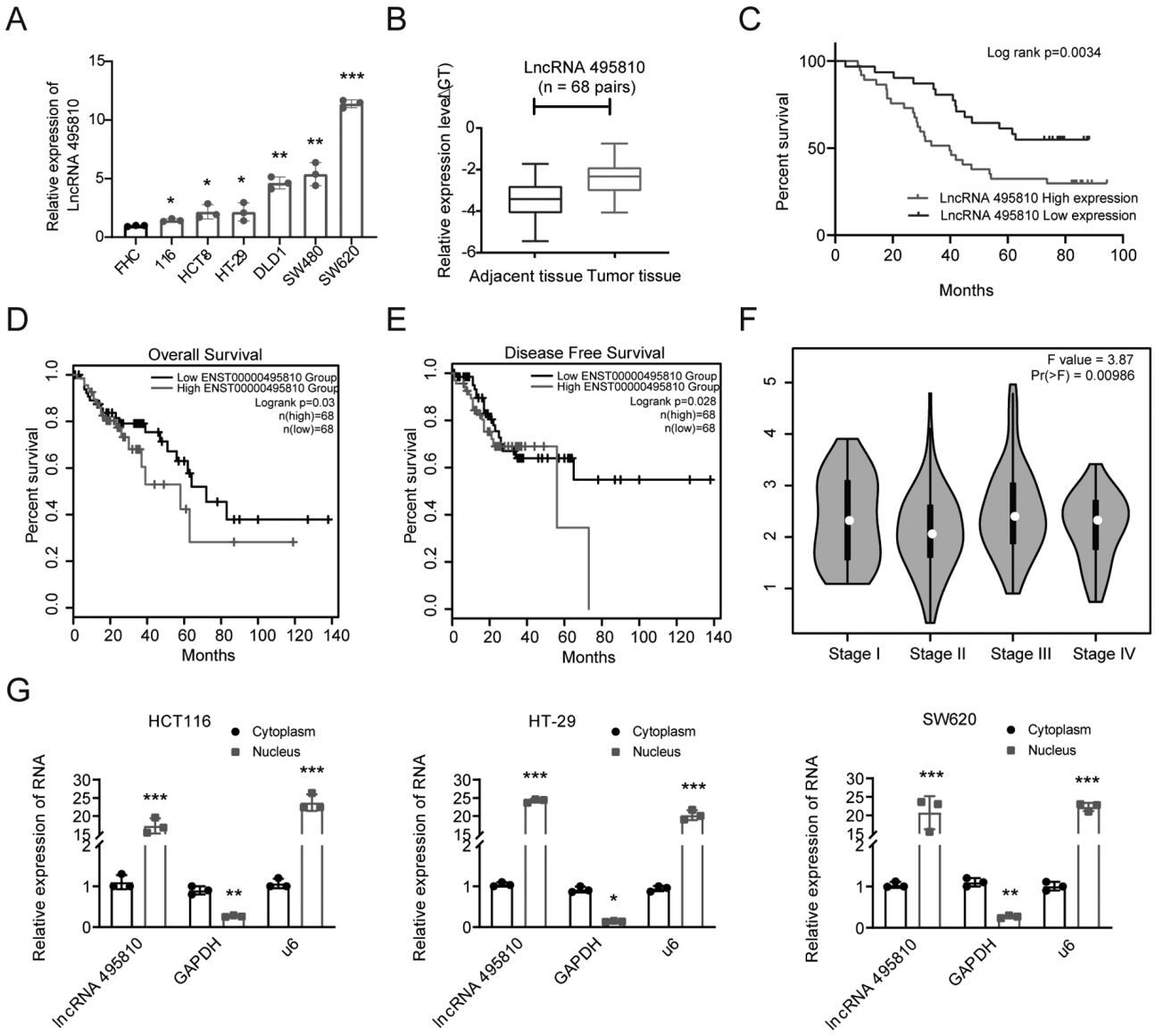

Figure 1. LncRNA 495810 is upregulated in colorectal cancer. (A) The relative expression level of lncRNA 495810 was determined by RT-qPCR in FHC and CRC cell lines. GAPDH was used as internal control. (B) Relative expression levels of lncRNA 495810 in 68 paired tumor tissues and adjacent tissues were detected. (C) Survival analysis according to lncRNA 495810 expression in CRC. Data are presented as the mean ± SEM. (D and E) Kaplan-Meier survival analysis of patient (D) overall survival and (E) disease-free survival according to 495810 levels based on the GEPIA 2 database. (F) The correlation between 495810 expression and pathological stage based on the GEPIA 2 database. (G) RT-qPCR was used to detect the expression level of lncRNA 495810 in the nucleus and cytoplasm. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001. lncRNA, long non-coding RNA; RT-qPCR, reverse transcription-quantitative PCR; CRC, colorectal cancer.

Moreover, the migration ability of the si-495810 group was significantly reduced compared with the NC (non-specific control) group in SW620 cells (Fig. 2E). Whereas in the gain-of-function assays, 495810 overexpression significantly increased cell migration ability of HCT116 cells (Fig. 2F). The aforementioned results collectively indicated that lncRNA 495810 was involved in colorectal tumorigenesis and progression as an oncogene.

LncRNA 495810 promotes aerobic glycolysis in CRC cells. To determine the signaling pathway affected by lncRNA 495810 in promoting CRC, RNA pull-down combined with mass spectrometry (Bioprofile) was used to identify targets directly regulated by lncRNA 495810. The aforementioned target proteins were imported into the Metascape database (www.metascape.org) for gene enrichment analysis. The results demonstrated that the pathways closely related to lncRNA 495810 were glycolysis and gluconeogenesis (Fig. 3A-C). Furthermore, metabolic assays revealed that silencing lncRNA 495810 expression level declined, whereas ectopic lncRNA 495810 expression enhanced glucose consumption, pyruvate acid production and ATP production (Fig. 3D-I). These data indicated that 495810 is a glycolysis-related lncRNA and promotes aerobic glycolysis in CRC.

PKM2 is the direct target of lncRNA 495810. To identify targets that are directly regulated by lncRNA 495810, the proteins bound to the sense strand of lncRNA 495810 were used to construct a PPI network (Fig. 4A). At the same time, the MCODE algorithm built into the Metascape database was

Table I. Association of lncRNA 495810 expression with clinical characters and overall survival in patients with colorectal cancer (n=68 pairs).

Factors	Subset	Hazard ratio (95% confidence interval)	P-value
Sex	Male/Female	0.83 (0.42-1.65)	0.600
Tumor size (cm)	>5/≤5	0.26 (0.09-0.75)	0.013
Differentiation	Poorly/Well, moderately	0.71 (0.35-1.45)	0.350
Tumor stage	(II/III/IV)/I	1.38 (0.84-2.28)	0.209
Expression level of lncRNA 495810	High/Low	2.26 (1.06-4.80)	0.034

lncRNA, long non-coding RNA.

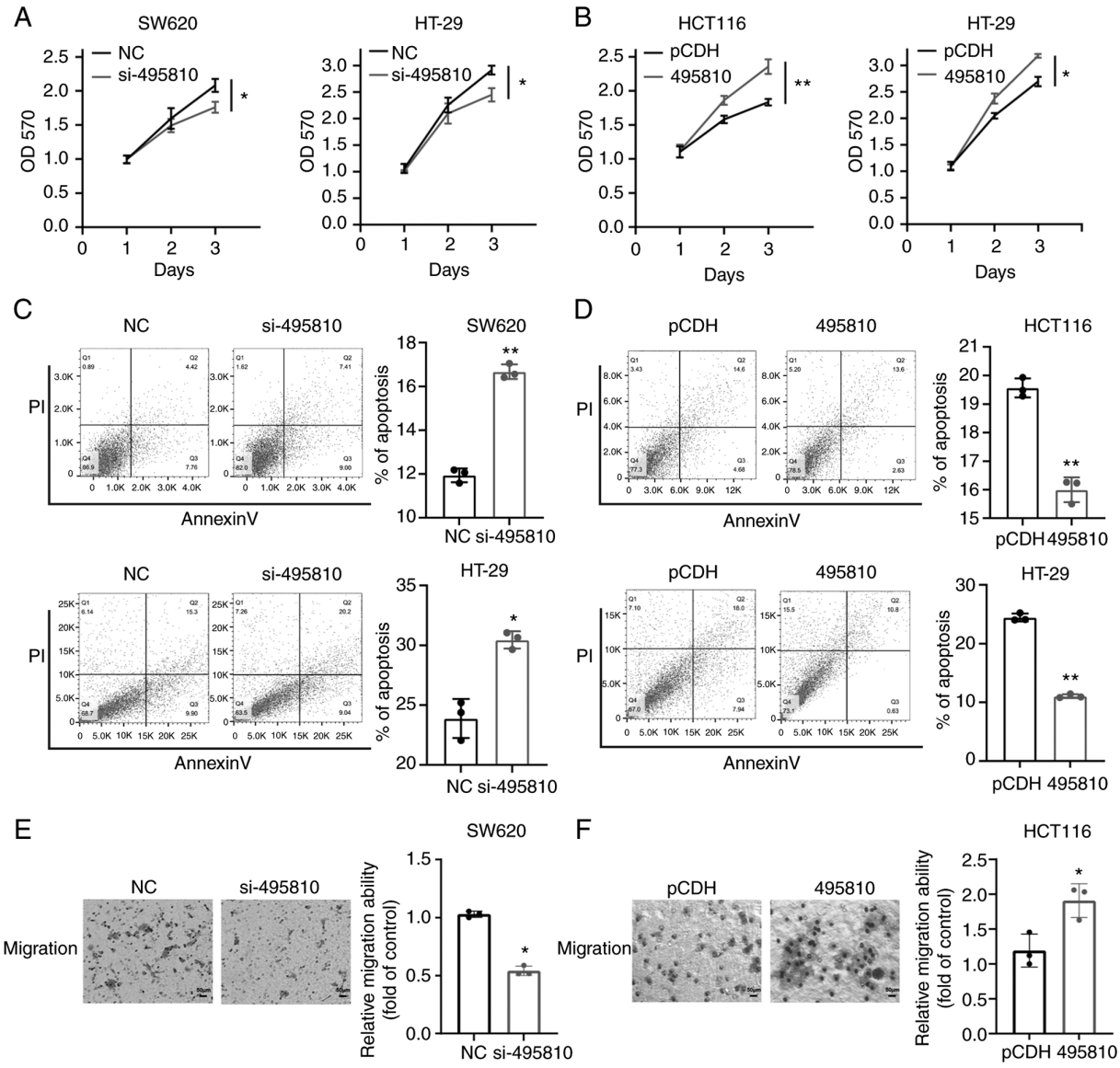

Figure 2. High expression of lncRNA 495810 promotes colorectal cancer growth. (A) After transient transfection of NC (non-specific control) and si-495810 for 48 h, the effects of lncRNA 495810 on cell proliferation were measured by MTT assays. (B) After transient transfection of pCDH or 495810, MTT assays were performed to detect the proliferation of cells. (C and D) The proportion of apoptotic cells was analyzed using PI and Annexin V staining after 495810 knockdown or overexpression. (E and F) The functions of 495810 knockdown or overexpression on metastasis were assessed by Transwell assays. Representative images (scale bar, 50 μm) were shown. *P<0.05 and **P<0.01. lncRNA, long non-coding RNA; si-, small interfering; NC, negative control.

used to cluster these proteins (Table II). Glycolysis-related enzymes, including PKM2, LDHB, ENO1, PGK1, LDHA and TPI1, attracted the authors' attention (Fig. 4B). Among which, PKM2 was positively correlated with lncRNA 495810

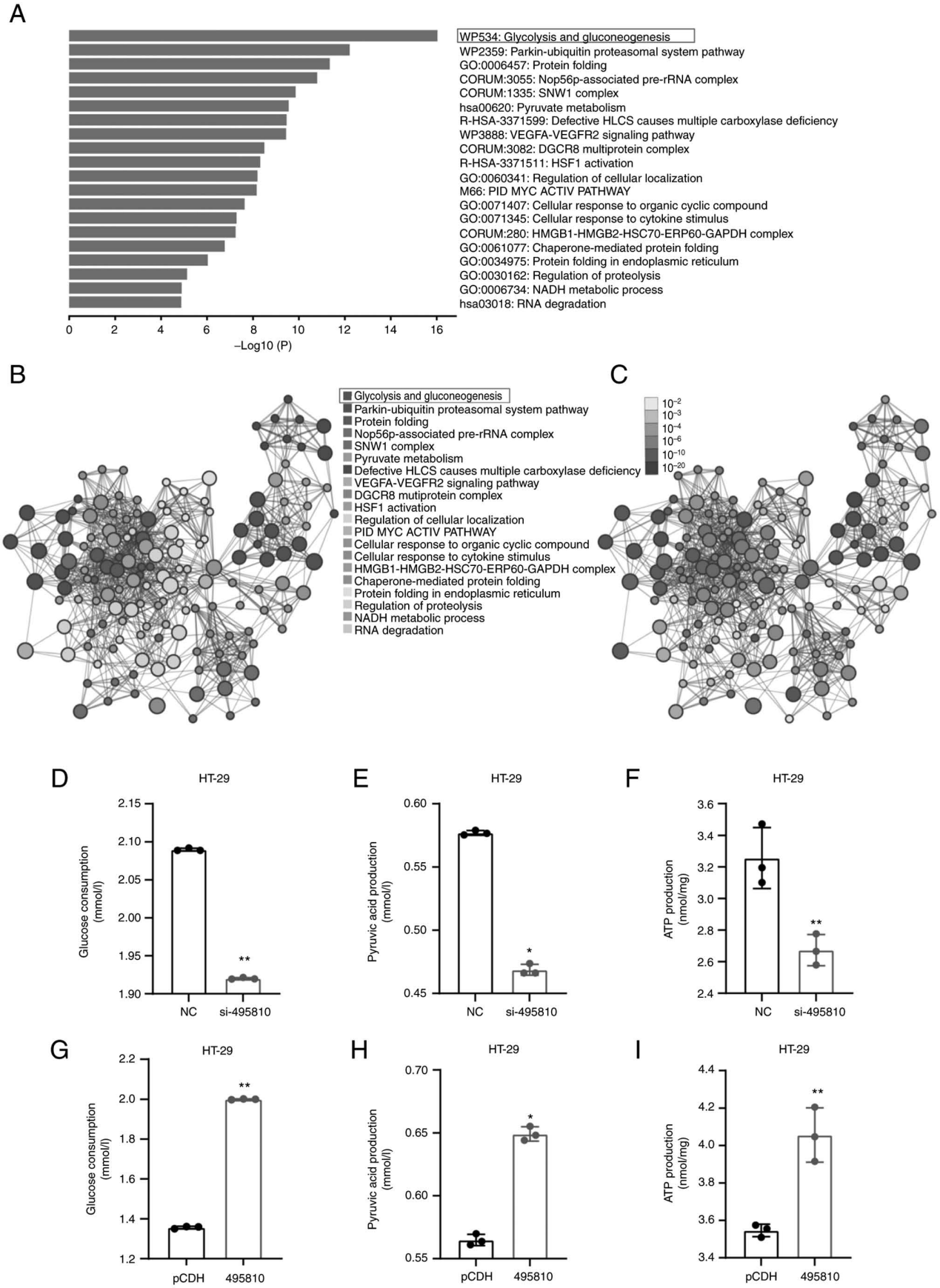

Figure 3. Long non-coding RNA 495810 promotes aerobic glycolysis in colorectal cancer cells. (A-C) The 495810 target genes were imported into the Metascape database for gene enrichment analysis; (A) Pathway and process enrichment analysis. (B) Network of enriched terms, colored by cluster ID, where nodes that share the same cluster ID are typically close to each other. (C) Network of enriched terms, colored by P-value, where terms containing more genes tend to have a more significant p-value. (D-F) After the HCT116 cells were transfected with NC or si-495810 for 48 h, the (D) glucose consumption, (E) ATP generation (F) and pyruvate acid production were determined. (G-I) After the HCT116 cells were transiently transfected with pCDH or 495810 for 48 h, the (G) glucose consumption, (H) ATP generation and (I) pyruvate acid production were determined. *P<0.05 and **P<0.01. si-, small interfering; NC, negative control.

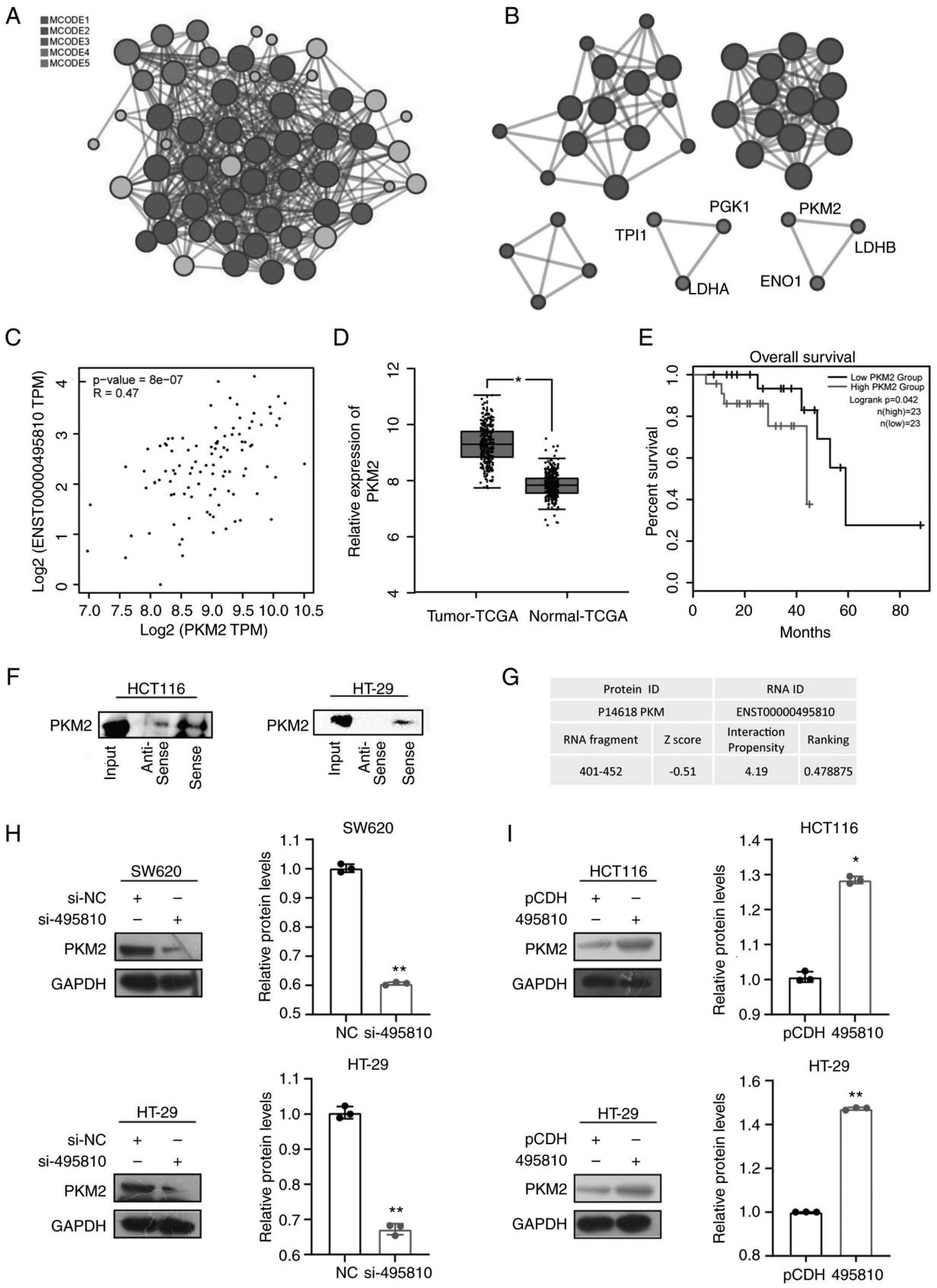

Figure 4. LncRNA 495810 interacts and positive correlates with PKM2. (A and B) Protein-protein interaction network and MCODE components identified in lncRNA 495810 targets. (C) The correlation analysis between 495810 and PKM2. (D) Relative expression of PKM2 in colorectal cancer tumor tissues and normal tissues based on the GEPIA 2 database. (E) Kaplan-Meier survival analysis of patient overall survival according to PKM2 levels based on the GEPIA 2 database. (F) Western blot analysis of the proteins retrieved from the 495810 pull-down assays using an anti-PKM2 antibody. (G) The predicted result of catRAPI. (H) After transient transfection of NC and si-495810 for 48 h, the expression of PKM2 protein was measured by western blotting in SW620 and HT-29 cells and the densitometric analysis of relative protein expression was performed. (I) The expression of PKM2 protein was detected by western blot analysis in HCT116 and HT-29 cells transfected with pCDH and 495810 for 48 h and the densitometric analysis of relative protein expression was performed. *P<0.05 and **P<0.01. lncRNA, long non-coding RNA; PKM2, pyruvate kinase isozyme M2; si-, small interfering; NC, negative control.

Table II. MCODE components identified in 495810 target genes.

MCODE	GO	Description	Log_{10}(P)
MCODE 1	R-HSA-3371556	Cellular response to heat stress	-9.4
	R-HSA-5336415	Uptake and function of diphtheria toxin	-8.8
	GO: 0006986	response to unfolded protein	-8.7
MCODE 2	GO: 0006457	protein folding	-7.5
	CORUM: 1335	SNW1 complex	-7.2
	R-HSA-389960	Formation of tubulin folding intermediates by CCT/TriC	-6.7
MCODE 3	GO:1903312	negative regulation of mRNA metabolic process	-6.9
	CORUM: 3055	Nop56p-associated pre-rRNA complex	-6.8
	WP411	mRNA processing	-6.5
MCODE 4	WP534	Glycolysis and gluconeogenesis	-8.5
	GO:0006090	pyruvate metabolic process	-8.1
	hsa00010	Glycolysis/Gluconeogenesis	-8
MCODE 5	WP4629	Aerobic glycolysis	-10.3
	WP1946	Cori cycle	-9.8
	GO: 0006096	glycolytic process	-8.7

GO, Gene Ontology.

(Fig. 4C), but not other proteins (Fig. S2A-E). Transcriptional and survival data for PKM2 were subsequently examined in the GEPIA 2 database. Consistent with lncRNA 495810, the expression of PKM2 was significantly upregulated in tumor tissues (Fig. 4D). The overall survival rates of the colon cancer patients with high levels of PKM2 were lower than those with low expression levels of PKM2 (Fig. 4E).

LncRNA 495810 interacts with PKM2 and promotes its expression. Next, the interaction between lncRNA 495810 and PKM2 was predicted via RPISeq analysis. The interaction probabilities of lncRNA 495810 and PKM2 were greater than 0.5 (RF=0.65, SVM=0.65). The data suggested that lncRNA 495810 may bind to PKM2 (Table SIV), which was also verified by RNA pull-down assay in HCT116 and HT-29 cells (Fig. 4F). In addition, a potential lncRNA 495810-binding region of PKM2 (401-452 nt) was predicted using catRAPI (Fig. 4G). To confirm the impact of lncRNA 495810 on PKM2, the expression of PKM2 was detected in CRC cells after depletion or overexpression of lncRNA 495810. The results identified that silencing lncRNA 495810 expression declined, whereas ectopic lncRNA 495810 expression enhanced the protein level of PKM2 (Fig. 4H and I).

LncRNA 495810 enhances PKM2 protein stability via the ubiquitin-proteasome pathway. Increasing evidence has demonstrated that lncRNAs regulate protein expression by post-translational modification (17,23-25). Its regulation mode of 495810 was then explored. The results showed that silencing lncRNA 495810 expression shortened, whereas ectopic lncRNA 495810 expression prolonged the half-life of PKM2 protein (Fig. 5A and B). In addition, suppression of proteasome activity by MG132, an inhibitor of proteasome, prevented the lncRNA-induced PKM2 expression (Fig. 5C and D), suggesting that PKM2 protein degradation was mediated by the ubiquitin-proteasome pathway.

Discussion

Patients with advanced CRC have a poor prognosis. Reliable prediction of recurrence risk would be of great benefit in improving outcomes for patients with colon cancer. However, the molecular mechanisms underlying its development and recurrence remain unclear, limiting the development of effective molecular biomarkers (26,27). Emerging evidence has revealed that dysregulation of lncRNAs plays a crucial role in cancer development and progression (28,29). In the present study, it was identified that a novel glycolysis-associated lncRNA 495810 was highly expressed in CRC cells and tissues. Moreover, lncRNA 495810 is a new candidate oncogene in the CRC. Clinical analysis revealed that high expression of lncRNA 495810 was associated with poor survival, and was correlated with an advanced clinical grade and stage. Thus, lncRNA 495810 could be used to evaluate prognosis in colon cancer. The COX regression analysis indicated that the expression of lncRNA 495810 was a prognostic factor for CRC and may be a biomarker of CRC. More notably, functional experiments presented that lncRNA 495810 significantly promoted proliferation and inhibited apoptosis in CRC cells. Therefore, it was indicated that lncRNA 495810 is a new candidate oncogene and promotes the occurrence of CRC. However, the potential molecular mechanism of lncRNA 495810 on CRC remains to be further investigated.

The involvement of lncRNAs in key oncogenic signaling pathways is an important piece of evidence demonstrating the functional role of lncRNAs in cancer (17,30). The present study demonstrated that the target proteins of lncRNA 495810, which were identified by RNA pull-down combined with mass spectrometry, were enriched in glycolysis pathway. It is well known that increased aerobic glycolysis is one of the hallmarks of cancer cells (31,32). Aerobic glycolysis not only provides ATP as an important energy source for cell growth, but also

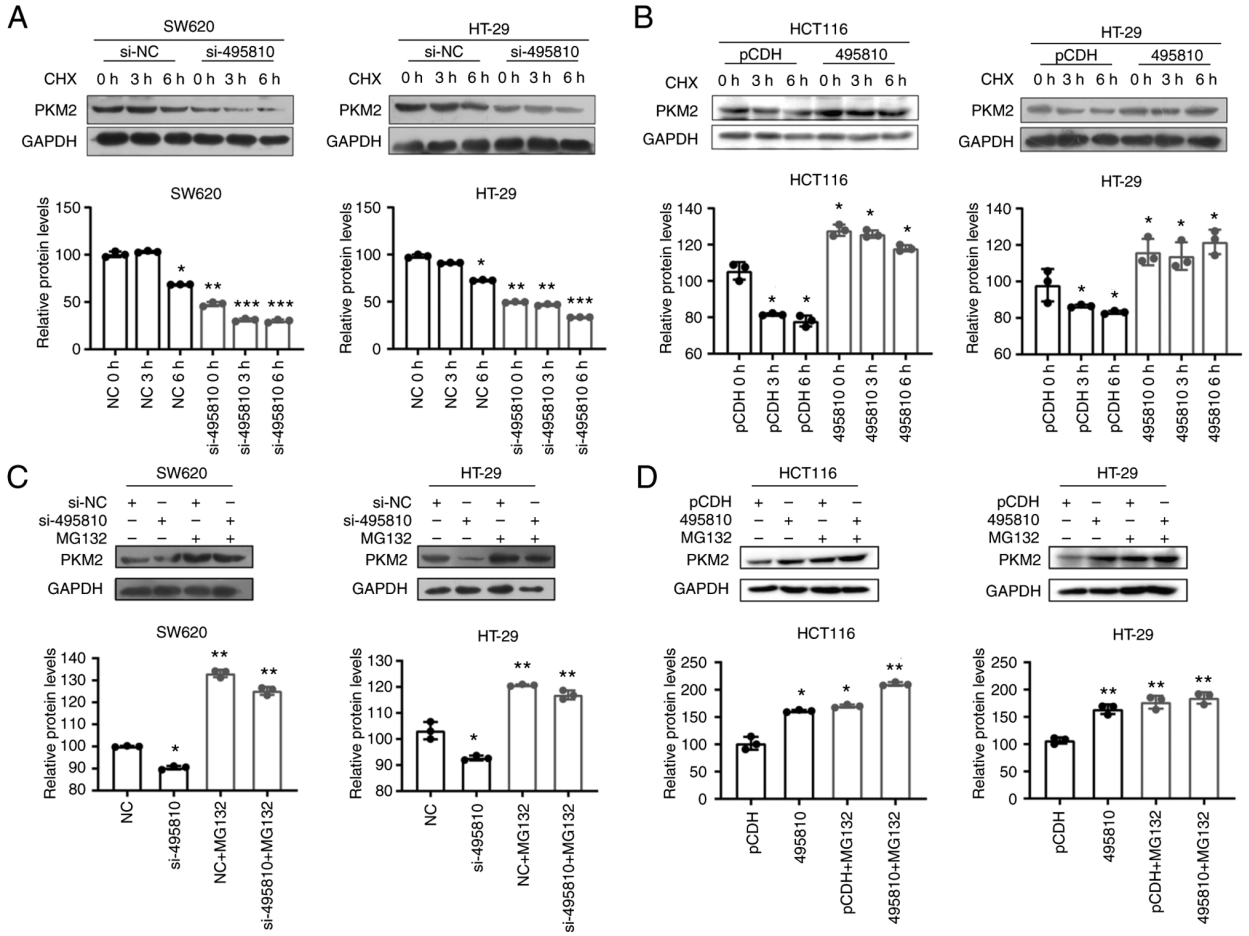

Figure 5. LncRNA 495810 enhances PKM2 protein stability by the ubiquitin-proteasome pathway. (A and B) The protein level of PKM2 was detected in (A) lncRNA 495810-depleted SW620 and HT-29 cells and (B) lncRNA 495810-overexpressing HT-29 and HCT116 cells by western blotting. Cells were treated with CHX (20 μg/ml) for 3 or 6 h before harvest. The densitometric analysis of relative protein expression is shown at the lower panel. (C and D) The protein level of PKM2 was determined in (C) lncRNA 495810-depleted SW620 and HT-29 cells and (D) lncRNA 495810-overexpressing HT-29 and HCT116 cells by western blot analysis. Cells were treated with MG132 (20 μM) for 6 h before harvest. The densitometric analysis of relative protein expression is shown at the lower panel. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001. lncRNA, long non-coding RNA; PKM2, pyruvate kinase isozyme M2; CHX, cycloheximide; si-, small interfering; NC, negative control.

provides raw materials for the synthesis of various biological macromolecules in tumor cells (33). Aberrantly expressed lncRNA in cancer cells is associated with aerobic glycolysis. Further study found that silencing lncRNA 495810 expression decreased, whereas overexpression of lncRNA 495810 increased the glycolysis-related indicators including glucose consumption, pyruvate acid production and ATP production. Collectively, the data suggested that 495810 could promote aerobic glycolysis and thereby provide energy for colon cancer proliferation.

Growing evidence suggests that lncRNAs are involved in cellular behavior, including metabolism, inflammation, cell differentiation and tumorigenesis, by interacting with specific proteins (34). The present findings indicated that PKM2, which positively correlated with lncRNA 495810, was identified as a key downstream of lncRNA 495810. Pyruvate kinase, which is a rate-limiting enzyme, catalyzes phosphoenolpyruvate to produce pyruvate in the last step of aerobic glycolysis. Pyruvate kinase isozyme M2 (PKM2) has been reported to be enriched in cancer cells and plays a key role in metabolic reprogramming and carcinogenesis (35-37). The ubiquitin-proteasome system is the main pathway of intracellular protein degradation (38) and is involved in the degradation of PKM2 proteins (39). In the present study, it was revealed that lncRNA 495810 may directly bind to PKM2 to protect it from ubiquitin-mediated degradation.

The expression levels of LncRNA 495810 are elevated in CRC cells and its tissues. However, the molecular mechanism of its abnormal expression in colon cancer remains unclear. Previous studies have shown that the regulation of key transcription factors can lead to abnormal expression of lncRNA in cancer cells. Chaudhary *et al* (40) reported that p53 as a transcription factor regulates the expression of lncRNA PINCR and promotes the development of CRC; transcription factor YY1 activates lncRNA-PVT1 and modulates lung cancer progression (41); transcription factors p53 and TP63 bind to enhancers in the promoter region of lincRNA00475 (linc-475) and lincRNA01503 respectively, resulting in the high expression of related lncRNA in tumor cells (42,43). Therefore, it was hypothesized that transcription factors play a key role in the aberrant expression of lncRNA 495810. The aforementioned findings will be further validated in a follow-up study.

Given the clinical, biochemical and functional significance of 495810 in CRC, the first evidence was provided of a novel

lncRNA 495810 in colorectal carcinogenesis as well as the glycolytic pathway, which may consist a novel biomarker and therapeutic target for CRC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZL and HW confirm the authenticity of all the raw data. KC conducted investigation, developed methodology and wrote the original draft. HW conducted investigation, wrote, reviewed and edited the manuscript, and provided resources. LZ performed software analysis and data validation. HL and GI wrote, reviewed and edited the manuscript, and performed software analysis. ZL conceptualized and supervised the study, wrote, reviewed and edited the manuscript, and acquired funding. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. SXULL2020014; approval date: 2020.04.08) by the Clinical Research Ethics Committees of the participating institutions (Taiyuan, China). Informed consent was provided by all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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