*Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)* *2023 24(3):221-231*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

**Research Article**

https://doi.org/10.1631/jzus.B2200221

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# Aerobic glycolysis in colon cancer is repressed by naringin via the *HIF1A* pathway

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**Abstract:** Metabolic reprogramming is a common phenomenon in cancer, with aerobic glycolysis being one of its important characteristics. Hypoxia-inducible factor-1α (*HIF1A*) is thought to play an important role in aerobic glycolysis. Meanwhile, naringin is a natural flavanone glycoside derived from grapefruits and many other citrus fruits. In this work, we identified glycolytic genes related to *HIF1A* by analyzing the colon cancer database. The analysis of extracellular acidification rate and cell function verified the regulatory effects of *HIF1A* overexpression on glycolysis, and the proliferation and migration of colon cancer cells. Moreover, naringin was used as an inhibitor of colon cancer cells to illustrate its effect on *HIF1A* function. The results showed that the *HIF1A* and enolase 2 (*ENO2*) levels in colon cancer tissues were highly correlated, and their high expression indicated a poor prognosis for colon cancer patients. Mechanistically, *HIF1A* directly binds to the DNA promoter region and upregulates the transcription of *ENO2*; ectopic expression of *ENO2* increased aerobic glycolysis in colon cancer cells. Most importantly, we found that the appropriate concentration of naringin inhibited the transcriptional activity of *HIF1A*, which in turn decreased aerobic glycolysis in colon cancer cells. Generally, naringin reduces glycolysis in colon cancer cells by reducing the transcriptional activity of *HIF1A* and the proliferation and invasion of colon cancer cells. This study helps to elucidate the relationship between colon cancer progression and glucose metabolism, and demonstrates the efficacy of naringin in the treatment of colon cancer.

**Key words:** Colon cancer; Naringin; Hypoxia inducible factor-1α (*HIF1A*); Enolase 2 (*ENO2*); Glycolysis; Metabolic reprogramming

## 1 Introduction

The most common gastrointestinal tumors are those of the colon and rectum. More than 95% of colorectal cancer patients are over 50 years of age at diagnosis, and the five-year relative survival rate in colorectal cancer is 65% (Miller et al., 2019). For earlier stages of colorectal cancer, surgery is still the main effective treatment. However, chemotherapy and radiotherapy are beneficial for patients with middle and advanced colon cancer (Denlinger and Barsevick, 2009; Cartwright, 2012). In addition, according to the molecular characteristics of colorectal cancer, some targeted drugs, such as inhibitors of B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) and Kirsten rat sarcoma viral oncogene homolog (*KRAS*), have better curative effects (André et al., 2015; Dienstmann et al., 2015; Taieb et al., 2017). Despite that our understanding of colon cancer is increasing, the current treatment efficacy remains unsatisfactory.

The Warburg effect is a specific means of metabolic reprogramming in cancer cells. Briefly, in an oxygen-rich environment, cancer cells still undergo

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Received Apr. 17, 2022; Revision accepted Sept. 6, 2022; Crosschecked Mar. 8, 2023

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aerobic glycolysis to break down glucose for energy (Warburg et al., 1927; Koppenol et al., 2011). The Warburg effect is the result of energy metabolism evolution in cancer cells to meet their substantial energy demands; thus, it is considered a hallmark of cancer (Hsu and Sabatini, 2008; Schwartz et al., 2017). Recent research has suggested that abnormally high expression and activation of multiple key kinases of the glycolytic metabolic pathway are the direct causes of the Warburg effect (Ramzy et al., 2023). Phosphoglycerate kinase 1 (PGK1) is the first rate-limiting enzyme in glycolysis and is highly expressed in various cancers (Ahmad et al., 2013; Hu et al., 2017). Previous research has shown that *O*-GlcNAcylation of PGK1 contributes to cancer progression by regulating the ratio of aerobic glycolysis to tricarboxylic acid cycle (Ahmad et al., 2013). Similarly, enolase 2 (*ENO2*) is highly expressed in lung, colon, and breast cancers and is strongly associated with poor cancer prognosis (Soh et al., 2011; Liu et al., 2020). However, the networks in which these genes function and their contributions to tumor progression remain unclear.

In recent years, a number of natural compounds have been shown to have apoptotic activity against a variety of cancers (Rajedadram et al., 2021; Sun et al., 2021; Du et al., 2022). Naringin, chemically known as 4',5,7-trihydroxyflavanone 7-*O*-rhamnoglucoside, is a natural flavanone glycoside derived from grapefruits and many other citrus fruits (Chen et al., 2016). It is one of the main active ingredients from the dried and immature fruit of *Citrus aurantium* L. (Zhang et al., 2014). Citrus peel extracts have been shown to reduce metallothionein activity in colon cancer cell lines, thereby inhibiting the progression of colon cancer (Ademosun et al., 2015). The consumption of grapefruit has also been shown to inhibit the development of colon cancer (Vanamala et al., 2006). Naringin is believed to inhibit the formation of inflammatory factors and inflammatory signaling pathways (Habauzit et al., 2011; Gopinath and Sudhandiran, 2012). In addition, in vivo experiments showed that naringin improves mitochondrial dysfunction by regulating the activity of metalloproteinases in mitochondria (Wang et al., 2015). Li et al. (2013) have indicated that naringin inhibits the proliferation of triple-negative breast cancer by blocking the cell cycle. In addition, it has a similar inhibitory effect on bladder cancer cells (Kim et al., 2008). However, the effect of naringin on aerobic glycolysis in colon cancer remains unexplored.

In our study, we identified glycolytic genes related to hypoxia-inducible factor-1α (*HIF1A*) by systematically analyzing the colon cancer database. The analyses of extracellular acidification rate and cell function verified the regulatory effects of *HIF1A* overexpression on glycolysis, and the proliferation and migration of colon cancer cells. The natural compound naringin was used as an inhibitor of colon cancer cells to illustrate its effect on *HIF1A* function. This study helps elucidate the relationship between colon cancer progression and glucose metabolism and shows the value of naringin in the treatment of colon cancer.

## 2 Materials and methods

### 2.1 Cell culture and chemical reagents

Colon cancer cell lines (LoVo CCL-229™ and HCT116 CCL-247™) were purchased from the American Type Culture Collection (ATCC; the Global Bioresource Center, Manassas, VA, USA). LoVo cells were cultured in F-12K medium (Life Technologies, Massachusetts, USA), and HCT116 cells were cultured in McCoy's 5A modified medium (Life Technologies) with 10% (0.1 g/mL) fetal bovine serum (FBS; Life Technologies). The cells were cultured under 5% carbon dioxide ($CO_2$) and a constant temperature of 37 °C. Trypsin-ethylenediamine tetraacetic acid (EDTA) solution was used to digest and passage these colon cancer cells. Naringin (HY-N0153, 99.79% purity) was purchased from MedChemExpress of China and dissolved in dimethyl sulfoxide (DMSO).

### 2.2 Vector construction

The dual luciferase reporter gene vector was used to clone the *HIF1A* motif sequence into the pGL3.0 vector. Specifically, the core binding sequence ACGTGC of *HIF1A* was obtained from the JASPAR website (http://jaspar.genereg.net), and the sequences were cloned into the pGL3.0 vector four times.

### 2.3 Cell transfection

Fresh medium was added when the cell confluence was 40%–60%. After 2 h, Neofect™ DNA transfection reagent (Neofect Biotech, Beijing, China) was used to treat the plasmid for 20 min. The plasmid: transfection reagent:Opti-MEM (Gibco, Grand Island, NY, USA) ratio was 1 μg:1 μL:200 μL. Cells were

cultured for 24–48 h after they were mixed with the medium.

### 2.4 Quantitative real-time PCR

In order to prepare colon cancer cells for treatment, we first used TRIzol (TaKaRa Biomedical Technology, Kyoto, Japan) to extract total cellular RNA. A total of 2 μg messenger RNA (mRNA) was reversely transcribed into complementary DNA (cDNA) by the PrimeScript RT Master Mix (TaKaRa Biomedical Technology). Then, specific amplification primers for *HIF1A*, *ENO2*, and β-actin (*ACTB*) were designed. Quantitative real-time polymerase chain reaction (qPCR) was performed on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Massachusetts, USA). The qPCR enzyme was a high-fidelity Taq enzyme (Vazyme Biotechnology, Nanjing, China), the final reaction volume was 10 μL, and the qPCR was carried out in a 96-well plate. The primers were as follows: *ACTB* primers (forward 5'-TCACCAACTGGGACGACA-3', reverse 5'-ACAGCCTGGATAGCAACG-3'); *HIF1A* primers (forward 5'-AGAGCAGGAAAAGGAGTC-3', reverse 5'-GCTTGAAAAAGTGAACCA-3'); and *ENO2* primers (forward 5'-AAGGACAAATACGGCAAG-3', reverse 5'-CTCTGAGGCAGCAACATC-3').

### 2.5 Chromatin immunoprecipitation (ChIP)

The prepared colon cancer cells were treated with a final concentration of 1% (volume fraction) paraformaldehyde at room temperature for 10 min. Next, glycine was added to a final concentration of 0.125 mol/L to stop the crosslinking, and the cells were collected. The cells were washed twice with cold phosphate-buffered saline (PBS) to obtain cell pellets, and then added with protease inhibitor complexes. The cell sonication parameters were as follows: 25% power, 4.5 s impact, and 9 s interval. The samples were centrifuged at 10 000*g* for 10 min at 4 ℃, and 300 μL of the supernatant was used to detect the ultrasonic effect by agarose gel electrophoresis. *HIF1A* antibody, ChIP dilution buffer, and protein A agarose were added to the lysate and inverted overnight at 4 ℃. After incubation overnight, 60 μL of protein A agarose/salmon sperm DNA was added to each tube, and the samples were inverted at 4 ℃ for 2 h. After 10 min, the samples were centrifuged at 700 r/min for 1 min, followed by removing the supernatant. The precipitated complex was washed sequentially with washing buffer. Subsequently, 250 μL of elution buffer was added to each tube and centrifuged to collect the supernatant. The washing step was repeated, and 20 μL of NaCl (5 mol/L) was added to each tube to decrosslink the sample at 65 ℃ overnight. After decrosslinking, 1 μL of RNase A was added to each tube and incubated at 37 ℃ for 1 h. Finally, 10 μL of 0.5 mol/L EDTA, 20 μL of 1 mol/L Tris-HCl (pH 6.5), and 2 μL of 10 mg/mL proteinase K were added to each tube, followed by the recovery of the DNA fragments.

### 2.6 Gene expression data analysis

We downloaded the colon cancer dataset (GSE14333) from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) and obtained the original expression values for many genes. We found 28 glycolysis-related enzymes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg), analyzed the genes related to *HIF1A* expression through the Morpheus website (https://software.broadinstitute.org/morpheus), and presented them in a heatmap. GraphPad 5.0 (GraphPad Software, San Diego, USA) was used to evaluate the correlation between the expression value of *HIF1A* and that of glycolytic genes, and then fit the correlation curve. After downloading the patient information, such as survival time and survival status, we used GraphPad 5.0 to draw the survival curve and calculate the *P* value.

The supplementary materials and methods described detailed information on JASPAR analysis, western blot, extracellular acidification rate (ECAR), Matrigel invasion, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and dual luciferase reporter assay.

## 3 Results

### 3.1 Inhibition of colon cancer cell proliferation by naringin

Naringin is a natural compound derived from certain citrus fruits, and is a traditional Chinese medicine mainly used to fight inflammation (Fig. 1a). Herein, we investigated its effect on the activity of colon cancer cells. Interestingly, the results showed that 10 mmol/L naringin effectively inhibited the proliferation of these

cells (Fig. 1b). Aerobic glycolysis is one of the characteristic energy metabolism reprogramming processes of cancer cells. Thus, we next tested the effect of naringin on the aerobic glycolysis of colon cancer cells. As expected, the rate of extracellular acidification confirmed that naringin attenuated the overall level and the reserve level of aerobic glycolysis (Figs. 1c and 1d). The detection results of glycolytic metabolites also demonstrated that naringin reduced glucose uptake and metabolism by colon cancer cells (Fig. 1e). These data suggested that naringin appears to be effective in colon cancer treatment.

### 3.2 Role of *HIF1A* in the effect of naringin on colon cancer

*HIF1A*, as a known regulator of aerobic glycolysis, has been reported to be involved in the progression of various cancers. We speculated that naringin has a certain effect on the activity of *HIF1A*. Thus, we cloned the DNA sequence containing the *HIF1A* binding site into the pGL3.0-Basic vector (Fig. 2a). The dual luciferase assay showed that naringin inhibited the transcriptional activity of *HIF1A* (Fig. 2b). To confirm the biological functions of *HIF1A* in colon cancer cells, we carried out our experiments using

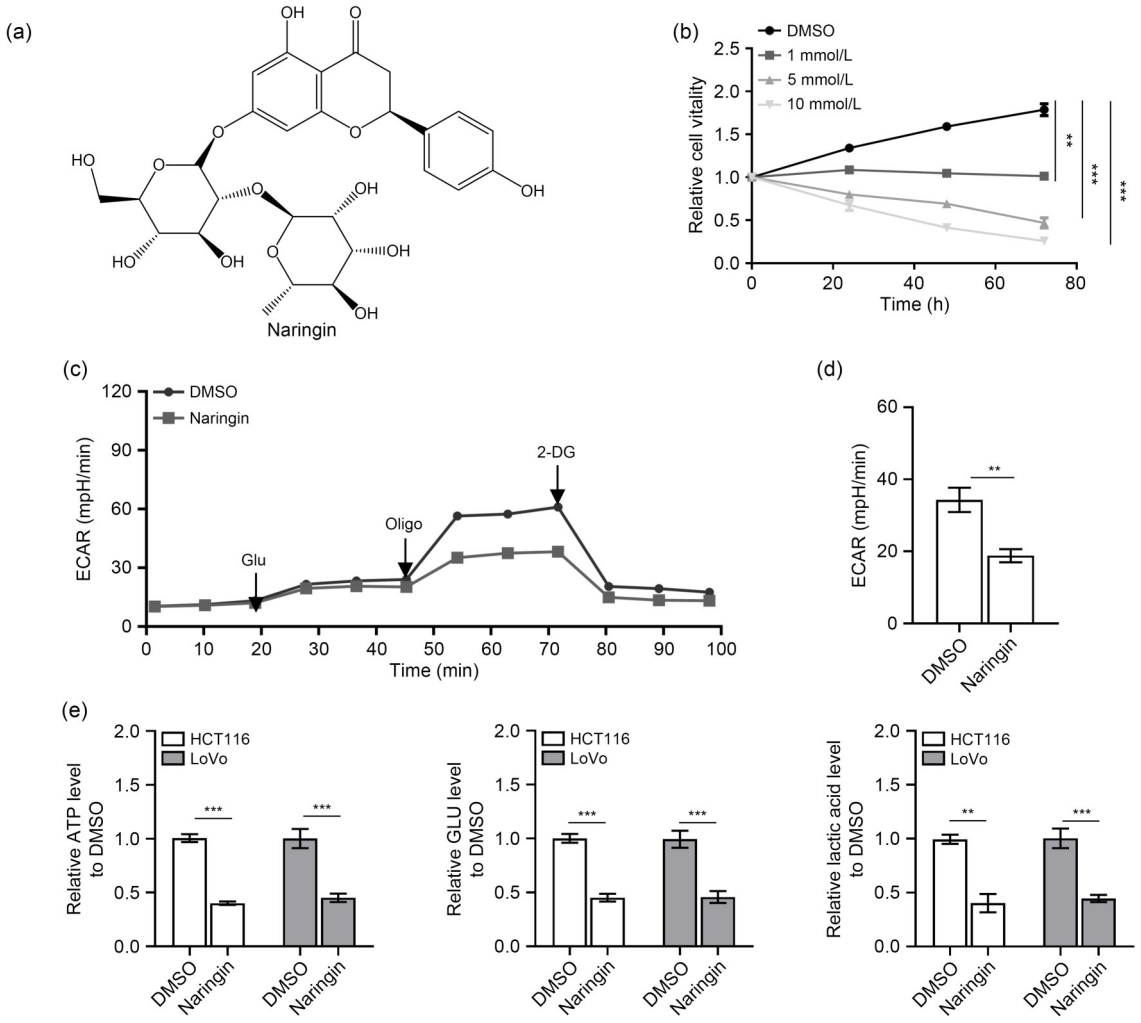


**Fig. 1 Reduction of the Warburg effect of colon cancer cells by naringin. (a) The chemical structure of naringin. (b) The MTT assay verified the effect of naringin on the proliferation of colon cancer cells. (c–e) The ECAR value and glycolytic substrate test verified the effect of naringin on the glycolytic ability of colon cancer cells. Data are presented as mean± SD, $n$=3. $^{**}$ $P$<0.01, $^{***}$ $P$<0.001. MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; ECAR: extracellular acidification rate; SD: standard deviation; DMSO: dimethyl sulfoxide; mpH: 1/1000 pH; Glu: glucose; Oligo: oligomycin; 2-DG: 2-deoxyglucose; ATP: adenosine triphosphate.**

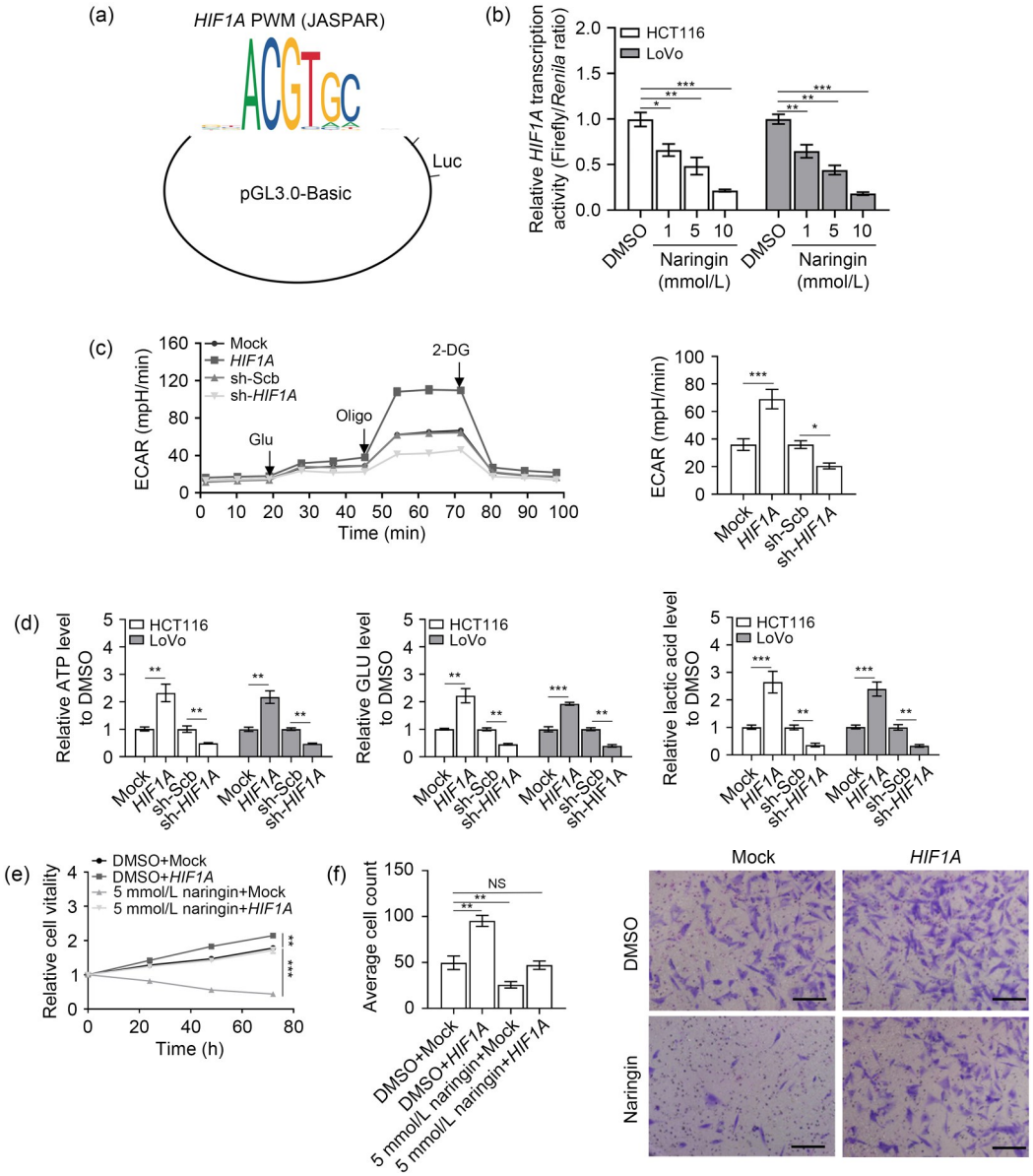


**Fig. 2 Role of *HIF1A* in the effect of naringin on colon cancer progression. (a) Schematic diagram of the construction of *HIF1A* PWM base sequence (http://jaspar.genereg.net). (b) The relative fluorescence intensity reflects the regulatory effect of naringin on *HIF1A* transcriptional activity. (c) The Seahorse assay demonstrated the effect of *HIF1A* on the ECAR value in colon cancer. (d) The relative intracellular ATP level, glucose uptake level, and lactic acid production level reflected the glycolytic ability of colon cancer cells. (e, f) MTT and Matrigel invasion assays were used to evaluate the effects of *HIF1A* and naringin on the proliferation and invasion of colon cancer cells. Scale bar: 100 μm. Data are presented as mean±SD, $n$=3. $^{*}$ $P$<0.05, $^{**}$ $P$<0.01, $^{***}$ $P$<0.001, $^{NS}$ no significance. *HIF1A*: hypoxia inducible factor-1α; PWM: position-specific weight matrix; ECAR: extracellular acidification rate; ATP: adenosine triphosphate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SD: standard deviation; DMSO: dimethyl sulfoxide; mpH: 1/1000 pH; Glu: glucose; Oligo: oligomycin; 2-DG: 2-deoxyglucose.**

stable colon cancer cell lines transfected with the mock vector, the *HIF1A* construct, sh-Scb, or sh-*HIF1A*. The ECAR value confirmed that *HIF1A* knockdown or overexpression had significant effects on the glycolytic flux (Fig. 2c). In addition, the ectopic expression or silencing of *HIF1A* increased or decreased the levels of adenosine triphosphate (ATP), glucose, and lactic acid, which all supported the hypothesis that *HIF1A* promoted glycolysis (Fig. 2c). Correspondingly, the key indicators of aerobic glycolysis, i.e., glucose uptake,

ATP production, and lactic acid production content, also changed with the expression of *HIF1A* (Fig. 2d). As expected, the overexpression of *HIF1A* significantly increased the proliferation and invasion of colon cancer cells, while the knockdown of *HIF1A* effectively inhibited these activities (Fig. S1). The above evidence indicated that *HIF1A* improved the aerobic glycolytic capacity of colon cells, which in turn promoted the progression of colon cancer.

Subsequently, we sought to explore the role of *HIF1A* in the effect of naringin on colon cancer cells. Correspondingly, naringin also counteracted the proliferation of colon cancer cells caused by *HIF1A* (Fig. 2e). Matrigel invasion experiments confirmed this conclusion (Fig. 2f). This means that *HIF1A* is necessary for naringin to exert its inhibitory effect on colon cancer progression.

### 3.3 Co-expression of glycolytic gene *ENO2* and *HIF1A* in colon cancer

*HIF1A* is a recognized key transcription factor related to glycolysis. To explore the possible mechanism by which *HIF1A* regulates aerobic glycolysis in colon cancer, we sought to identify *HIF1A*-regulated genes associated with aerobic glycolysis. We established a dataset of validated genes corresponding to glycolysis derived from the KEGG pathway database. Then, we analyzed the human colorectal cancer dataset (GSE14333) by utilizing R2 (Genomics Analysis and Visualization Platform), and generated five candidate genes positively correlated with *HIF1A*: *ENO2*, phosphoglycerate mutase 1 (*PGAM1*), bisphosphoglycerate mutase (*BPGM*), pyruvate kinase (*PKM*), and adenosine diphosphate (ADP)-dependent glucokinase (*ADPGK*) ($R$>0.3, $P$<0.05; Fig. S2). By comparing the correlation between *HIF1A* and the expression levels of candidate genes, we identified *ENO2* as the target gene of glucose metabolism regulated by *HIF1A* (Fig. 3a). In addition, Kaplan-Meier survival analysis indicated that a higher expression of both *HIF1A* and *ENO2* was significantly associated with poor patient survival (Fig. 3b). DepMap website analysis (https://depmap.org/portal/interactive) also showed that the expression of *HIF1A* and *ENO2* in different colon cancer cells was positively correlated (Fig. 3c). These data showed that the expression of the glucose metabolism-related gene *ENO2* is positively correlated with *HIF1A*, and both can promote cancer.

### 3.4 Transactivation of *ENO2* in colon cancer mediated by *HIF1A*

We next investigated the potential effects and regulatory mechanism of *HIF1A* on the expression of *ENO2*. The mining of JASPAR revealed that as a key transcription factor, *HIF1A* had a 6-nt enrichment peak (ACGTGC) within the promoter of *ENO2* (Fig. 4a). Immunofluorescence staining assays revealed that *HIF1A* was localized in the nucleus and was not affected by naringin (Fig. 4b). Dual luciferase assays indicated that the overexpression or silencing of *HIF1A* enhanced or reduced the activity of the *ENO2* promoter in HCT116 and LoVo cells, respectively (Fig. 4c). Consistently, chromatin co-immunoprecipitation demonstrated that transfection with *HIF1A* or sh-*HIF1A* increased or decreased *HIF1A* binding to the promoter of *ENO2*, respectively (Fig. 4d). qPCR and western blot analyses further confirmed that *HIF1A* promoted the expression of *ENO2* at the mRNA and protein levels in HCT116 and LoVo cells (Figs. 4e–4g). Correspondingly, the expression of other genes (*PGAM1*, *BPGM*, *PKM*, and *ADPGK*) did not change significantly (Fig. S3a). To further confirm that the *HIF1A* regulation of *ENO2* expression was based on transcriptional regulation, we deleted the binding site of *HIF1A* in the *ENO2* promoter region. The results proved that the *ENO2* promoter region lacking a binding site was no longer regulated by *HIF1A* (Fig. S3b).

Subsequently, we overexpressed *HIF1A* and knocked down *ENO2* to further confirm the necessity of *ENO2* for *HIF1A* in colon cancer aerobic glycolysis. The results showed that the knockdown of *ENO2* partially counteracted the increase in aerobic glycolytic capacity caused by *HIF1A* in colon cancer cells (Fig. S4). We believe that other aerobic glycolytic genes exist in colon cancer that are regulated by *HIF1A*, and therefore, knocking down *ENO2* does not completely suppress the effect of *HIF1A*, albeit it is a highly critical player. In addition, we found that naringin did not affect the expression of *HIF1A*, but attenuated the transcriptional regulation of *ENO2* by *HIF1A* (Figs. 4h–4j, S3c, and S3d). These data suggested that the transcription factor *HIF1A* increased colon cancer progression by facilitating the expression of *ENO2* at the transcriptional level.

In general, naringin at an appropriate concentration was taken up by colon cancer cells, thereby reducing the transcriptional activity of the transcription

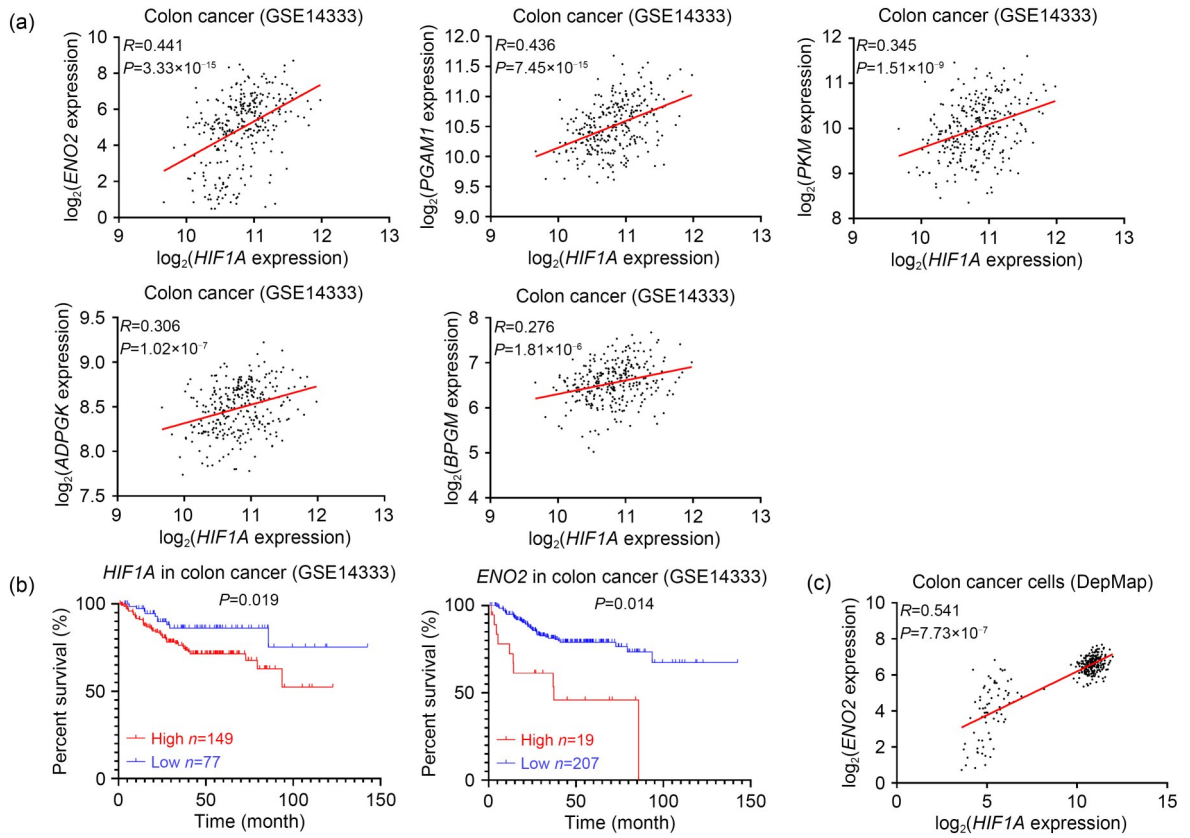


**Fig. 3 Co-expression of glycolytic gene *ENO2* and *HIF1A* in colon cancer. (a) The correlation curves (GSE14333, *n*=226) show the degree of correlation between these glycolytic genes and *HIF1A* in colon cancer. (b) The Kaplan-Meier survival curves (GSE14333, *n*=226) show the roles of *ENO2* and *HIF1A* in colon cancer patients. (c) The correlation curve of *HIF1A* and *ENO2* in colon cancer cells. *ENO2*: enolase 2; *HIF1A*: hypoxia inducible factor-1α; *PGAM1*: phosphoglycerate mutase 1; *PKM*: pyruvate kinase; *ADPGK*: adenosine diphosphate (ADP)-dependent glucokinase; *BPGM*: bisphosphoglycerate mutase.**

factor *HIF1A*. In this way, this molecule blocked aerobic glycolysis in colon cancer cells. Finally, the proliferation and invasion of colon cancer cells were inhibited by naringin.

## 4 Discussion

Metabolic reprogramming occurs in cancer cells as an adaptation to the substantial energy consumption due to their rapid proliferation. Compared with normal cells, cancer cells obtain ATP faster through glycolysis instead of oxidative phosphorylation in the mitochondria. Even in an environment with sufficient oxygen supply, cancer cells still preferentially utilize aerobic glycolysis, which is known as the Warburg effect (Warburg et al., 1927; vander Heiden et al., 2009; Yu et al., 2017). In this study, we found that abnormally elevated levels of aerobic glycolysis existed in colon cancer cells, and this effect was positively correlated with the proliferation and invasion of these cells. Accordingly, the inhibition of aerobic glycolysis effectively reduced the growth of these cells, which constitutes an important finding to understand the metabolic disorders of colon cancer cells.

*ENO2* is one of the three enolase isoenzymes in mammals and is mainly expressed in mature neurons and cells of neuronal origin (Kim and Dang, 2005; Andreou et al., 2021). Recent studies have found that *ENO2* is highly expressed in various tumor tissues, such as prostate cancer and Ewing sarcoma, which is closely related to the prognosis of cancer patients (Kim et al., 2017; Jiang et al., 2022). As a key enzyme in glycolysis, *ENO2* promotes the conversion of β-glycerophosphate to dihydroxyacetone phosphate. Therefore, the enzymatic activity of *ENO2* is crucial for glucose metabolism in cancer cells. Zheng et al. (2020) demonstrated that histone deacetylase 3

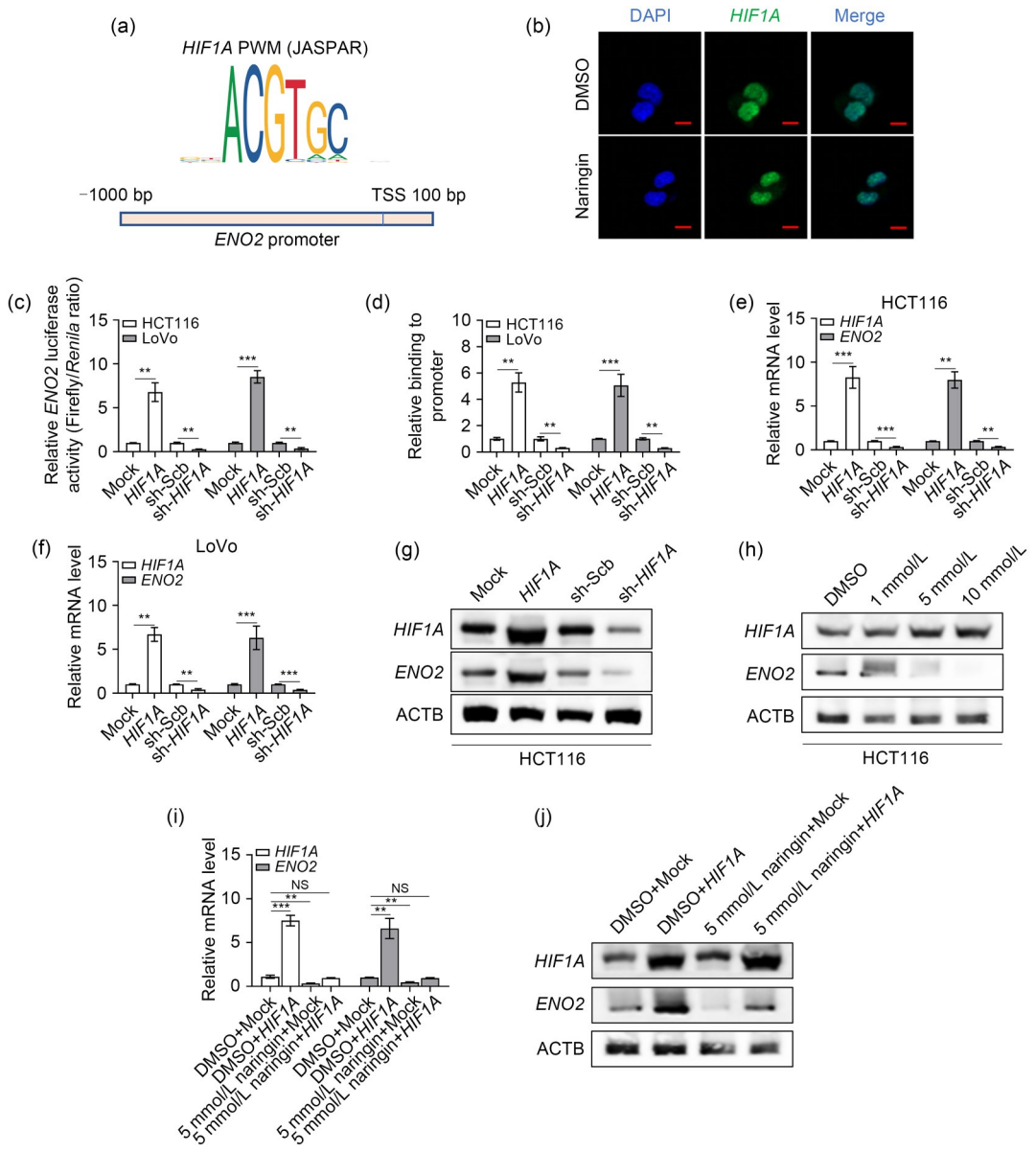


**Fig. 4 Effect of *HIF1A* on the transactivation of *ENO2* in colon cancer. (a) The *HIF1A* PMW site was analyzed using the JASPAR website to predict the binding region of *HIF1A* in the *ENO2* promoter region. (b) Immunofluorescence results showed that *HIF1A* was localized in the nucleus of colon cancer cells (scale bar=10 μm). (c) Dual luciferase reporter vectors containing the *ENO2* promoter were transfected, and *HIF1A* was overexpressed or knocked down. The relative fluorescence intensity reflected the change in the transcriptional activity of *ENO2*. (d–g) ChIP, qPCR, and western blot assays detected the transcription levels, mRNA levels, and protein levels of *HIF1A* and *ENO2*, respectively. (h–j) The expression of *HIF1A* and *ENO2* in colon cancer cells treated by naringin and *HIF1A*. Data are presented as mean±SD, *n*=3. $^{**}$ *P*<0.01, $^{***}$ *P*<0.001, $^{NS}$ no significance. *HIF1A*: hypoxia inducible factor-1α; *ENO2*: enolase 2; PWM: position-specific weight matrix; ChIP: chromatin immunoprecipitation; qPCR: quantitative real-time polymerase chain reaction; mRNA: messenger RNA; SD: standard deviation; TSS: transcription start site; DAPI: 4',6-diamidino-2-phenylindole; ACTB: β-actin; DMSO: dimethyl sulfoxide.**

(HDAC3)-induced deacetylation of *ENO2* enhances the activity of *ENO2* in glycolysis. A recent study found that the BRAF V600E mutation, which was closely associated with colon cancer progression, exhibited a significant dependence on *ENO2*, and mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signalings were also positively correlated with the expression level of *ENO2* (Yukimoto et al., 2021). Our findings suggested that *HIF1A* upregulated the *ENO2* expression levels and

enhanced the aerobic glycolytic capacity of colon cancer cells. Although we had no direct evidence that the enzymatic activity of each *ENO2* molecule was altered, the effect of *ENO2* on aerobic glycolysis was enhanced. *ENO2* not only plays an enzymatic activity in glycolysis, but also it has been shown to be associated with the activation of other intracellular signaling pathways and the mutation of oncogenes. Unfortunately, limited by the research direction and funding support, we did not further deepen this direction of research in this paper. This should be the task of follow-up studies.

Naringin, as a natural compound, has been found to regulate various intracellular signaling pathways and physiological processes. Current research suggested that naringin mainly affects the process of inflammation (Habauzit et al., 2011; Gopinath and Sudhandiran, 2012). Appropriate concentrations of naringin solutions inhibit nuclear factor-κB (NF-κB) nuclear translocation and reduce inflammation (Ansari et al., 2022). This molecule can also suppress inflammation by reducing the production of inflammation-related factors (Habauzit et al., 2011; Luo et al., 2012). Experiments have shown that naringin inhibits the liver 3-hydroxy-3-methyl CoA (HMG-CoA) reductase activity of low-density lipoprotein receptor (LDLR) knockout mice, thereby reducing the plasma lipid concentration of mice (Luo et al., 2012). However, it is not yet known how naringin affects glycolysis. Our data proved that naringin reduces the transcriptional activity of *HIF1A* in colon cancer cells, thereby downregulating the expression of *ENO2*, a key gene for glycolysis. Overexpression of *HIF1A* counteracted this inhibitory effect. These findings suggested that naringin may potentially inhibit the progression of colon cancer.

## 5 Conclusions

In this study, we found that the transcriptional activity of the *HIF1A* is abnormally increased, and it is closely related to the expression of the key gene of glycolysis, *ENO2*. Further investigation showed that *HIF1A* directly binds to the promoter region of *ENO2* to enhance its transcription. The natural compound naringin inhibits the transcriptional activity of *HIF1A* in colon cancer, thereby suppressing the Warburg effect. In general, naringin blocks the transcriptional regulation of *ENO2* by *HIF1A*, thus preventing the proliferation and invasion of colon cancer cells.

## Acknowledgments

This work was supported by the Fund of Hubei Provincial Health Commission (No. ZY2021M080), the Medical Research Project of Jiangsu Provincial Health Commission (No. Z2021068), the Yancheng Medical Science and Technology Development Plan Project (No. YK2021004), and the Young Scientific and Technological Talents Support Project by Jiangsu Association for Science and Technology (No. TJ-2022-097), China.

## Author contributions

Guangtao PAN and Ping ZHANG performed the experiments and prepared the manuscript. Aiying CHEN, Yu DENG, Zhen ZHANG, Han LU, Aoxun ZHU, and Cong ZHOU performed the experiments and statistical analyses. Sen LI and Yanran WU designed the draft of the research process and provided funding for the experiment. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

## Compliance with ethics guidelines

Guangtao PAN, Ping ZHANG, Aiying CHEN, Yu DENG, Zhen ZHANG, Han LU, Aoxun ZHU, Cong ZHOU, Yanran WU, and Sen LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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**Supplementary information**

Figs. S1–S4; Materials and methods