

***LGALS4* inhibits glycolysis and promotes apoptosis of colorectal cancer cells via β -catenin signaling**

SHENGJIE LI¹, KAIFENG YANG¹, JIAYOU YE¹, CHENGFAN XU¹, ZHIXIANG QIN¹,
YING CHEN², LANJIAN YU¹, TIANYU ZHOU¹, BIN SUN² and JUN XU¹

¹Department of Gastroenterology Surgery, Yichang Central People's Hospital, The First College of Clinical Medical Science, China Three Gorges University, Yichang, Hubei 443000, P.R. China; ²Center for Clinical Research and Translational Medicine, Yangpu Hospital, School of Medicine, Tongji University, Shanghai 200438, P.R. China

Received June 26, 2024; Accepted October 31, 2024

DOI: 10.3892/ol.2025.14873

Abstract. Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. Glycolysis serves a crucial role in the development of CRC. The aim of the present study was to investigate the function of lectin galactoside-binding soluble 4 (*LGALS4*) in the regulation of glycolysis and its therapeutic potential in CRC. In the present study, 175 overlapping differentially expressed genes were identified by comprehensive analysis of The Cancer Genome Atlas database and the GSE26571 CRC dataset from the Gene Expression Omnibus database. *LGALS4* was identified as the central gene by prognostic analysis using the mimetic map construction method and least absolute shrinkage and selection operator Cox regression. *In vitro* experiments were performed to evaluate the effects of *LGALS4* overexpression on CRC cell phenotype and aerobic glycolysis, as well as its relationship with β -catenin signaling. *LGALS4* was significantly down-regulated in CRC, with an average 3-fold decrease compared with *LGALS4* expression levels in normal tissues. *LGALS4* was also significantly associated with patient survival. *LGALS4* overexpression inhibited CRC cell growth, induced cell cycle arrest and enhanced 5-fluorouracil (5-FU)-induced apoptosis. Specifically, *LGALS4* overexpression resulted in a ~50% decrease in cell proliferation and a ~2-fold increase in apoptosis. In addition, *LGALS4* overexpression inhibited

aerobic glycolysis and reduced glucose-dependent and glycolytic activity in CRC cells. The downregulatory effect of *LGALS4* on glycolysis-related genes was further enhanced by the addition of the β -catenin inhibitor XAV-939. *LGALS4* expression decreased CRC progression by inhibiting glycolysis and affecting β -catenin signaling. Overexpression of *LGALS4* reduced the proliferation and glycolytic capacity of CRC cells and also enhanced their sensitivity to 5-FU. These results may potentially provide new perspectives for CRC treatment and targets for future clinical intervention strategies.

Introduction

According to data from 2024, colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide and is the third most frequently diagnosed cancer (1). The development of CRC is a complex, multistep process influenced by a combination of environmental and genetic factors (2). Key risk factors include advanced age, family history, inflammatory bowel disease, dietary habits and lifestyle factors such as smoking and physical inactivity (3). The incidence and mortality rates of CRC vary globally, with higher rates typically observed in developed countries (4); these differences may be due to lifestyle variables, such as diets heavy in fat and poor in fiber, a lack of physical activity and greater screening rates that result in more frequent diagnoses. Despite advances in screening programs and the introduction of various therapeutic interventions, including radiation, chemotherapy, surgery and targeted therapy, CRC remains a significant public health issue. Patient prognosis largely depends on the tumor stage and molecular profile of the cancer (5). Although progress has been made, many questions remain unanswered regarding the understanding of the molecular mechanisms of CRC, particularly in terms of early diagnosis and therapeutic strategies. For example, aberrant activation of the Wnt/ β -catenin signaling pathway is a common molecular event in CRC, but its specific role in disease progression has not been fully elucidated (6). The heterogeneity of CRC presents challenges in achieving optimal clinical outcomes, emphasizing the need for innovative diagnostic biomarkers, therapeutic strategies and prognostic indicators to enhance patient management and outcomes in CRC.

Correspondence to: Professor Bin Sun, Center for Clinical Research and Translational Medicine, Yangpu Hospital, School of Medicine, Tongji University, 450 Tengyue Road, Yangpu, Shanghai 200438, P.R. China
E-mail: binsun@tongji.edu.cn

Professor Jun Xu, Department of Gastroenterology Surgery, Yichang Central People's Hospital, The First College of Clinical Medical Science, China Three Gorges University, 183 Yiling Avenue, Yichang, Hubei 443000, P.R. China
E-mail: 26720772@qq.com

Key words: lectin galactoside-binding soluble 4, colorectal cancer, glycolysis, β -catenin signaling, apoptosis

Lectin galactoside-binding soluble 4 (*LGALS4*), a member of the galactose lectin family, serves a role in a variety of biological processes (7). In CRC, the expression level of *LGALS4* is closely associated with tumor progression, invasiveness and drug resistance. It has been reported that *LGALS4* expression is high in normal colonic epithelial cells and significantly lower in CRC tissues, suggesting that it may function as a tumor suppressor (7). Loss of function of *LGALS4* has been associated with enhanced proliferation, migration and invasion of tumor cells (7). At the molecular level, *LGALS4* is able to induce cell cycle arrest by regulating the levels of cell cycle-related proteins, such as Cyclin D1, p21 and p15, thereby inhibiting the proliferation of tumor cells (8). In addition, *LGALS4* can inhibit tumor development by affecting the Wnt/ β -catenin signaling pathway, an aberrant activation pathway commonly found in CRC (9). Increased expression of *LGALS4* levels reduces the expression level of β -catenin and upregulates inhibitory factors of the Wnt signaling pathway, such as Ephrin B1, which suppresses the proliferation and invasion of tumor cells. At the clinical level, the expression level of *LGALS4* correlates with the prognosis of patients with CRC and the decrease in its expression may predict the severity of the disease and the response to treatment (10). In addition, the expression level of *LGALS4* correlates with tumor resistance to the chemotherapeutic agent oxaliplatin, suggesting its potential for therapeutic application (10).

Glycolysis, a crucial metabolic pathway, produces pyruvate from glucose conversion while producing ATP and NADH (11). In CRC, glycolysis is significantly upregulated, an occurrence known as the Warburg effect, where cancer cells predominantly rely on aerobic glycolysis even when oxygen is abundant (12). This metabolic reprogramming supports rapid cell proliferation and tumor growth, making glycolysis a focal point in CRC research. Zuo *et al* (13) investigated the impact of the long non-coding RNA maternally expressed gene 3 (MEG3) on glycolysis in CRC, reporting that MEG3 suppresses glycolysis by promoting the ubiquitin-dependent degradation of c-Myc, a key regulator of glycolytic genes. Overexpression of MEG3 significantly reduces glycolysis, glycolytic capacity and lactate production in CRC cells; conversely, MEG3 knockdown produces the opposite result. Additionally, MEG3 activation by vitamin D suggests a potential therapeutic value in CRC treatment through glycolysis modulation. Similarly, Zhu *et al* (14) reported that microRNA (miR)-146b-5p enhances cell proliferation, glycolysis and invasiveness in CRC by specifically targeting the pyruvate dehydrogenase E1 subunit b. Overexpression of miR-146b-5p enhanced these processes, while knockdown inhibited them, underscoring its oncogenic role. Zhu *et al* (15) identified five glycolysis-related genes (enolase 3, glypican 1, prolyl 4-hydroxylase subunit 1, sperm associated antigen 4 and stanniocalcin 2) that form a prognostic model, highlighting the significant influence of aerobic glycolysis on CRC development. These studies underscore the critical role of glycolysis and its potential for CRC therapy, offering valuable insights into tumor metabolism and potential therapeutic strategies.

Despite the established role of glycolysis in CRC and the known tumor-suppressive function of *LGALS4*, the precise mechanisms by which *LGALS4* regulates glycolysis and its potential as a therapeutic target in CRC remain understudied.

The present study aimed to address these gaps by investigating the role of *LGALS4* in modulating glycolysis and its interplay with the β -catenin signaling pathway in CRC cells. These findings introduced a novel perspective for CRC treatment, highlighting the potential of *LGALS4* as a therapeutic target and prognostic indicator, which has not been extensively reported in previous research.

Materials and methods

Data acquisition and analysis of differentially expressed genes (DEGs). COAD samples from the TCGA were obtained via the Clinical Bioinformatics Assistant website (<https://www.aclbi.com/static/index.html#/>). The GSE26571CRC microarray dataset was obtained from the GEO database (<https://www.ncbi.nlm.nih.gov/gds/>). The TCGA-COAD dataset comprised 455 tumor samples and 41 normal control samples, while the GSE26571 dataset included 12 CRC samples and 5 control samples. Probe IDs were transformed into gene symbols and differential expression analysis was employed using the 'Limma' package (version 3.46.0) in R (version 4.1.2; Posit Software). According to previous literature, genes with a fold change (FC) >2 were identified as upregulated DEGs and those with an FC <0.5 as downregulated DEGs (16,17).

Identification and enrichment analysis of overlapping DEGs. The overlapping DEGs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using the Database for Annotation, Visualization and Integrated Discovery. The GO analysis included the biological process (BP), molecular function (MF) and cellular component (CC) categories to comprehensively explore the functional roles of the identified overlapping DEGs.

Construction of prognostic risk model for overlapping DEGs. The Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression model was used to identify genes associated with patient prognosis. The λ parameter in the LASSO model was used to adjust the strength of selection of the variables in the model to prevent overfitting. Smaller values of λ , which allowed more variables to enter the model but may lead to excessive model complexity and larger values of λ , which increased the penalization strength and retained only the most significant variables, simplified the model structure. The optimal λ value, $\lambda_{\min}=0.0382$ was determined, through 10-fold cross-validation to ensure that the model achieved the best balance between predictive accuracy and simplicity. This process of optimizing λ enabled the model to select only those genes with the strongest associations with patient survival outcomes, which included ribosomal protein S17 (*RPS17*), G2/mitotic-specific Cyclin B1 (*CCNB1*), karyopherin subunit $\alpha 2$ (*KPNA2*), TNF receptor associated protein 1 (*TRAP1*), complement C8 chain (*C8G*), *LGALS4*, lectin galactoside-binding soluble-2 (*LGALS2*), carbonic anhydrase 4 (*CA4*), and solute carrier family 6 member 8 (*SLC6A8*). The risk score was calculated using the following formula: Risk score = $\sum(\text{coefficient} \times \text{gene expression})$. Where the coefficient represented the coefficient of each gene in the LASSO model and gene expression was the expression level of the corresponding gene in the sample. The risk score showed the effect of each gene on patient prognosis, with positive

coefficients indicating positive correlation and negative coefficients indicating negative correlation. A model capable of assessing the prognostic risk of patients was constructed, with high risk scores predicting poorer prognostic outcomes. The score of risk for each sample was calculated according to the following formula: Risk score = $(0.1219) \times RPS17 + (-0.0582) \times CCNB1 + (-0.0442) \times KPNA2 + (-0.0137) \times TRAP1 + (0.0885) \times C8G + (-0.1236) \times LGALS4 + (-0.009) \times LGALS2 + (-0.0063) \times CA4 + (0.0012) \times SLC6A8$. The COAD cohort from the TCGA database were stratified into low- and high-risk groups according to the expression patterns of the aforementioned genes. Kaplan-Meier (KM) analysis was employed to ascertain the overall survival (OS) probability for the two risk groups. The median survival time was calculated and the statistical significance of survival differences between the groups was evaluated using the log-rank test. To clarify relative risk, hazard ratios (HRs) were calculated for the high-risk group. Additionally, Receiver Operating Characteristic (ROC) curves were generated using the timeROC package (<https://CRAN.R-project.org/package=timeROC>) and the Area Under the Curve (AUC) values were calculated to evaluate the predictive ability of the prognostic models for patient survival at 1, 3 and 5 years. Higher AUC values indicated stronger prognostic prediction capabilities.

Construction of prognostic nomogram and expression analysis of prognostic significant genes. Univariate and multivariate Cox regression analyses were performed on signature genes and specific clinical predictive variables, such as patient age and sex, using the 'forestplot' package (version 2.0.1; <https://cran.r-project.org/web/packages/forestplot/vignettes/forestplot.html>). For each variable, the HRs, 95% CIs and P-values were calculated. Key prognostic factors were identified based on variables with $P < 0.05$. Nomograms were created to predict the 1-, 3- and 5-year survival probabilities using the rms software (version 3.6.1; <https://cran.r-project.org/web/packages/rms/index.html>; provided by R Foundation for Statistical Computing) (18). The consistency index (C-index) was determined to assess the prediction accuracy of the model. The performance of the model was then assessed by predicting survival curves for 1, 3 and 5 years and by generating a calibration curve to evaluate the accuracy of these predictions. The expression levels of *C8G*, *LGALS4* and *RPS17* in different samples were examined in the TCGA-COAD and GSE26571 datasets using the SangerBox platform (version 3.0; <http://vip.sangerbox.com/home.html>).

Cell lines and culture. Based on previous literature, the LoVo, HCT-116 and SW480 cell lines were selected and NCM460 cells were used as the control cell line (19-21). LoVo, HCT-116 and SW480 cells were obtained from the Cell Bank of the Chinese Academy of Sciences. NCM460 cells were purchased from Jennio Biotech Co., Ltd. All cells were cultured in DMEM, 1% penicillin-streptomycin and 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). Cells were maintained at 37°C with 5% CO₂ and 20% O₂.

Vector construction and transfection. Total RNA was isolated from NCM460 cells using the TRIzol® kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

cDNA was synthesized using the PrimeScript RT kit (Takara Bio Inc.). cDNA was amplified using the CloneAmp™ HiFi PCR Premix (Takara Bio, Inc.) to amplify the corresponding *LGALS4* fragment, which was cloned into the pcDNA™3.1⁽⁺⁾ vector (Thermo Fisher Scientific, Inc.). The correct insertion of the gene was verified by DNA sequencing and the recombinant plasmid was transformed and amplified in *Escherichia coli* to prepare plasmid DNA for cell transfection. For transfection, 1 µg plasmid DNA was used per well, and transfection was performed at 37°C for 24 h. The *LGALS4* overexpression plasmid was introduced into LoVo and HCT-116 cells using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) as the transfection reagent, and cells were incubated for 48 h post-transfection before further experimentation. Cells stably expressing *LGALS4* were screened by resistance. The selection method involved puromycin at a concentration of 1 µg/ml for selection and 0.5 µg/ml puromycin for maintenance. The amplification primer sequences used were as follows: Forward (F), 5'-CTCGAGATGGCCTATGTCCCC-3' and reverse (R), 5'-TCTAGATTAGATCTGGACATAGGA CAAGG-3'.

Cell treatment. CRC cells were treated with various agents to investigate their effects on glucose metabolism and cell viability. Glucose was administered at concentrations of 10.0, 1.0 and 0.5 mM. To inhibit glucose transport and hexokinase activity, cells were treated with cytochalasin B (Cyto-B) at 20 µM and 3-bromopyruvate (3-BrPA) at 10 µg/ml. Additionally, the 5-fluorouracil (5-FU) anticancer drug was used at 50 µg/ml as an and XAV-939, a β-catenin inhibitor, was applied at 10 µM to examine its impact on CRC cells. All treatments were administered for 48 h.

Reverse transcription-quantitative PCR (RT-qPCR). TRNzol reagent (cat. no. DP424; Tiangen Biotech Co., Ltd.) was used to extract the total RNA from CRC cells following the manufacturer's instructions. cDNA synthesis was performed using a PrimeScript RT kit (Takara Bio Inc.). RT-qPCR was conducted on the StepOnePlus Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR Green PCR Master Mix (Takara Biotechnology Co., Ltd.). The thermocycling conditions were as follows: Initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. Gene expression levels were quantified, normalized to GAPDH and calculated using the 2^{-ΔΔC_q} method (22). Table I lists the primer sequences for the genes investigated in the present study.

Western blot (WB) assay. Protease and phosphatase inhibitors (CoWin Biosciences) were added to RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd) to facilitate the preparation of protein lysates from CRC cells. The BCA Protein Assay Kit (Beyotime Institute of Biotechnology) was used to measure the protein concentration. Proteins in equal quantities (20 µg/lane) were separated using 10% SDS-PAGE and then transferred onto PVDF membranes (Beyotime Institute of Biotechnology). The membranes were blocked with 5% skim milk at room temperature for 1 h. After blocking, the membranes were washed three times with

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Sequence (5'-3')
<i>LGALS4</i>	F: CCGGACATTGCCATCAACAG R: CAAAGCTCTTGCCTGTGGGA
<i>BAX</i>	F: GAGCTAGGGTCAGAGGGTCA R: CCCCATTTCATCTACCCTGC
<i>BCL2</i>	F: ACCTACCCAGCCTCCGTTAT R: GAACTGGGGGAGGATTGTGG
<i>CASP3</i>	F: GCTGGATGCCGTCTAGAGTC R: ATGTGTGGATGATGCTGCCA
<i>CASP9</i>	F: ATTGCACAGCACGTTTCACAC R: TATCCCATCCCAGGAAGGCA
<i>CTNNB1</i>	F: AAAGCGGCTGTTAGTCACTGG R: CGAGTCATTGCATACTGTCCAT
<i>MYC</i>	F: CACCACCAGCAGCGACTCT R: GGCACCTCTTGAGGACCAGT
<i>PKM</i>	F: GACTGCCTTCATTACAGACCCA R: GGGTGGTGAATCAATGTCCAG
<i>SLC2A1</i>	F: ATGCGGGAGAAGAAGGTCAC R: GTTGACGATACCGGAGCCAA
<i>LDHA</i>	F: GGCCTGTGCCATCAGTATCT R: GGAGATCCATCATCTCTCCC
<i>HK2</i>	F: CCTGTGAATCGGAGAGGTCC R: ATTTTGGCGTCACAACGTCT
<i>GAPDH</i>	F: ACCACAGTCCATGCCATCAC R: TCCACCACCCTGTTGCTGTA

F, forward; R, reverse; *LGALS4*, lectin galactoside-binding soluble 4; *CTNNB1*, catenin beta 1; *MYC*, Myc proto-oncogene; *PKM*, pyruvate kinase M1/M2; *SLC2A1*, solute carrier family 2 member 1; *LDHA*, lactate dehydrogenase A; *HK2*, hexokinase 2.

Tris-buffered saline containing 0.1% Tween-20. They were then incubated with primary antibodies against *LGALS4* (1:1,000; cat. no. ab175185), Cyclin dependent kinase 1 (CDK1) (1:1,000; cat. no. ab265590), Cyclin A2 (1:1,000; cat. no. ab227277), Cyclin B1 (1:2,000; cat. no. ab181593), Bax (1:1,000; cat. no. ab32503), Bcl-2 (1:1,000; cat. no. ab194583), caspase-9 (1:1,000; cat. no. ab32539), caspase-3 (1:1,000; cat. no. ab90437), β -catenin (1:1,000; cat. no. ab227499), c-myc (1:1,000; cat. no. ab32072), solute carrier family 2 member 1 (GLUT1) (1:5,000; cat. no. ab195021), pyruvate kinase M2 (PKM2) (1:1,000; cat. no. ab154816), lactate dehydrogenase A (LDHA) (1:1,000; cat. no. ab300637) and hexokinase 2 (HK2) (1:1,000; cat. no. ab209847) (all from Abcam) overnight at 4°C. After rinsing, the membranes were incubated with secondary antibodies conjugated with HRP (1:5,000; cat. no. D110011-0100; Sangon Biotech Co., Ltd) for 1 h at room temperature. GAPDH (1:5,000; cat. no. KC-5G4; Aksamies Inc.) was used as an internal reference. Protein bands were visualized using an ECL kit (Tiangen Biotech Co., Ltd.) and imaged using a ChemiDoc system (Bio-Rad Laboratories, Inc.). Densitometric analysis was performed

using ImageJ software (version 2.0.0; National Institutes of Health).

Cell counting kit-8 (CCK-8) assay. The CCK-8 assay kit (cat. no. KGA317s; Nanjing KeyGen Biotech Co., Ltd) was used to evaluate cell viability. CRC cells were seeded in 96-well plates at a density of 5×10^3 cells/well. Following transfection with either the vector control or *LGALS4* overexpression plasmid, CCK-8 reagent was added to each well and incubated for 2 h at 37°C. A microplate reader (Shanghai Kehua Bio-Engineering Co., Ltd.) was used to measure the absorbance of each sample at 450 nm after 0, 1, 2, 3, 4 and 5 days.

Cell invasion and migration assays. Transwell assays were used to assess cell invasion and migration. Transfected CRC cells (5×10^4 cells) were suspended in serum-free DMEM in the upper chamber of the plate. Subsequently, DMEM supplemented with 10% FBS was added to the lower chamber of the plate. Cells were incubated at 37°C in a 5% CO₂ incubator for 24 h to allow migration. After the incubation period, cells that had migrated to the underside of the Transwell membrane were fixed using 4% paraformaldehyde at room temperature for 15 min. Cells were then stained using DAPI for 10 min at room temperature to visualize their nuclei. Finally, the number of migrating cells in the field of view were visualized using an inverted fluorescence microscope. For the cell invasion experiments, the top chamber of the Transwell was coated with Matrigel (BD Biosciences) at 37°C for 20 min, as previously reported (23).

Flow cytometry. For the assessment of cellular phenotypes via flow cytometry, CRC cells were enzymatically dissociated using 0.25% trypsin-EDTA (Thermo Fisher Scientific, Inc.) at 37°C for 5 min to obtain a single-cell suspension. The cells were then lysed with 100 μ l ice-cold RIPA buffer (containing protease and phosphatase inhibitors) on ice for 30 min at 4°C to ensure complete cell lysis. The cells were then subjected to dual staining with propidium iodide (PI) for DNA content analysis and annexin V conjugated to FITC (BD Biosciences) for the detection of apoptotic cells, following the manufacturer's recommended protocols. Staining was performed at room temperature for 15 min in the dark. This procedure allowed for the discrimination of viable, early apoptotic and necrotic cells based on their distinct fluorescence characteristics. The PI staining also facilitated the evaluation of the cell cycle distribution by reflecting the DNA content at various stages (24-26). For cell cycle analysis, cells were also treated with RNase A (10 μ g/ml) for 15 min at 37°C to ensure accurate DNA content analysis. The stained cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, Inc.) and the resulting data were processed with FlowJo software (version 10.6.0; FlowJo LLC) to quantify the apoptotic rate and to determine the cell cycle phase distribution. The percentage of cells in the G1, S and G2 phases were calculated and statistically compared across different experimental conditions [transfection with either *LGALS4* overexpression plasmid or vector control, followed by treatment with 5-FU (5.0 μ M) or 0.5 mM glucose] to assess the modulation of cell cycle progression by the treatments administered.

Glucose uptake assay. 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG; cat. no. 186689-07-6; Anjiekai Biological Medicine Technology) was used as a glucose tracer. Cells were seeded at 1×10^5 cells/well in 6-well plates in four replicates and incubated overnight at 37°C and 5% CO₂. The following day, cells were subjected to glucose starvation for 4 h. Then, one well was treated with DMEM containing glucose as a negative control, while the other wells were incubated with 2-NBDG for 2 h at 37°C. After the incubation, cells were collected by trypsinization, followed by centrifugation at 4°C for 5 min at 300 x g, and rinsed twice with PBS. Subsequently, flow cytometry was performed using a CytoFLEX flow cytometer). The geometric mean fluorescence intensity of the cells was measured, with emission at 530 nm and excitation at 488 nm. Data analysis was conducted using FlowJo software, version 10.6.0.

Colony formation assay. A colony formation assay was used to assess the colony formation capacity of cells. Cells were seeded at a concentration of 2×10^3 in 60 mm plates and cultured for 2 weeks at 37°C with 5% CO₂, under varying glucose concentrations (10.0, 1.0 and 0.5 mM). After fixation in methanol at room temperature for 30 min, cells were stained using alkali nitro tetrazolium blue chloride at room temperature for 20 min. A chemiluminescence imager (Clinx) was used to image the cells, and the colonies were counted manually. Colonies were defined as cell clusters containing ≥ 50 cells. Each experiment was performed thrice to ensure accuracy.

Lactate and ATP assays. To measure lactate production, 1×10^5 cells/well were seeded in 24-well plates and cultured at 37°C with 5% CO₂ for 24 h. This process was repeated three times. For ATP production measurement, 1×10^5 cells/well were seeded in 96-well plates and incubated at 37°C with 5% CO₂ for 24 h in five replicates. Subsequently, the medium in both assays was replaced with DMEM containing 1 mM glucose and the cells were incubated overnight at 37°C with 5% CO₂. The following day, lactate in the culture media was measured using a commercial lactate assay kit (cat. no. A109-2; Nanjing Jiancheng Bioengineering Institute). The relative ATP concentration was also measured using a commercial ATP assay kit (cat. no. S0026; Beyotime Institute of Biotechnology). Both kits were used according to the manufacturers' instructions.

Statistical analysis. Statistical analysis was conducted using R software. Each experiment was carried out in triplicate and the results were presented as the mean $\pm$ SD. For survival curve analysis, Kaplan-Meier analysis was used to evaluate the overall survival, and the statistical significance between survival curves was assessed using the log-rank test. Differences between groups were evaluated using a one-way ANOVA when there were more than two experimental groups, followed by post-hoc analysis with Tukey's test. When comparing only two groups, an unpaired Student's t-test was applied $P < 0.05$ was used to indicate a statistically significant difference.

Results

Identification and enrichment analysis of overlapping DEGs in TCGA-COAD and GSE26571 datasets. A total of 1,171 downregulated DEGs and 1,521 upregulated DEGs were

identified in COAD samples compared with normal samples in the TCGA database (Fig. 1A). From the GSE26571 dataset, 356 upregulated DEGs and 338 downregulated DEGs were identified in colon cancer samples compared with normal samples (Fig. 1B). Subsequently, using bioinformatics platforms, a cross-analysis of the upregulated and downregulated DEGs from the TCGA-COAD dataset and the GSE26571 dataset identified a total of 175 overlapping DEGs (Fig. 1C and D). The enrichment analysis demonstrated significant enrichment in terms related to 'DNA replication' (GO: 0006260, BP), 'response to zinc ion' (GO: 0010043, BP), 'CMG complex' (GO: 0071162, CC), 'small ribosomal subunit' (GO: 0015935, CC), 'RNA binding' (GO: 0003723, MF) and 'cadherin binding' (GO: 0045296, MF) (Fig. 1E), as well as pathways involved in the 'cell cycle', 'DNA replication' and 'ribosome' (Fig. 1F).

Prognostic analysis of nine signature genes in the risk model. A total of 175 overlapping DEGs (Table SI) were analyzed using the LASSO Cox regression method. By employing cross-validation to identify the optimal lambda value of 0.0382, nine genes (Table SII) were identified that served as significant predictors of patient outcomes (Fig. 2A and B). These genes, identified through their ability to discriminate between high-risk and low-risk groups, were characterized by their expression patterns that strongly associated with survival rates. The high-risk group, as determined by the risk model, exhibited increased mortality and diminished overall survival compared with the low-risk group, a finding further supported by the Kaplan-Meier survival analysis (Fig. 2C). A median survival time of 5 years was identified for both groups, with a HR of 2.537, indicating a pronounced impact on survival probability (Fig. 2D). Moreover, the model's predictive accuracy was validated by the ROC curve, which demonstrated an area under the curve of 0.696 at the 5 year mark, signifying a reliable prediction of survival outcomes. This selection process and subsequent analysis underscored the robustness of the risk model and suggested a potential role for the identified genes in CRC prognosis.

Nomogram analysis of key prognostic variables and screening of hub genes. A total of three factors were identified as statistically significant after analyzing 9 genes and 2 clinical variables in the risk model: *C8G*, *LGALS4* and *RPS17* (Fig. 3A and B). Using these insights, a predictive model with a C-index of 0.634 was constructed (Fig. 3C). The calibration curve showed the highest consistency with model predictions at 1 year, followed by 3 and 5 years, indicating that these variables have predictive power for patient survival (Fig. 3D). The expression of the three prognostic significant genes was assessed in both the TCGA-COAD and GSE26571 datasets. These findings indicated that *C8G* was significantly under-expressed in tumor samples in the TCGA-COAD dataset, but was not significantly expressed in the GSE26571 dataset. *LGALS4* was notably under-expressed in the tumor samples of both datasets, while *RPS17* was significantly over-expressed (Fig. 3E and F). A previous study reported that *LGALS4* is a potential prognostic factor in CRC patients, but its role in CRC glycolysis remains unclear (27). Therefore, *LGALS4* was selected as a hub gene for further investigation.

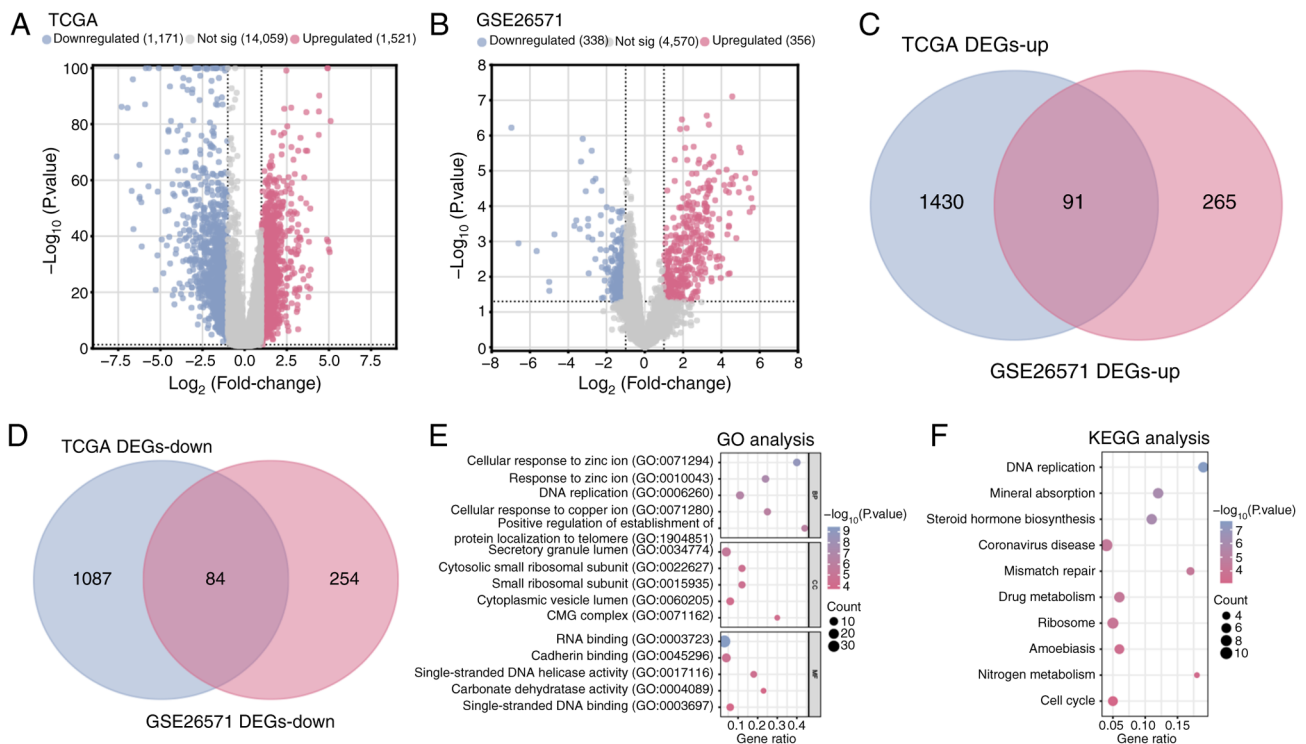


Figure 1. Identification and enrichment analysis of overlapping DEGs in TCGA and GSE26571 datasets. Volcano plots for screening DEGs in the (A) TCGA-COAD and (B) GSE26571 datasets. Venn diagrams showing the cross-analysis of upregulated and downregulated DEGs in the (C) TCGA-COAD and (D) GSE26571 datasets. (E) GO and (F) KEGG enrichment analysis of the overlapping DEGs. DEGs, differentially expressed genes; TCGA, The Cancer Genome Atlas; COAD, colon adenocarcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; sig, significant; up, upregulated; down, downregulated.

Overexpression of LGALS4 inhibited the proliferation, migration and invasion of CRC cells. The expression of *LGALS4* was assessed in normal cells (NCM460) and CRC cells (LoVo, HCT-116 and SW480) using RT-qPCR and WB. These results demonstrated that *LGALS4* was significantly under-expressed in LoVo and HCT-116 cells compared with normal cells (Fig. 4A-C). Therefore, these two cell lines were selected for further experiments. The RT-qPCR and WB analyses showed efficient overexpression of *LGALS4* in LoVo and HCT-116 cells (Fig. 4D-F). Based on the results of the CCK-8 assay, on day 5 of cell culture, a significant decrease in cell viability of cells overexpressing *LGALS4* was observed in the LoVo cell line, which was ~25% compared with the control cells. For the HCT-116 cell line, the cell viability of cells overexpressing *LGALS4* also showed a significant decrease to ~50% compared with the control (Fig. 4G-H). These results suggested that *LGALS4* may inhibit cell proliferation by modulating the expression of cell cycle-related proteins or their activities, which in turn inhibits cell proliferation. The Transwell assays demonstrated that the invasive and migratory abilities of LoVo cell lines were significantly decreased upon *LGALS4* overexpression compared with control cells (Fig. 4I-L). Specifically, the invasion capacity was decreased 4-fold and the migration capacity was decreased 2.5-fold compared with the control. A similar effect was demonstrated in the HCT-116 cell line, in which the invasive capacity was reduced by 3-fold and the migratory capacity by 2-fold compared with control cells. This effect may stem from the effects of *LGALS4* on cell adhesion, reorganization of the cytoskeleton or degradation of the

extracellular matrix, which are all critical aspects of the cell migration and invasion process (28).

LGALS4 overexpression induces cell cycle arrest in CRC cells. To explore the effect of *LGALS4* overexpression on the cell cycle progression of CRC cells, flow cytometric analysis was performed on LoVo and HCT-116 cells that were transfected with either an *LGALS4* overexpression vector or a control vector. These results demonstrated a significant effect of overexpression of *LGALS4* on the cell cycle distribution of LoVo and HCT-116 cells. Compared with control cells, the proportion of cells overexpressing *LGALS4* was significantly higher in the G1 phase and significantly lower in the S phase. In LoVo cells, overexpression of *LGALS4* resulted in a ~1.5-fold increase in the proportion of cells in G1 phase and a ~10-fold decrease in the proportion of cells in S phase compared with the control cells. For HCT-116 cells, overexpression of *LGALS4* resulted in a ~1.8-fold increase in the proportion of G1-phase cells and a ~15-fold decrease in the proportion of S-phase cells compared with control cells (Fig. 5A-D). The expression levels of cell cycle-related proteins Cyclin B1, CDK1 and Cyclin A2 were further analyzed as these proteins serve a key role in driving the cell cycle transition from G1 to S phase (29). These results demonstrated that *LGALS4* overexpression significantly decreased the protein expression levels of CDK1, Cyclin B1 and Cyclin A2 in CRC cells compared with the control group (Fig. 5E-G). These results suggested that *LGALS4* overexpression may cause a G1 phase cell cycle arrest by downregulating the levels of cell cycle regulatory proteins.

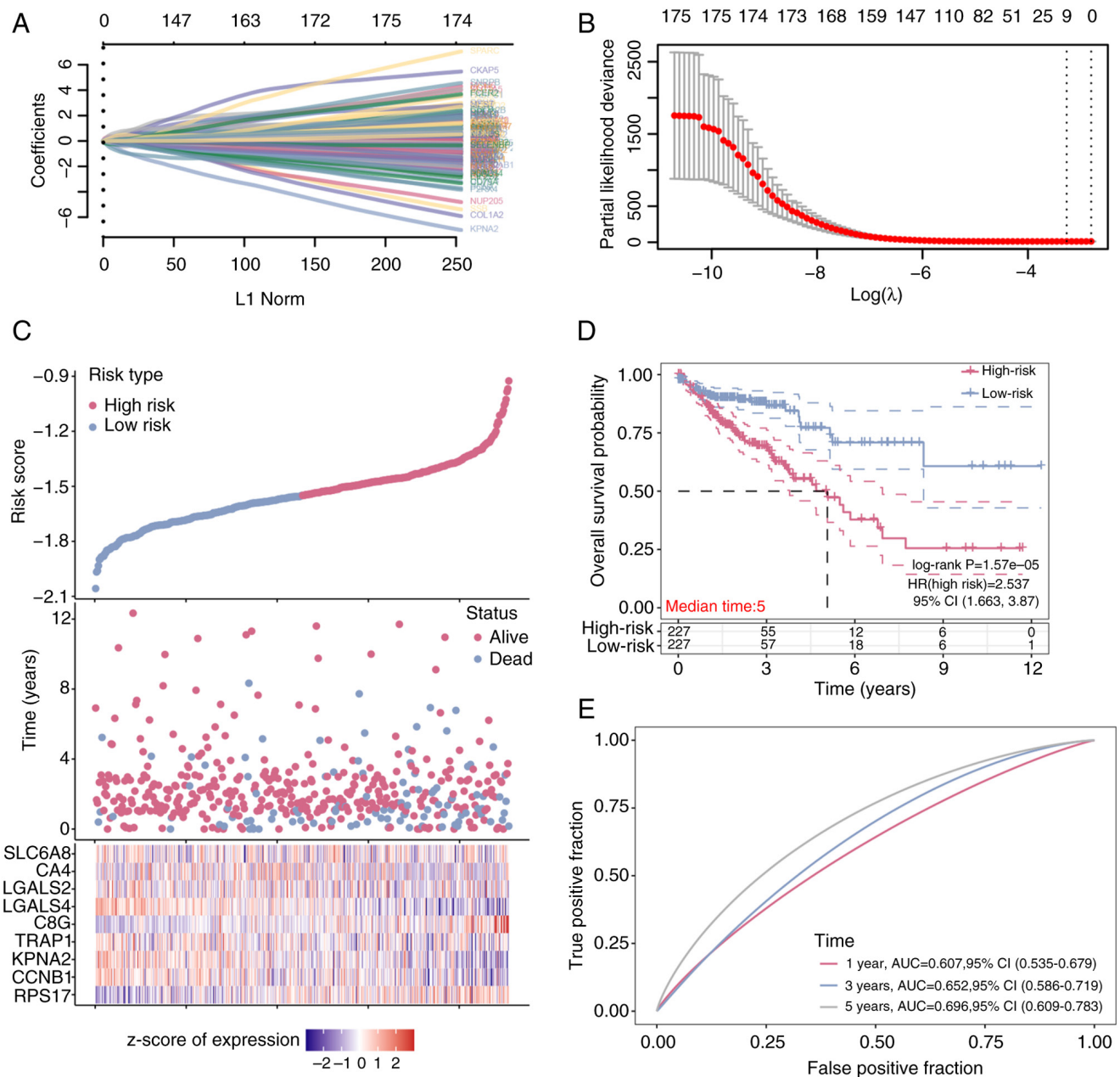


Figure 2. Prognostic analysis of nine signature genes in overlapping DEGs. (A) Least Absolute Shrinkage and Selection Operator-Cox regression model analysis on 175 overlapping differentially expressed genes. The absolute values of the coefficients for the different genes were plotted against their corresponding L1 regularization norm values. (B) The relationship between 10-fold cross-validation partial likelihood deviation and $\log(\lambda)$. (C) Risk model analysis of the selected sample data. (D) Kaplan-Meier survival curve analysis of the high-risk group and the low-risk group in the risk model (E) Receiver operating characteristic curve analysis of the risk model at 1, 3 and 5 years post-treatment. AUC, area under the curve; *RPS17*, ribosomal protein S17; *CCNB1*, G2/mitotic-specific Cyclin-B1; *KPNA2*, karyopherin subunit 2; *TRAP1*, TNF receptor associated protein 1; *C8G*, complement C8 chain; *LGALS*, lectin galactoside-binding soluble 4; *CA4*, carbonic anhydrase 4; *SLC6A8*, solute carrier family 6 member 8.

Overexpression of *LGALS4* promoted CRC cell apoptosis. Flow cytometry was used to analyze the effect of *LGALS4* overexpression on the apoptosis of LoVo and HCT-116 cells. These results demonstrated that the apoptosis rate of CRC overexpressing *LGALS4* was significantly increased by ~2.5-fold compared with control cells (Fig. 6A-C). RT-qPCR results showed that *LGALS4* overexpression significantly increased the expression levels of *CASP3*, *BAX* and *CASP9* and decreased the expression level of *BCL2* (Fig. 6D and E). These results were also confirmed by WB assay results (Fig. 6F-H).

Overexpression of *LGALS4* inhibited aerobic glycolysis in CRC. Cancer cells typically rely on higher glucose concentrations to maintain their rapid glycolytic processes. By depriving glucose, the nutrient-limited conditions in the tumor microenvironment can be mimicked and the adaptation and survival of cancer cells to stressful conditions can be studied (30). Colony formation assays delineated the glucose dependency of *LGALS4*-overexpressing cells, demonstrating an inherent reliance on exogenous glucose for optimal proliferation. Compared with control cells, *LGALS4* overexpressing cells showed enhanced survival under glucose-scarce conditions,

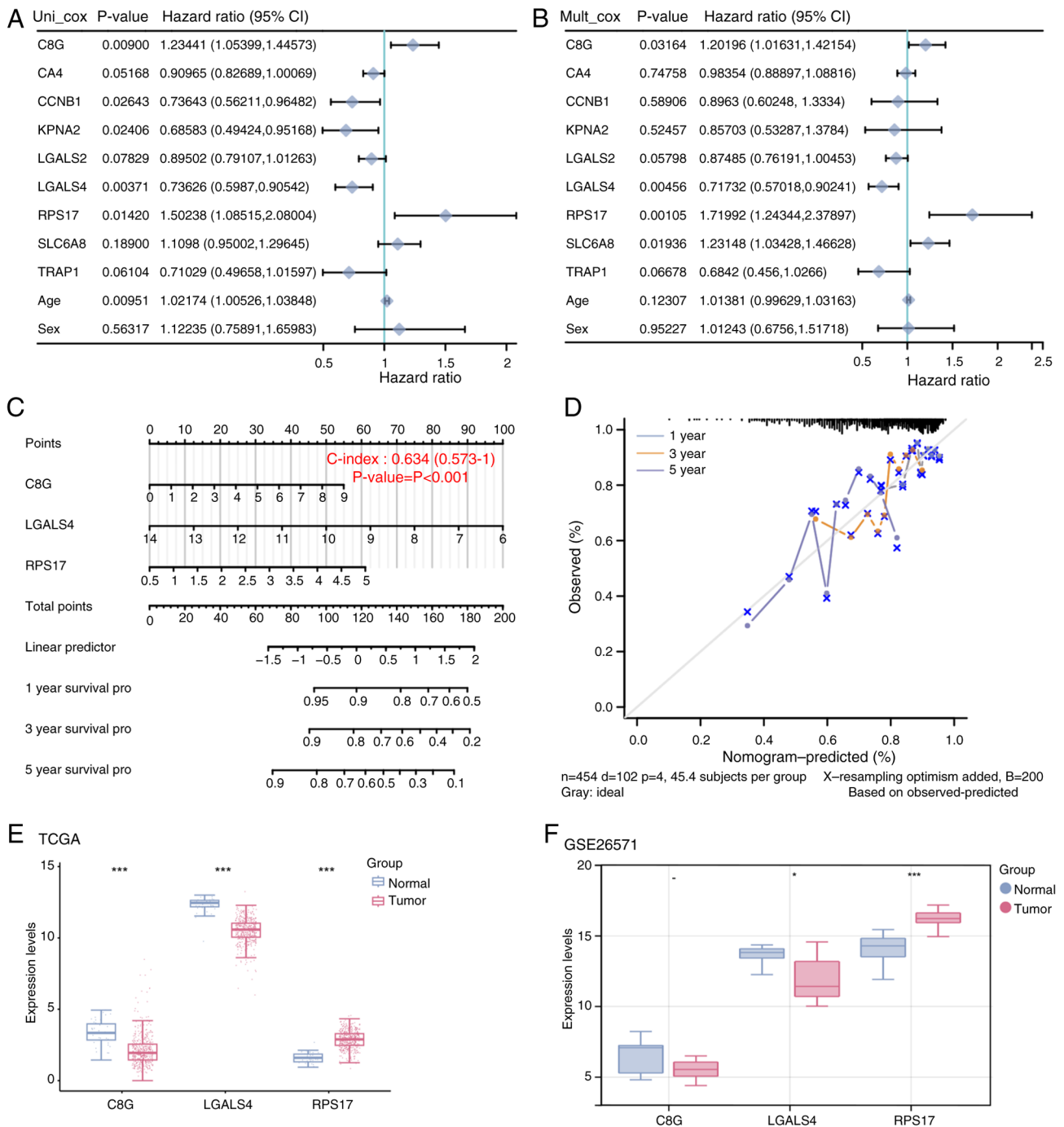


Figure 3. Nomogram analysis of key prognostic variables and screening of hub genes. (A) Univariate and (B) multifactorial Cox regression analysis of signature genes and clinical prognostic variables. (C) Nomograms of key prognostic variables, along with the C-index, were used to evaluate the model's predictive power. A C-index close to 1 indicated high predictive accuracy, while a C-index of 0.5 indicated no predictive power. (D) The dashed lines represented the ideal calibration curves of the nomogram Expression levels of *C8G*, *LGALS4* and *RPS17* in the (E) TCGA-colon adenocarcinoma and (F) GSE26571 datasets. Purple represented normal samples and red represented tumor samples. *P<0.05, ***P<0.001 vs. normal group. TCGA, The Cancer Genome Atlas; pro, probability.

particularly at a glucose concentration of 0.5 mM, suggesting that they may have acquired metabolic adaptations to the glucose-deficient microscopic environment (Fig. 7A-D). Subsequent flow cytometric analysis, utilizing Annexin V-FITC staining, corroborated the diminished apoptotic propensity of *LGALS4*-overexpressing CRC cells subjected to glucose deprivation at 0.5 mM, underscoring their enhanced survival kinetics relative to control cell populations (Fig. 7E-G). Cyto-B and 3-BrPA target the initiation steps

of the glycolytic pathway such as glucose uptake and the first phosphorylation reaction, respectively (31). The use of these inhibitors could aid in the understanding of the metabolic adaptations of *LGALS4* overexpressing cells when the glycolytic pathway is inhibited. Targeted inhibition of glucose transport and hexokinase activity was performed using Cyto-B at a concentration of 20 μ M and 3-BrPA at a concentration of 10 μ g/ml, respectively. The cytotoxic effects of these inhibitors on *LGALS4*-overexpressing cells were ascertained

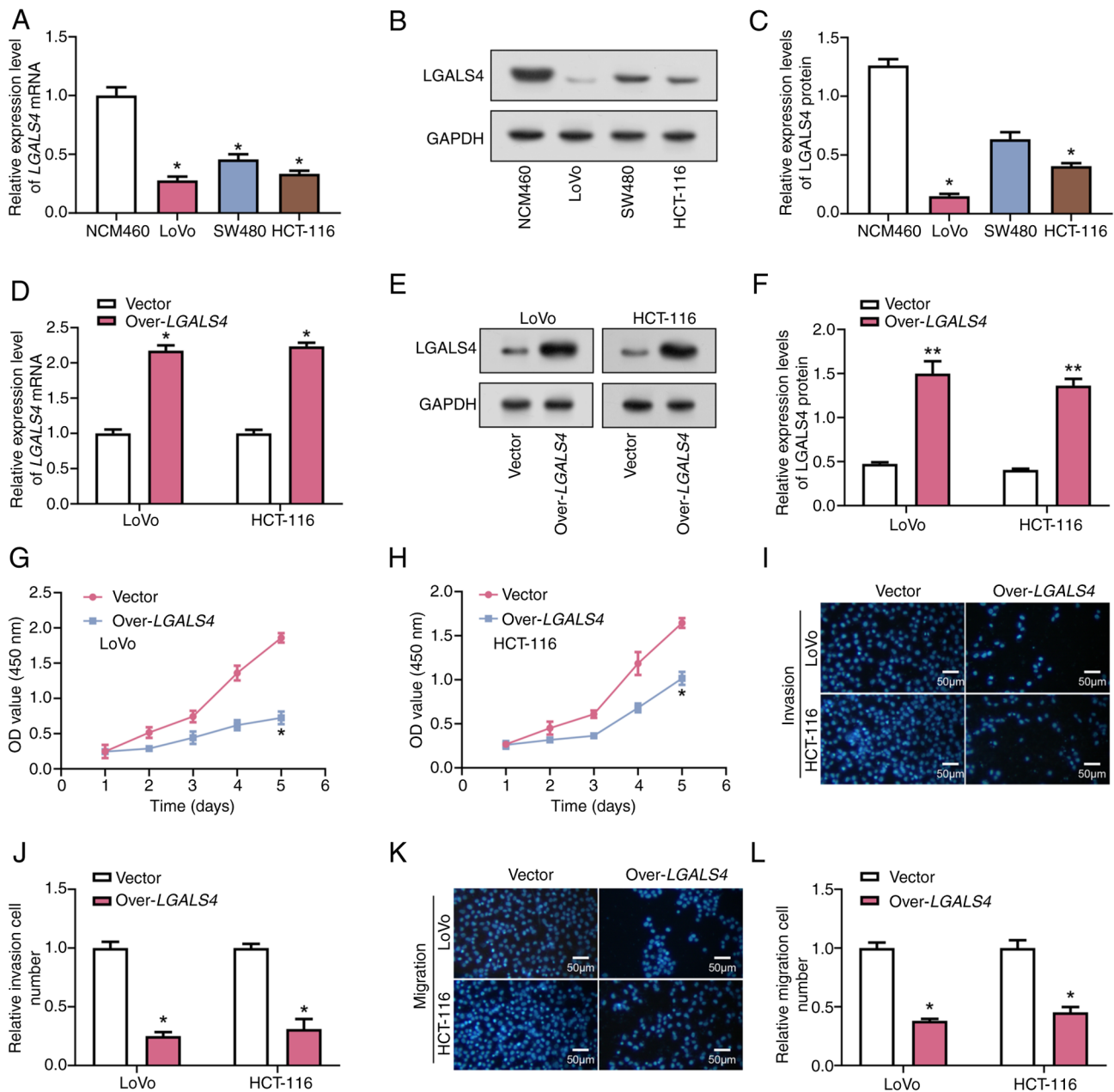


Figure 4. Overexpression of *LGALS4* inhibits the proliferation, migration and invasion of CRC cells. (A) RT-qPCR was used to detect the expression levels of *LGALS4* in normal cells (NCM460) and CRC cells (LoVo, HCT-116 and SW480). (B) WB analysis was performed to detect the protein expression levels of *LGALS4* in normal cells (NCM460) and CRC cells (LoVo, HCT-116, SW480). (C) Semi-quantification of the WB analysis showing the protein expression levels of *LGALS4* in normal cells (NCM460) and CRC cells (LoVo, HCT-116, SW480). * $P < 0.05$ vs. NCM460. (D) RT-qPCR was used to assess the overexpression efficiency of *LGALS4* in LoVo and HCT-116 cells. (E) WB analysis further confirmed the overexpression efficiency of *LGALS4* in LoVo and HCT-116 cells. (F) Semi-quantification of the WB analysis further confirming the overexpression efficiency of *LGALS4* in LoVo and HCT-116 cells. The Cell Counting Kit-8 assay was used to detect the effect of *LGALS4* overexpression on the viability of (G) LoVo and (H) HCT-116 cells. The Transwell assay was used to detect the effect of *LGALS4* overexpression on the (I and J) invasion and (K and L) migration capacities of CRC cells. * $P < 0.05$, ** $P < 0.01$ vs. Vector. RT-qPCR, reverse transcription-quantitative PCR; WB, western blot; *LGALS4*, lectin galactoside-binding soluble 4; over, overexpression; CRC, colorectal cancer.

through CCK-8 assays following a 48 h incubation. These results demonstrated a preservation of cell viability among *LGALS4*-overexpressing cells, compared with control cells, suggesting the potential presence of a recalibrated metabolic phenotype conferring resistance to glycolytic inhibition (Fig. 7H and I). The increased survival of cyto-B-treated control cells may be due to the fact that the inhibitory effect of cyto-B on glucose transport did not completely block the energy supply of the cells, and the cells may be sustained by other metabolic pathways such as fat oxidation or amino acid

metabolism. In addition, the concentration of cyto-B may not be sufficient to completely inhibit glucose uptake, or the cells may be somewhat adapted to cyto-B treatment.

LGALS4 overexpression enhanced 5-FU-induced apoptosis and inhibited glucose metabolism in CRC cells. 5-FU is an anticancer drug used to treat various types of cancer, including CRC. It typically inhibits DNA synthesis by interfering with the biosynthesis of pyrimidine nucleotides (32). Flow cytometry analysis showed that overexpression of *LGALS4* significantly

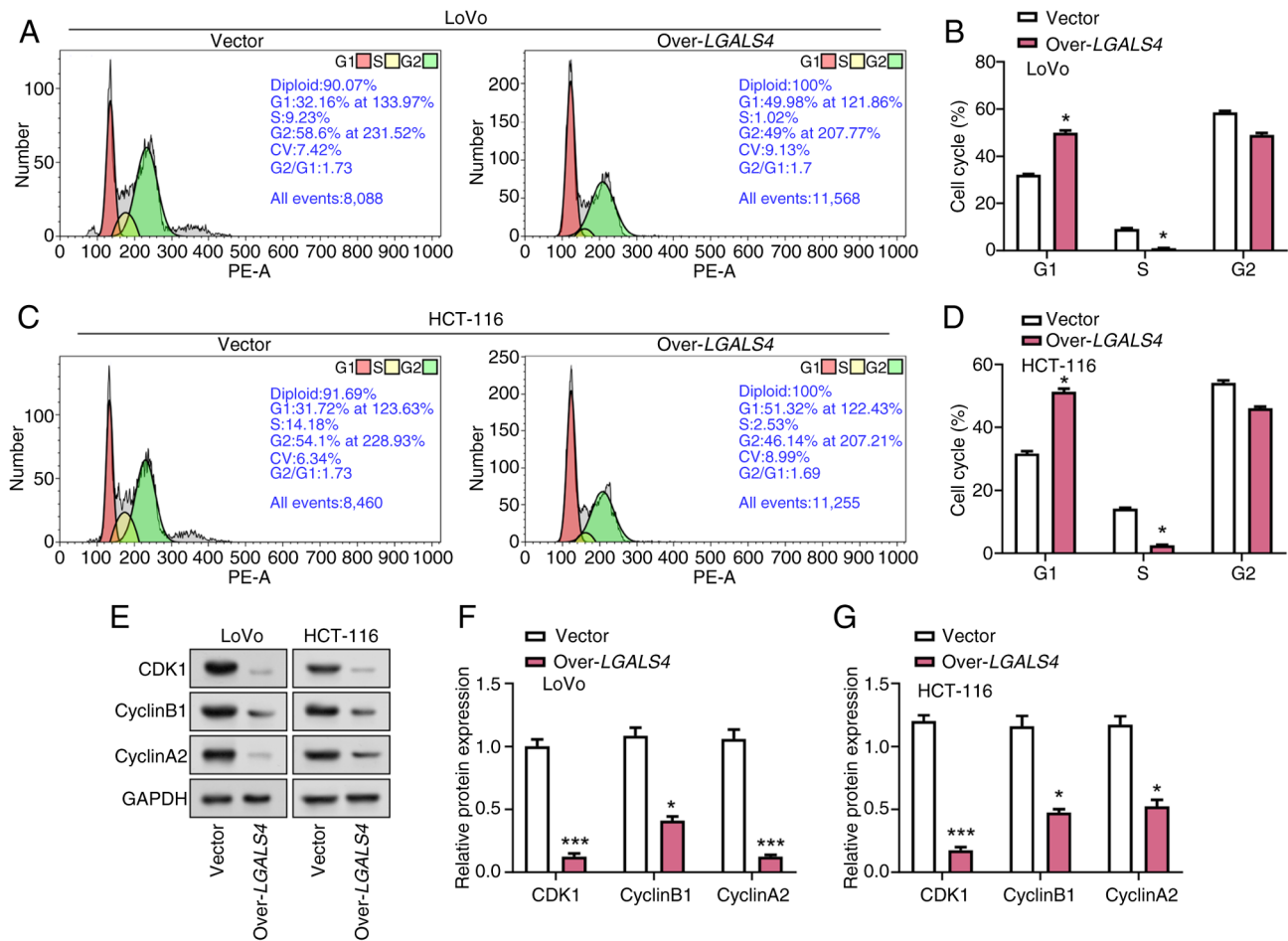


Figure 5. *LGALS4* overexpression induces cell cycle arrest in CRC cells. (A) Flow cytometric analysis showing the effect of *LGALS4* overexpression on the cell cycle in LoVo cells. (B) Quantification of flow cytometry data showing the effect of *LGALS4* overexpression on the cell cycle in LoVo cells. (C) Flow cytometric analysis showing the effect of *LGALS4* overexpression on the cell cycle in HCT-116 cells. (D) Quantification of flow cytometry data showing the effect of *LGALS4* overexpression on the cell cycle in HCT-116 cells. (E) Western blot analysis showing the protein expression levels of cell cycle-related proteins CDK1, Cyclin B1 and Cyclin A2 in LoVo and HCT-116 cells following *LGALS4* overexpression. Semi-quantification of protein expression levels of CDK1, Cyclin B1 and Cyclin A2 in (F) LoVo and (G) HCT-116 cells following *LGALS4* overexpression, presented as bar graphs. * $P < 0.05$, *** $P < 0.001$ vs. vector. CRC, colorectal cancer; *LGALS4*, lectin galactoside-binding soluble 4; over, overexpression; CDK1, Cyclin dependent kinase 1.

increased apoptosis of CRC cells compared with control cells, and the pro-apoptotic effect was significantly increased when combined with 5-FU treatment compared with the control (Fig. 8A-C). WB analysis demonstrated significantly increased protein expression levels of apoptosis markers caspase-9, caspase-3 and Bax, and significantly decreased protein expression levels of Bcl-2 in CRC cells overexpressing *LGALS4* compared with controls. The effects of *LGALS4* overexpression on these markers were significantly enhanced by 5-FU treatment (Fig. 8D-F). Additionally, 2-NBDG uptake, ATP production and lactate levels were measured in LoVo and HCT-116 cells. These results showed that overexpression of *LGALS4* significantly decreased ATP production, lactate levels and glucose uptake in CRC cells compared with the control (Fig. 8G-8K). These results suggest that *LGALS4* overexpression may enhance 5-FU-induced apoptosis in CRC cells and disrupt glucose metabolism, further inhibiting cell viability.

LGALS4 modulated β -catenin signaling to inhibit glycolysis in CRC cells. To ascertain the impact of *LGALS4* overexpression on glycolysis-related proteins in CRC cells, conducted

RT-qPCR analysis was performed on CRC cells that were transfected with either an *LGALS4* overexpression vector or a control vector. These findings showed that overexpression of *LGALS4* significantly downregulated the expression levels of key glycolysis-related factors, such as *CTNNB1*, *MYC*, solute carrier family 2 member 1 (*SLC2A1*), pyruvate kinase M1/2 (*PKM*), *HK2* and *LDHA* compared with control cells (Fig. 9A and B). WB analysis showed similar results, with a significant decrease in the expression levels of these proteins following *LGALS4* overexpression compared with the controls (Fig. 9C-E). HCT-116 and LoVo cells were treated with the β -catenin inhibitor XAV-939, in addition to inducing *LGALS4* overexpression. These results demonstrated that inhibition of β -catenin significantly enhanced the downregulation of the glycolysis-related factors that were induced by *LGALS4* overexpression (Fig. 9F-J). These findings suggest that *LGALS4* overexpression may inhibit the expression of key glycolysis-related proteins in CRC cells and this effect is further potentiated by β -catenin inhibition. The Wnt/ β -catenin signaling pathway serves a central role in cell proliferation, migration and invasion and *LGALS4* potentially inhibits the

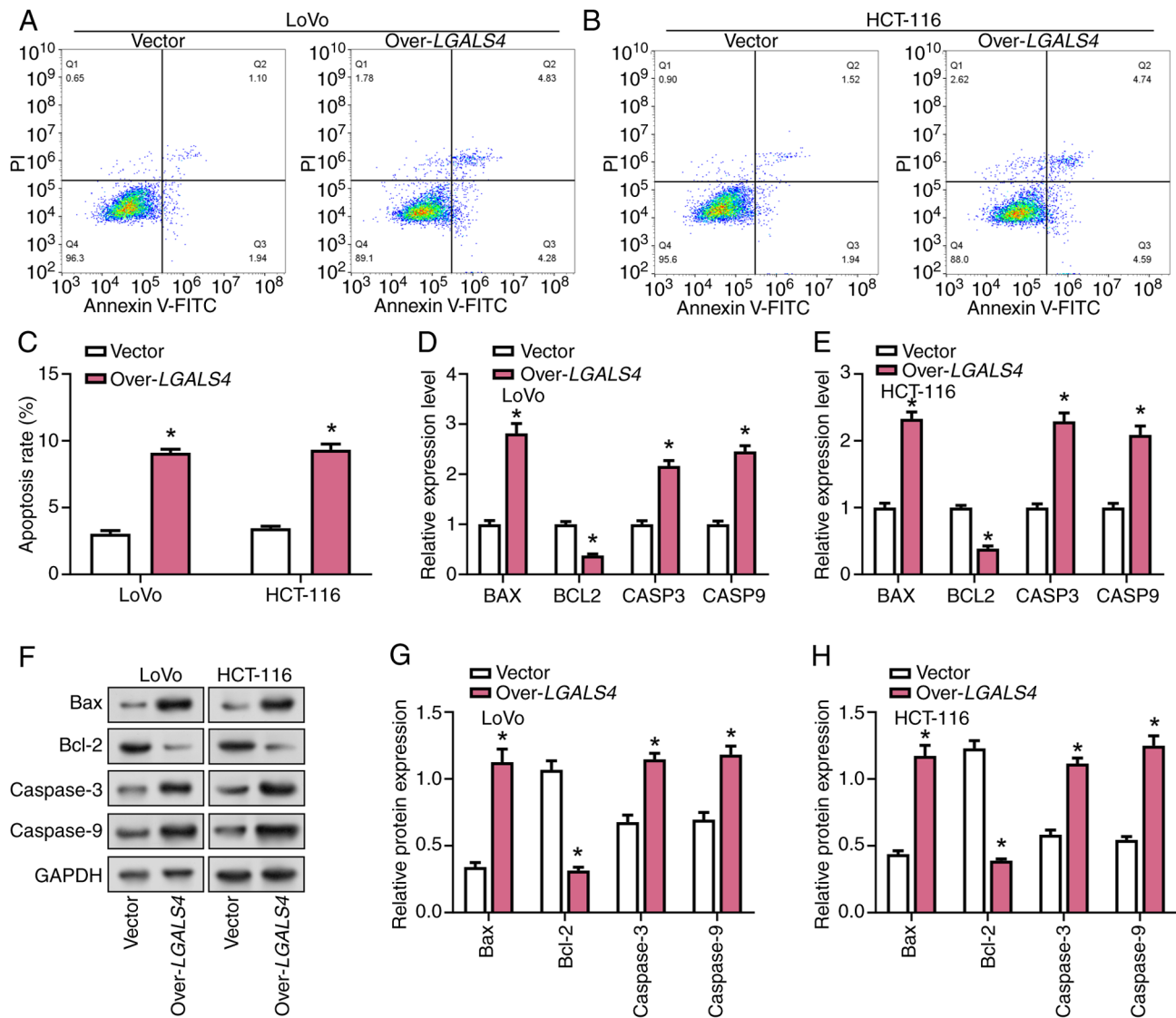


Figure 6. Overexpression of *LGALS4* promotes CRC cell apoptosis. Flow cytometric analysis showing the effect of *LGALS4* overexpression on apoptosis in (A) LoVo and (B) HCT-116 cells. (C) Quantification of apoptosis in LoVo and HCT-116 cells following *LGALS4* overexpression. RT-qPCR analysis showing the relative mRNA expression levels of apoptosis-related genes (*BAX*, *BCL2*, *CASP3*, and *CASP9*) in (D) LoVo and (E) HCT-116 cells following *LGALS4* overexpression. (F) Western blot analysis showing the protein expression of Bax, Bcl-2, caspase-3, and caspase-9 in LoVo and HCT-116 cells following *LGALS4* overexpression. Semi-quantification of protein expression levels of Bax, Bcl-2, caspase-3 and caspase-9 in (G) LoVo and (H) HCT-116 cells following *LGALS4* overexpression. * $P < 0.05$ vs. vector. CRC, colorectal cancer; RT-qPCR, reverse transcription-quantitative PCR; *LGALS4*, lectin galactoside-binding soluble 4; *CASP3*, caspase-3; *CASP9*, caspase-9.

malignant behavior of tumor cells by inhibiting this signaling pathway and reducing the expression of glycolysis-related genes.

Discussion

CRC is a multifaceted disease characterized by genetic and molecular alterations that drive its progression and impact patient outcomes (33). To better understand the molecular underpinnings of CRC, the present study performed a bioinformatics analysis of DEGs using the TCGA-COAD and GSE26571 datasets. This analysis showed overlapping DEGs, primarily involved in processes such as DNA replication, cell cycle and ribosome function. Genes involved in DNA replication and cell cycle pathways may be associated with rapid proliferation and tumor development in CRC. CRC is closely

associated with aberrant DNA replication, as evidenced by the prevalence of DNA polymerase ϵ , catalytic subunit mutations such as p.S297F in colorectal and endometrial carcinomas (34). Furthermore, Zurlo *et al* (35) demonstrated that Cladosporol A induces G1-phase cell cycle arrest in CRC cells, particularly HT-29 cells, by upregulating the expression of *p21(waf1/cip1)*, which reduces Cyclin levels and inhibits CDK activity. Zinc is a cofactor for many enzymes and transcription factors and is essential for cell proliferation and differentiation (36). Genes responsive to zinc ion levels may be involved in regulating cellular adaptation to changes in the microenvironment (37). Ribosomes are sites of protein synthesis and their biosynthesis is closely linked to the metabolic requirements of cell growth and tumor cells (38). Calcineurin is a cell adhesion molecule involved in cell-cell interactions and maintenance of tissue structure. Changes in the expression of its related genes may

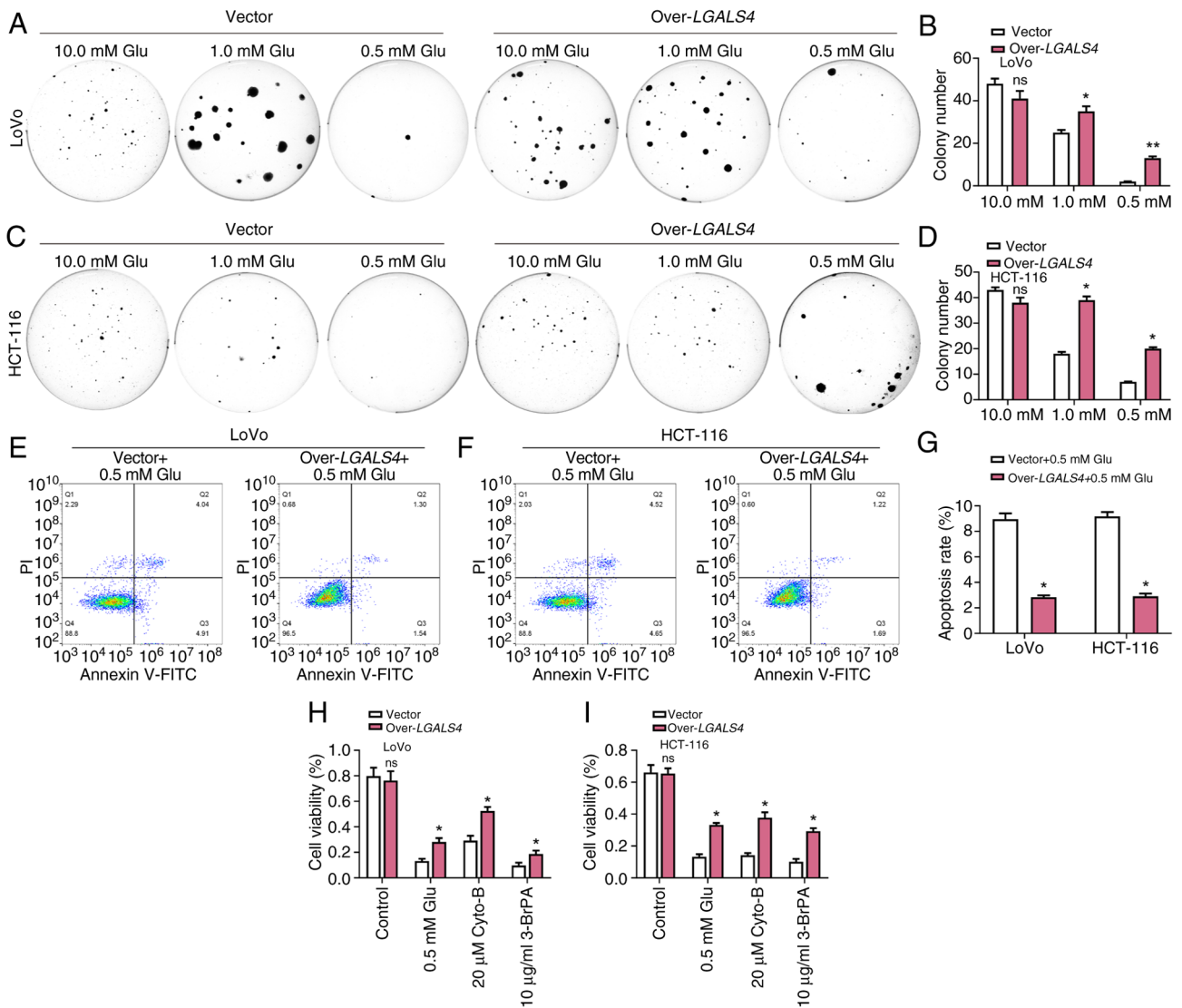


Figure 7. Overexpression of *LGALS4* inhibits aerobic glycolysis in CRC cells. (A) Colony formation assays were performed on LoVo cells after overexpression of *LGALS4* and treatment with 10.0, 1.0 and 0.5 mM of Glu. (B) Semi-quantification of colony formation in LoVo cells overexpressing *LGALS4*. (C) Colony formation assay showing the effect of *LGALS4* overexpression on HCT-116 cells following treatment with 10.0, 1.0 and 0.5 mM Glu. (D) Semi-quantification of colony formation in HCT-116 cells overexpressing *LGALS4*. Flow cytometric analysis showing the apoptosis of (E) LoVo and (F) HCT-116 cells treated with 0.5 mM Glu after *LGALS4* overexpression. (G) Quantification of apoptosis in LoVo and HCT-116 cells treated with 0.5 mM Glu after *LGALS4* overexpression. Quantification of cell viability in (H) LoVo and (I) HCT-116 cells overexpressing *LGALS4* or a control vector under treatment with 0.5 mM Glu, 20 μ M Cyto-B, and 10 μ g/ml 3-BrPA. * P <0.05, ** P <0.01 vs. vector. CRC, colorectal cancer; Glu, glucose; Cyto-B, cytochalasin B; 3-BrPA, 3-Bromopyruvate; *LGALS4*, lectin galactoside-binding soluble 4; over, overexpression; ns, not significant.

affect the invasiveness and metastatic ability of tumor cells (39). Another study by Zurlo *et al* (40) reported that Cladosporol A inhibits CRC proliferation by enhancing the expression of *p21(waf1/cip1)* through Sp1-peroxisome proliferator activated receptor γ interaction. This compound also induces β -catenin degradation, thereby suppressing the β -catenin/T-cell factor (TCF) pathway and promoting E-cadherin expression, which impedes cell cycle progression. Prognostic analysis of overlapping DEGs identified significant prognostic genes, including *C8G*, *LGALS4* and *RPS17*. Expression analysis demonstrated significantly decreased expression levels of *C8G* and *LGALS4* in tumor samples from the TCGA-COAD and GSE26571 datasets. Yu *et al* (41) identified *RPS17* as a hub gene in the co-expression network of differentially expressed genes in CRC with microsatellite instability, suggesting its significant role in the ribosome pathway's involvement in CRC.

LGALS4 is a protein encoded by the *LGALS4* gene in humans. It belongs to the galectin family and has the ability to bind and recognize β -galactoside sugars (42). *LGALS4* is primarily expressed in the gastrointestinal tract, where it serves essential roles in cell-cell adhesion, epithelial differentiation and mucosal immunity (43). Its involvement in various physiological processes, including intestinal homeostasis, inflammation and cancer progression has previously been reported. Watanabe *et al* (44) reported that elevated levels of circulating *Galectin-4* in patients with CRC correlate with disease progression, suggesting its potential as a follow-up marker post-surgery. *Galectin-1* may be useful for patient screening and *Galectin-4* can complement CEA/CA19-9 in enhancing CRC monitoring. Additionally, Zhou *et al* (45) demonstrated that surface profiles of CRC cells and tumor-infiltrating lymphocytes from surgical samples align

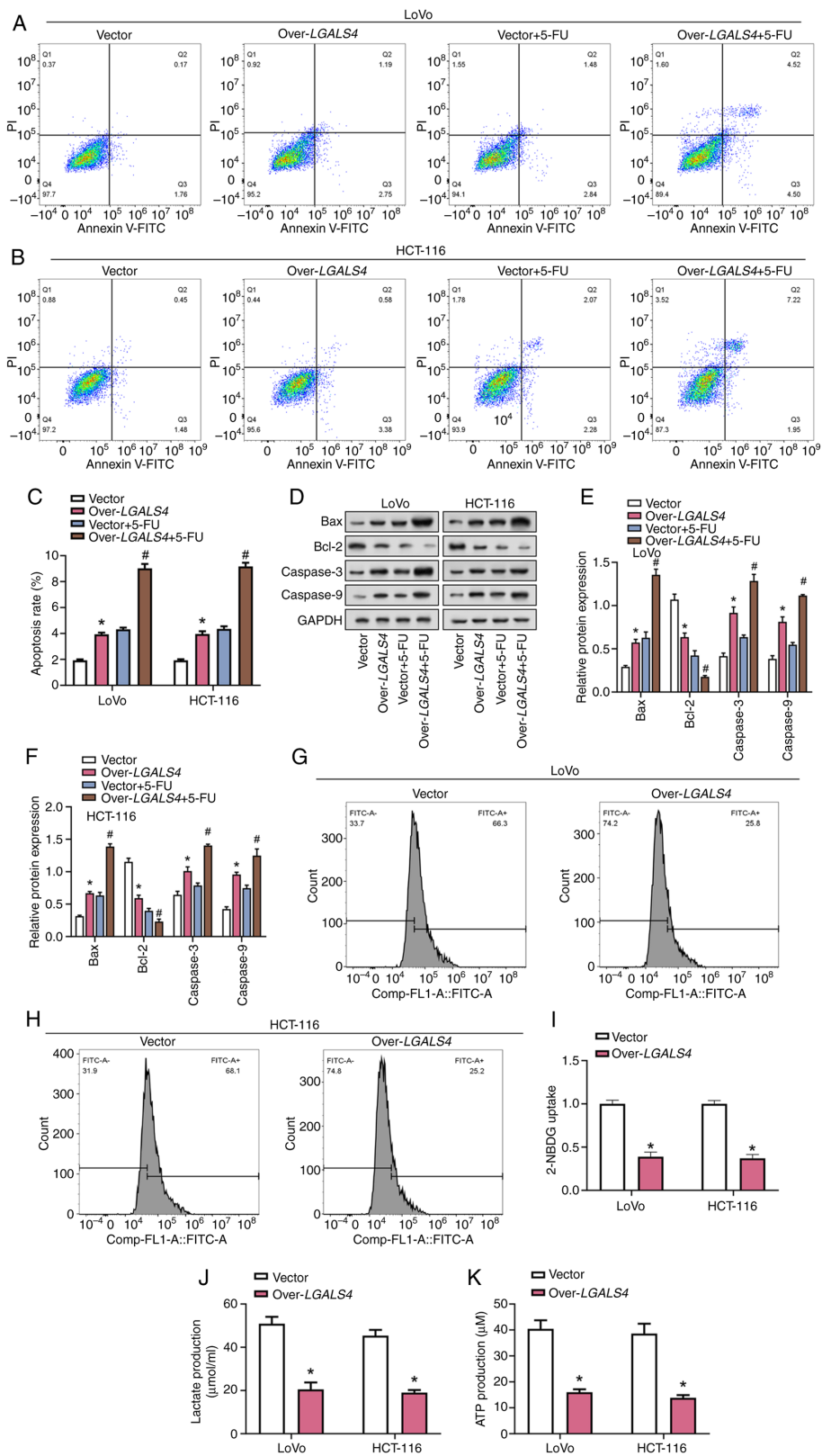


Figure 8. *LGALS4* overexpression enhances 5-FU-induced apoptosis and inhibits glycolysis in colorectal cancer cells. Flow cytometric analysis was performed to assess apoptosis in (A) LoVo and (B) HCT-116 cells following *LGALS4* overexpression, with or without treatment with 50 $\mu\text{g/ml}$ 5-FU. (C) Quantification of apoptosis in LoVo and HCT-116 cells following *LGALS4* overexpression, with or without 50 $\mu\text{g/ml}$ 5-FU treatment. (D) Western blot analysis was performed to detect the expression of apoptotic proteins (caspase-3, caspase-9, Bax and Bcl-2) in LoVo and HCT-116 cells following *LGALS4* overexpression, with or without 50 $\mu\text{g/ml}$ 5-FU treatment. Semi-quantification of the western blot analysis results for apoptotic proteins (caspase-3, caspase-9, Bax and Bcl-2) in (E) LoVo and (F) HCT-116 cells following *LGALS4* overexpression, with or without 50 $\mu\text{g/ml}$ 5-FU treatment. * $P<0.05$ vs. vector; # $P<0.05$ vs. vector + 5-FU. (G and H) Flow cytometry was used to measure the uptake of 2-NBDG in (G) LoVo and (H) HCT-116 cells following *LGALS4* overexpression. (I) Quantification of 2-NBDG uptake in LoVo and HCT-116 cells transfected with Vector or over-*LGALS4*. (J) A lactate detection kit was used to measure lactate release from LoVo and HCT-116 cells following *LGALS4* overexpression. (K) An ATP detection kit was used to measure ATP production in LoVo and HCT-116 cells following *LGALS4* overexpression. * $P<0.05$ vs. vector. 5-FU, 5-Fluorouracil; *LGALS4*, lectin galactoside-binding soluble 4; over, overexpression; 2-NBDG, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose.

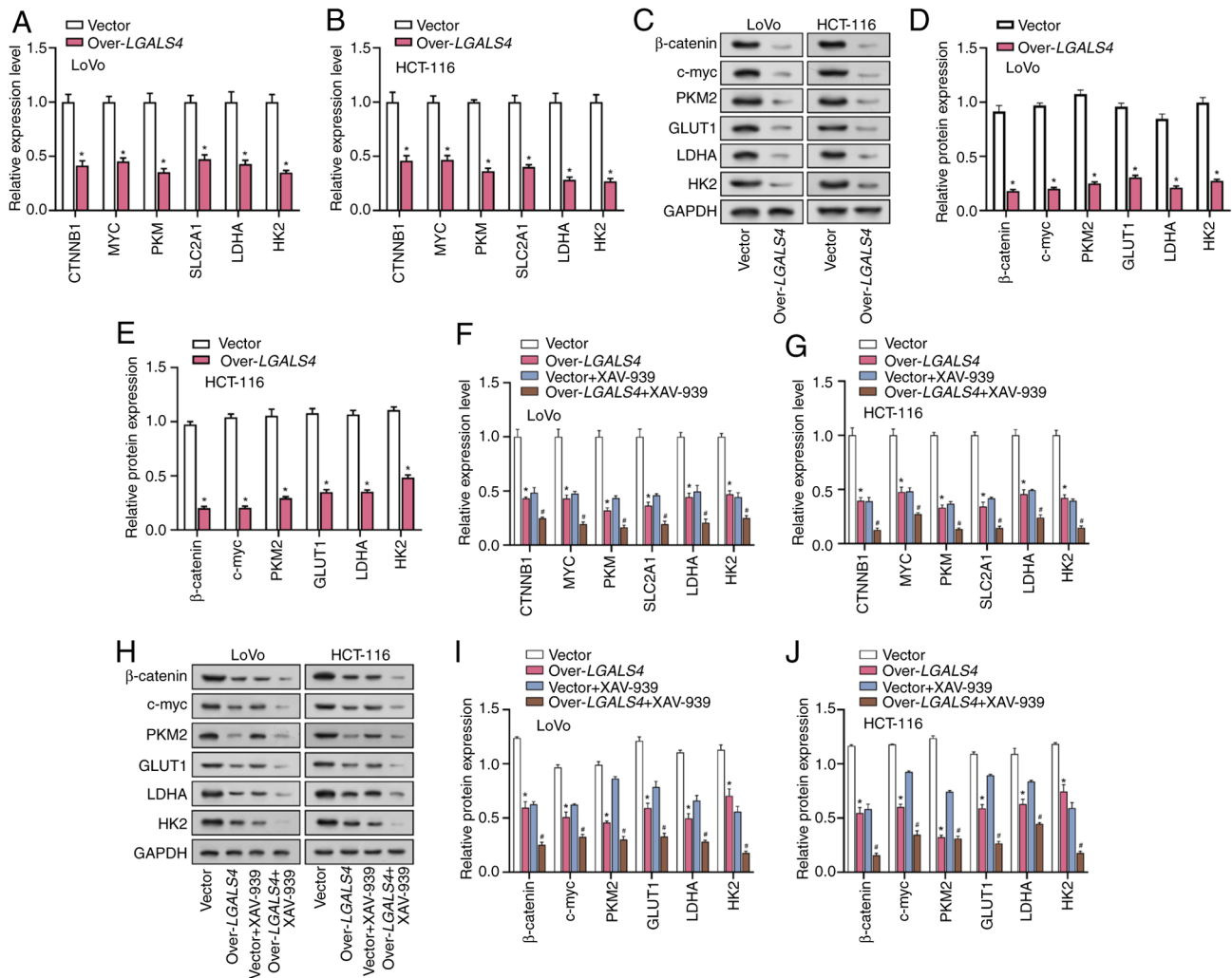


Figure 9. *LGALS4* affects β -catenin signaling to inhibit glycolysis. RT-qPCR analysis of glycolysis-related factors, including *CTNNB1*, *MYC*, *PKM*, *SLC2A1*, *LDHA*, and *HK2*, in (A) LoVo and (B) HCT-116 cells following *LGALS4* overexpression. (C) WB analysis showing the protein expression of glycolysis-related factors in LoVo and HCT-116 cells following *LGALS4* overexpression. Semi-quantification of the WB analysis results for glycolysis-related factors (β -catenin, c-myc, PKM2, GLUT1, LDHA and HK2) in (D) LoVo and (E) HCT-116 cells following *LGALS4* overexpression. *P<0.05 vs. vector. RT-qPCR analysis of glycolysis-related factors in (F) LoVo and (G) HCT-116 cells treated with or without the β -catenin inhibitor XAV-939 following *LGALS4* overexpression. (H) WB analysis showing the protein expression of glycolysis-related factors in LoVo and HCT-116 cells treated with or without the β -catenin inhibitor XAV-939 following *LGALS4* overexpression. Semi-quantification of the WB analysis results for glycolysis-related factors in (I) LoVo and (J) HCT-116 cells treated with or without the β -catenin inhibitor XAV-939 following *LGALS4* overexpression. *P<0.05 vs. vector; #P<0.05 vs. vector + XAV-939. RT-qPCR, reverse transcription-quantitative PCR; WB, western blot; *LGALS4*, lectin galactoside-binding soluble 4; *CTNNB1*, catenin beta 1; *MYC*, Myc proto-oncogene; *PKM*, pyruvate kinase M1/M2; *SLC2A1*, solute carrier family 2 member 1; CRC, colorectal cancer; PKM2, pyruvate kinase type M2; GLUT1, solute carrier family 2 member 1; *LDHA*, lactate dehydrogenase A; *HK2*, hexokinase 2; over, overexpression.

with prognostic categories and minimal antigenic panels, including *Galectin-4*, providing potential predictors for disease relapse and patient survival. Furthermore, Satelli *et al* (7) reported that *Galectin-4* acts as a potential tumor suppressor in CRC, with its downregulation observed in adenomas and invasive carcinomas. Overexpression induces cell cycle arrest, reduces migration and sensitizes cells to apoptosis, suggesting its significance in CRC biology through interaction with Wnt signaling proteins and downregulation of Wnt target genes. In the present study, overexpression of *LGALS4* limited the capacity of CRC cells to migrate, proliferate and invade and disrupted CRC cell cycle distribution. Meanwhile, overexpression of *LGALS4* promoted apoptosis in CRC cells. This suggests that *LGALS4* could potentially serve as both a therapeutic target and a potential prognostic marker, underscoring its importance in cancer research and clinical applications.

Glucose is a primary energy source for cells and a simple sugar. It is vital for cellular metabolism and is transported into cells through glucose transporters. Once inside the cell, glucose undergoes glycolysis, a metabolic process known as the energy investment phase and the energy payback phase, which produces ATP and NADH by converting glucose to pyruvate (46). Glycolysis, which takes place in the cytoplasm, consists of 10 enzyme-catalyzed stages. ATP is consumed during the energy investment phase to phosphorylate glucose and its intermediates. The energy payback phase involves the production of NADH and ATP. Glycolysis is an anaerobic process, meaning it does not require oxygen and is essential for energy production under both aerobic and anaerobic conditions (47). This pathway is crucial for cells with high energy demands, such as muscle and cancer cells. Zhou *et al* (48) reported that *Dioscin* inhibits aerobic glycolysis in CRC

cells by degrading S-phase kinase-associated protein 2 via *Cadherin 1*, consequently reducing CRC proliferation. Additionally, Zhou *et al* (49) showed that increased expression of PKM2 in CRC promotes aerobic glycolysis, cell proliferation and migration, suggesting PKM2 may act as a promising therapeutic target for CRC. Moreover, Zhao *et al* (50) reported that Sam68, an RNA-binding protein, promotes aerobic glycolysis in CRC by regulating the alternative splicing of PKM2, enhancing glycolysis and cell proliferation. This underscores the potential of Sam68 as a target for therapy in CRC. In the present study, overexpression of *LGALS4* significantly enhanced the survival of CRC cells under low glucose conditions. Although this phenomenon appears contradictory to the effect of *LGALS4* downregulating key factors of glycolysis, this may potentially be due to the reprogramming of metabolic pathways in CRC cells facilitated by *LGALS4*. *LGALS4* may have maintained the metabolic demand and energy supply of cells under low-glucose conditions either by activating non-glycolytic energy-generating pathways or by enhancing the efficiency of cellular utilization of nutrients. In addition, *LGALS4*-overexpressing CRC cells exhibited relative resistance to glucose deprivation and glycolytic inhibition, which may be related to its effects on cell cycle and apoptotic pathways, making the cells more tolerant to metabolic stress. *LGALS4* may enhance cellular adaptation during glycolytic inhibition by regulating signaling pathways related to cell survival, such as the β -catenin signaling pathway. The decreased expression level of *LGALS4* observed in CRC tissues may be related to its role as a tumor suppressor. Downregulation of *LGALS4* expression in a variety of cancers correlates with a better prognosis, whereas in the present study, the downregulation of *LGALS4* may be related to metabolic reprogramming of the tumor cells to adapt to rapid proliferation and to evade immune surveillance. The present study showed that *LGALS4*-overexpressing CRC cells exhibited increased survival under glucose deprivation conditions and showed tolerance to glycolytic inhibition compared with negative controls. Flow cytometry results confirmed that *LGALS4* significantly reduced apoptosis induced by glucose deprivation. These findings suggest that therapeutic pathways targeting aerobic glycolysis may provide new strategies for CRC treatment in the future.

5-FU is an anticancer drug used to treat various cancers, such as breast cancer, hepatocellular carcinoma (HCC) and CRC (51). A previous study by Zou *et al* (52) reported that 5-FU induces cytotoxicity and apoptosis in cancer cells through a ROS-mediated mitochondrial pathway and allicin enhances the antitumor activity of 5-FU by increasing apoptosis and reducing mitochondrial membrane potential in HCC cells. Similarly, Zou *et al* (53) showed that insulin pretreatment enhances the anticancer effects of 5-FU in esophageal and colonic cancer cells by increasing 5-FU uptake, promoting apoptosis and upregulating the expression of cleaved caspase-3, thereby inhibiting cell proliferation more effectively. Additionally, Zuo *et al* (54) showed that 5-FU inhibits cell proliferation and induces apoptosis in HepG2 liver cancer cells, similar to the impacts of chikusetsusaponin IV and V, which also promote cell cycle arrest and enhance apoptotic protein activation, underscoring their potential in cancer treatment. The present study demonstrated that

overexpression of *LGALS4* promoted apoptosis and inhibited aerobic glycolysis in CRC cells. Based on this finding, it was hypothesized that 5-FU may have a synergistic effect with *LGALS4* overexpression by further enhancing the inhibitory effect on tumor cells. Specifically, *LGALS4* overexpression in CRC cells enhances the apoptotic response to 5-FU treatment and induces metabolic changes, reducing glycolytic activity and energy production. This may reduce the metabolism and elimination of 5-FU, thereby accumulating 5-FU in tumor cells and enhancing its anticancer effect. 2-NBDG, a glucose analog, is used to measure glucose uptake (55). Glycolysis, a metabolic process, transforms glucose into energy and lactate (56). Lactate release is a product of glycolysis and ATP, the main energy source within the cell, is produced through glycolysis (57). Therefore, inhibition of glycolysis by 5-FU will affect ATP production, leading to energy deficiency, while reducing lactate release and 2-NBDG uptake (58). Zhou *et al* (59) reported that Gefitinib causes A549 and H1975 non-small cell lung cancer cells to undergo programmed cell death and inhibit glycolysis, as evidenced by reduced glucose uptake, lowered ATP levels and increased apoptosis rates. Additionally, Zu *et al* (60) showed that by linking glucose metabolism with lipid synthesis, ATP citrate lyase (ACL) catalyzes the conversion of citrate to acetyl-CoA and oxaloacetate by utilizing ATP and CoA. ACL, overexpressed in various types of cancer, including CRC, serves as a possible target for therapeutic cancer by disrupting glycolysis-driven lipogenesis. Moreover, Zuo *et al* (61) reported that miR-4443 downregulates *TRIM14*, suppressing energy metabolism and metastasis in papillary thyroid carcinoma (PTC). miR-4443 inhibits ATP production and aerobic glycolysis by targeting *TRIM14*, indicating its role in PTC progression and energy regulation. In the present study, this was evidenced by increased apoptotic markers, decreased 2-NBDG uptake and decreased lactate and ATP levels. These results suggested that *LGALS4* may exert anti-CRC effects by inhibiting glycolysis.

The β -catenin signaling pathway is essential for a number of cellular functions, including cell proliferation, differentiation and migration. β -catenin is phosphorylated by a destruction complex when Wnt ligands are not present, leading to its degradation (62). Wnt activation inhibits the destruction complex, enabling β -catenin to accumulate and move into the nucleus (63). There, it interacts with *TCF*/lymphoid enhancer-binding factor (*LEF*) transcription factors to activate target gene expression, such as Cyclin D1 and c-Myc, promoting cell growth and survival. Dysregulation of this pathway is associated with various diseases, including cancer, making it an important target for therapeutic intervention (64). Zou *et al* (65) reported that elevated circular (circ) RNA circ_0068464 levels in CRC contribute to cell migration, proliferation and activation of the Wnt/ β -catenin signaling pathway. Its interaction with miR-383 further exacerbates CRC progression, suggesting therapeutic potential in targeting this pathway. Similarly, a study by Zou *et al* (66) showed that increased circCASK in CRC promotes tumor growth and invasion by upregulating six homeobox 1 expression. Forkhead box c2 transcriptionally induces circCASK expression, thereby activating the Wnt/ β -catenin signaling pathway and accelerating the development of CRC. In addition, there is a close connection

between β -catenin and glycolysis. Zhou *et al* (67) reported that Dihydrolipoamide S-acetyltransferase (*DLAT*), a glycolysis-related gene, is overexpressed in HCC, contributing to poor prognosis. Its downregulation inhibits PI3K/Akt and Wnt/ β -catenin signaling pathways, highlighting the role of *DLAT* as a potential therapeutic target in HCC. Additionally, Zhou *et al* (68) reported that cryptotanshinone suppresses breast cancer cell migration, invasion and proliferation by targeting glycolysis-related proteins, such as PKM2. This suggests a potential connection between β -catenin and glycolysis in breast cancer, suggesting PKM2 may be a promising therapeutic avenue. The interaction between *LGALS4* and the β -catenin signaling pathway may impact the metabolic properties of CRC cells. Overexpression of *LGALS4* may interfere with the intranuclear accumulation of β -catenin or its interaction with *TCF/LEF* and inhibit the transcription of genes downstream of the Wnt signaling pathway such as c-Myc, PKM2 and GLUT1. In addition, *LGALS4* may regulate the expression of the metabolic enzymes *LDHA* and *HK* and activate the AMPK signaling pathway by altering intracellular ATP levels or AMP/ATP ratios, thereby inhibiting metabolic enzyme expression. The effect of *LGALS4* on the expression or function of the glucose transporter protein GLUT1 may reduce glucose uptake, thereby affecting glucose-dependent metabolic pathways. As a tumor suppressor protein, overexpression of *LGALS4* inhibits tumor cell growth and metabolism through a variety of mechanisms, including downregulation of genes closely related to tumor metabolism. *LGALS4*-induced cell cycle arrest and promotion of apoptosis may also indirectly affect the expression of metabolism-related genes as these cellular processes are closely related to the metabolic status of cells. The RT-qPCR and WB analyses in the present study showed that *LGALS4* overexpression significantly reduced the levels of glycolysis-related proteins in CRC cells. When CRC cells were subjected to 10 μ M β -catenin inhibitor XAV-939 for 72 h, the expression levels of glycolysis-related proteins further decreased. This indicated that *LGALS4* may affect β -catenin signaling to inhibit glycolysis, thereby potentially inhibiting CRC development.

The present study investigated the role of *LGALS4* in CRC glycolysis. Although *LGALS4* has been identified as a potential prognostic factor for patients with CRC, its specific impact in tumor glycolysis has not been fully elucidated. Through analysis of the TCGA-COAD and GSE26571 databases, it was demonstrated that *LGALS4* expression was significantly downregulated in CRC tissues and strongly correlated with patient survival, suggesting a potentially important role in CRC development. Glycolysis is a major energy source for cancer cells and its aberrant activation is tightly linked to rapid proliferation and invasiveness of tumors. It was hypothesized that *LGALS4* may affect the metabolic properties of CRC cells by regulating the glycolytic pathway. The present preliminary data suggested that *LGALS4* overexpression inhibited glycolytic activity in CRC cells and that this effect may be associated with changes in the β -catenin signaling pathway. Given the role of β -catenin in the regulation of glycolytic gene expression, it could be suggested that *LGALS4* may regulate glycolysis through this signaling pathway. Therefore, the function of *LGALS4*

in CRC glycolysis and its associated molecular mechanism were analyzed.

In summary, overexpression of *LGALS4* exerted a multifaceted inhibitory effect in CRC cells, significantly affecting key biological properties of tumor cells. Firstly, it inhibited the glycolytic process, reducing the cell's dependence on glucose and decreasing lactate production and ATP generation, thereby directly limiting the cell's energy supply. Second, *LGALS4* caused cell cycle arrest in the G1 phase, which prevented normal cell cycle progression by decreasing the expression levels of cell cycle-related proteins such as CDK1, Cyclin B1 and Cyclin A2. In addition, *LGALS4* overexpression promoted apoptosis, which was closely related to the changes in the expression of apoptosis-related proteins such as Bax, Bcl-2, caspase-3 and caspase-9, increasing the rate of apoptosis. Meanwhile, *LGALS4* affected the β -catenin protein signaling pathway, decreasing the expression of glycolysis-related factors such as β -catenin protein, c-Myc, GLUT1, PKM2, HK2 and LDHA. A previous study has shown that the β -catenin protein signaling pathway is a signaling pathway that serves a central role in cell proliferation, migration and invasion (69). *LGALS4* may also cause metabolic reprogramming, which further reduced aerobic glycolysis in CRC cells and inhibited the rapid proliferation and invasiveness of tumor cells. Notably, *LGALS4* overexpressing cells showed increased sensitivity to the chemotherapeutic drug 5-FU, which may be achieved by enhancing 5-FU-induced apoptosis. *LGALS4* reduced 2-NBDG uptake and decreased ATP production and lactate release, suggesting a potential direct effect on cellular energy metabolism.

The present study suggested that *LGALS4* may provide a new potential target for CRC therapy. As a protein whose expression is downregulated in CRC and is associated with patient survival, *LGALS4* has the ability to regulate glycolysis and promote apoptosis in tumor cells, which provides a scientific basis for the development of new therapeutic approaches. By inhibiting glycolysis, *LGALS4* is able to reduce the energy supply of tumor cells, directly targeting their metabolic needs for rapid proliferation. In addition, *LGALS4* overexpression promoted apoptosis, providing a new therapeutic avenue for inducing tumor cell death. More importantly, *LGALS4* increased the sensitivity of tumor cells to chemotherapeutic agents such as 5-FU, which may help to improve the efficacy of existing therapeutic regimens, particularly in drug-resistant tumors. Meanwhile, the regulatory effect of *LGALS4* on the β -linker protein signaling pathway provided a new perspective on the control of tumor cell proliferation and invasion. These properties not only demonstrate the potential of *LGALS4* in individualized medicine, but also highlight its advantages in overcoming existing therapeutic limitations. With further research, *LGALS4* may be a key factor in improving treatment outcomes for patients with CRC.

Although the present study demonstrated the potential role of *LGALS4* in CRC in *in vitro* experiments and bioinformatics analyses, there were a number of limitations. First, the findings need to be further validated by *in vivo* models to ensure the accuracy and reliability of the biological effects. Second, the sample size and population representation may limit the generalizability of the findings. In addition, the long-term effects and specific molecular mechanisms of *LGALS4* need

to be explored in depth. To address these limitations, future studies should conduct *in vivo* experiments in animal models to assess the actual therapeutic potential and safety of *LGALS4*. The mechanism of *LGALS4* downregulation of the expression of factors such as β -catenin, c-Myc, PKM2, GLUT1, LDHA and HK is currently unknown. Techniques such as chromatin immunoprecipitation sequencing and RNA sequencing should be used to investigate how *LGALS4* affects the transcriptional activity of the β -catenin signaling pathway and its associated target genes. Through methods such as immunoprecipitation and mass spectrometry, the direct or indirect interactions between *LGALS4* and proteins such as β -catenin and c-Myc can be explored in addition to how these interactions affect their functions. In addition, metabolomics approaches should be used to analyze the metabolic changes in *LGALS4* overexpressing cells to understand how *LGALS4* regulates cellular metabolic pathways, particularly glycolytic processes. By observing the effects of *LGALS4* overexpression on the cell cycle and apoptosis, how these cellular events are linked to the regulation of the expression of metabolism-related genes could be determined. In the present study, apoptosis and cell cycle distribution of CRC cells were quantified using PI staining combined with flow cytometry. Although this method provided valuable information on cell cycle status and apoptosis rates, it also has its inherent limitations. For example, PI staining may not be able to distinguish between the various stages of the cell cycle and there may be some bias in the assessment of cell survival status. In addition, due to the limitations of the present study conditions, 5-bromo-2-deoxyuridine (BRDU) staining was not performed to further validate cell proliferation. However, BRDU staining should be performed in future studies to complement the results of PI staining and provide additional validation of the findings of the present study. By combining these a more comprehensive understanding of the biology of CRC cells could be expected and these results could potentially provide a more solid experimental basis for future studies.

The present study highlighted the role of *LGALS4* in CRC and its potential as a therapeutic target. Bioinformatics analysis demonstrated that *LGALS4* was significantly downregulated in CRC and was associated with patient survival. Overexpression of *LGALS4* resulted in a significant upregulation of caspase-3 and caspase-9, a phenomenon that may be achieved through multiple mechanisms. First, *LGALS4* overexpression promoted apoptosis in CRC cells. Caspase-3 and caspase-9 act as key executors in the apoptotic pathway and caspase-9 acts as an initiating caspase to activate effector caspase-3, thereby triggering apoptosis. Second, *LGALS4* overexpression inhibited aerobic glycolysis in CRC cells and affected the β -catenin signaling pathway, which serves a crucial role in cell survival and apoptosis. By decreasing the activity of the β -catenin signaling pathway, *LGALS4* may promote the upregulation of caspase-3 and caspase-9, which in turn drives the apoptotic process. In addition, G1-phase cell cycle arrest caused by *LGALS4* overexpression may have triggered a cellular stress response that activated apoptotic pathways including caspase family proteins. *LGALS4* may also directly or indirectly regulate the expression of caspase-3 and caspase-9, which acted as post-transcriptional modifiers affecting the stability or translational efficiency of specific genes. Furthermore, possible intracellular feedback mechanisms may upregulate

the expression of apoptosis-related genes upon detection of survival stress or abnormal signals to remove damaged cells. Taken together, the upregulation of caspase-3 and caspase-9 levels in CRC cells by *LGALS4* overexpression may be due to its direct effect on apoptotic pathways and its potential inhibitory effect on the β -catenin signaling pathway, which exerts an antitumor effect in CRC.

The present study demonstrated the important role of *LGALS4* in CRC and its value as a potential future therapeutic target. These findings showed that *LGALS4* expression was downregulated in CRC tissues and correlated with poor patient prognosis, suggesting its role as a tumor suppressor. Functionally, overexpression of *LGALS4* inhibited glycolysis and inhibited cell cycle progression in CRC cells, leading to G1 phase arrest while promoting apoptosis. In addition, the regulatory effect of *LGALS4* on the β -catenin signaling pathway may have an inhibitory effect on the proliferation, migration and invasion of tumor cells. Notably, *LGALS4* overexpressed cells increased higher sensitivity to the commonly used chemotherapeutic drug 5-FU, which potentially provides a novel research avenue to improve the efficacy of chemotherapy. The present study also highlighted the impact of *LGALS4* in the metabolic reprogramming of tumors, indicating potential new perspectives for metabolically targeted therapies. These results further the current understanding of the molecular mechanisms of CRC and provide directions for future individualized treatment strategies and the development of new drugs, which may improve the treatment outcome of patients with CRC.

Acknowledgments

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

SL, KY and JY were responsible for conception and design of the study. CX, KY, ZQ and YC were responsible for data acquisition. SL, LY and BS were responsible for data analysis and interpretation. SL, TZ, JX and YC were responsible for statistical analysis. SL and BS drafted the manuscript. SL and KY confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Roshandel G, Ghasemi-Kebria F and Malekzadeh R: Colorectal cancer: Epidemiology, risk factors, and prevention. *Cancers* 16: 1530, 2024.
- Parmar S and Easwaran H: Genetic and epigenetic dependencies in colorectal cancer development. *Gastroenterol Report* 10: goac035, 2022.
- Yu J, Feng Q, Kim JH and Zhu Y: Combined effect of healthy lifestyle factors and risks of colorectal adenoma, colorectal cancer, and colorectal cancer mortality: Systematic review and meta-analysis. *Front Oncol* 12: 827019, 2022.
- Wong MC, Huang J, Lok V, Wang J, Fung F, Ding H and Zheng ZJ: Differences in incidence and mortality trends of colorectal cancer worldwide based on sex, age, and anatomic location. *Clin Gastroenterol Hepatol* 19: 955-966.e61, 2021.
- Biller LH and Schrag D: Diagnosis and treatment of metastatic colorectal cancer: A review. *JAMA* 325: 669-685, 2021.
- Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, Tao Q and Xu H: Wnt signaling in colorectal cancer: Pathogenic role and therapeutic target. *Mol Cancer* 21: 144, 2022.
- Satelli A, Rao PS, Thirumala S and Rao US: Galectin-4 functions as a tumor suppressor of human colorectal cancer. *Int J Cancer* 129: 799-809, 2011.
- Michalak M, Golde V, Helm D, Kaltner H, Gebert J and Kopitz J: Combining Recombinase-mediated cassette exchange strategy with quantitative proteomic and Phosphoproteomic analyses to inspect intracellular functions of the tumor suppressor galectin-4 in colorectal cancer cells. *Int J Mol Sci* 23: 6414, 2022.
- Zhang GL, Pan LL, Huang T and Wang JH: The transcriptome difference between colorectal tumor and normal tissues revealed by single-cell sequencing. *J Cancer* 10: 5883-5890, 2019.
- Cheraghi-Shavi T, Jalal R and Minuchehr Z: TGM2, HMGA2, FXYD3, and LGALS4 genes as biomarkers in acquired oxaliplatin resistance of human colorectal cancer: A systems biology approach. *PLoS One* 18: e0289535, 2023.
- Chandel NS: Glycolysis. *Cold Spring Harb Perspect Biol* 13: a040535, 2021.
- Jaiswara PK, Gupta VK, Rawat SG, Tiwari RK, Sonker P, Maurya RP and Kumar A: Targeting of Aerobic Glycolysis: An Emerging Therapeutic Approach Against Colon Cancer. *Colon Cancer Diagnosis and Therapy: Vol 2*. Springer, pp225-244, 2021.
- Zuo S, Wu L, Wang Y and Yuan X: Long non-coding RNA MEG3 activated by vitamin D suppresses glycolysis in colorectal cancer via promoting c-Myc degradation. *Front Oncol* 10: 274, 2020.
- Zhu Y, Wu G, Yan W, Zhan H and Sun P: miR-146b-5p regulates cell growth, invasion, and metabolism by targeting PDHB in colorectal cancer. *Am J Cancer Res* 7: 1136, 2017.
- Zhu J, Wang S, Bai H, Wang K, Hao J, Zhang J and Li J: Identification of five glycolysis-related gene signature and risk score model for colorectal cancer. *Front Oncol* 11: 588811, 2021.
- Xiong Y, Lei J, Zhao J, Lu Q, Feng Y, Qiao T, Xin S, Han Y and Jiang T: A gene-based survival score for lung adenocarcinoma by multiple transcriptional datasets analysis. *BMC Cancer* 20: 1046, 2020.
- He R, Zhang M, He L, Huang J, Man C, Wang X, Lang Y and Fan Y: Integrated analysis of Necroptosis-related genes for prognosis, immune microenvironment infiltration, and drug sensitivity in colon cancer. *Front Med (Lausanne)* 9: 845271, 2022.
- Hou S, Li L, Hou H, Zhou T and Zhou H: Establishment of nomogram to predict overall survival and cancer-specific survival of local tumor resection in patients with colorectal cancer liver metastasis with unresectable metastases: A large population-based analysis. *Discover Oncol* 15: 315, 2024.
- Chen S, Su T, Zhang Y, Lee A, He J, Ge Q, Wang L, Si J, Zhuo W and Wang L: *Fusobacterium nucleatum* promotes colorectal cancer metastasis by modulating KRT7-AS/KRT7. *Gut Microbes* 11: 511-525, 2020.
- Xu J, Xiao Y, Liu B, Pan S, Liu Q, Shan Y, Li S, Qi Y, Huang Y and Jia L: Exosomal MALAT1 sponges miR-26a/26b to promote the invasion and metastasis of colorectal cancer via FUT4 enhanced fucosylation and PI3K/Akt pathway. *J Exp Clin Cancer Res* 39: 54, 2020.
- Tang Q, Chen J, Di Z, Yuan W, Zhou Z, Liu Z, Han S, Liu Y, Ying G, Shu X and Di M: TM4SF1 promotes EMT and cancer stemness via the Wnt/ β -catenin/SOX2 pathway in colorectal cancer. *J Exp Clin Cancer Res* 39: 232, 2020.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Aslan M, Hsu EC, Liu S and Stoyanova T: Quantifying the invasion and migration ability of cancer cells with a 3D Matrigel drop invasion assay. *Biol Methods Protoc* 6: bpab014, 2021.
- Mao Z, Xiao H, Shen P, Yang Y, Xue J, Yang Y, Shang Y, Zhang L, Li X, Zhang Y, *et al*: KRAS(G12D) can be targeted by potent inhibitors via formation of salt bridge. *Cell Discov* 8: 5, 2022.
- Chen H, Chen H, Zhang J, Wang Y, Simoneau A, Yang H, Levine AS, Zou L, Chen Z and Lan L: cGAS suppresses genomic instability as a decelerator of replication forks. *Sci Adv* 6: eabb8941, 2020.
- Liu Y, Zhang X, Wang W, Liu T, Ren J, Chen S, Lu T, Tie Y, Yuan X, Mo F, *et al*: Ammonia promotes the proliferation of bone marrow-derived mesenchymal stem cells by regulating the Akt/mTOR/S6k pathway. *Bone Res* 10: 57, 2022.
- Acharjee A, Agarwal P, Nash K, Bano S, Rahman T and Gkoutos GV: Immune infiltration and prognostic and diagnostic use of LGALS4 in colon adenocarcinoma and bladder urothelial carcinoma. *Am J Transl Res* 13: 11353, 2021.
- Principe M, Borgoni S, Cascione M, Chattaragada MS, Ferri-Borgogno S, Capello M, Bulfamante S, Chapelle J, Di Modugno F, Defilippi P, *et al*: Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. *J Hematol Oncol* 10: 16, 2017.
- Ding L, Cao J, Lin W, Chen H, Xiong X, Ao H, Yu M, Lin J and Cui Q: The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. *Int J Mol Sci* 21: 1960, 2020.
- Sun L, Suo C, Li ST, Zhang H and Gao P: Metabolic reprogramming for cancer cells and their microenvironment: Beyond the Warburg Effect. *Biochim Biophys Acta Rev Cancer* 1870: 51-66, 2018.
- Fang Y, Shen ZY, Zhan YZ, Feng XC, Chen KL, Li YS, Deng HJ, Pan SM, Wu DH and Ding Y: CD36 inhibits β -catenin/c-myc-mediated glycolysis through ubiquitination of GPC4 to repress colorectal tumorigenesis. *Nat Commun* 10: 3981, 2019.
- Zhang M, Song H, Yang S, Zhang Y, Tian Y, Wang Y and Liu D: Deciphering the antibacterial mechanisms of 5-fluorouracil in *Escherichia coli* through biochemical and transcriptomic analyses. *Antibiotics* 13: 528, 2024.
- Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S and Al Moustafa AE: Molecular mechanisms of colon cancer progression and metastasis: Recent insights and advancements. *Int J Mol Sci* 22: 130, 2020.
- Zou Y, Liu FY, Liu H, Wang F, Li W, Huang MZ, Huang Y, Yuan XQ, Xu XY, Huang OP and He M: Frequent POLE1 p. S297F mutation in Chinese patients with ovarian endometrioid carcinoma. *Mutat Res* 761: 49-52, 2014.
- Zurlo D, Leone C, Assante G, Salzano S, Renzone G, Scaloni A, Foresta C, Colantuoni V and Lupo A: Cladosporol a stimulates G1-phase arrest of the cell cycle by up-regulation of p21waf1/cip1 expression in human colon carcinoma HT-29 cells. *Mol Carcinog* 52: 1-17, 2013.
- Patil R, Sontakke T, Biradar A and Nalage D: Zinc: An essential trace element for human health and beyond. *Food Health* 5: 13, 2023.
- Haase H and Rink L: Zinc signals and immune function. *Biofactors* 40: 27-40, 2014.
- Pelletier J, Thomas G and Volarević S: Ribosome biogenesis in cancer: New players and therapeutic avenues. *Nat Rev Cancer* 18: 51-63, 2018.
- Villalobo A: Ca²⁺ signaling and Src functions in tumor cells. *Biomolecules* 13: 1739, 2023.
- Zurlo D, Assante G, Moricca S, Colantuoni V and Lupo A: Cladosporol A, a new peroxisome proliferator-activated receptor γ (PPAR γ) ligand, inhibits colorectal cancer cells proliferation through β -catenin/TCF pathway inactivation. *Biochim Biophys Acta* 1840: 2361-2372, 2014.
- Yu C, Hong H, Zhang S, Zong Y, Ma J, Lu A, Sun J and Zheng M: Identification of key genes and pathways involved in microsatellite instability in colorectal cancer. *Mol Med Rep* 19: 2065-2076, 2019.

42. Günther J and Galuska SP: A brief history of galectin evolution. *Front Immunol* 14: 1147356, 2023.
43. Ferlizza E, Solmi R, Miglio R, Nardi E, Mattei G, Sgarzi M and Lauriola M: Colorectal cancer screening: Assessment of CEACAM6, LGALS4, TSPAN8 and COL1A2 as blood markers in faecal immunochemical test negative subjects. *J Adv Res* 24: 99-107, 2020.
44. Watanabe M, Takemasa I, Kaneko N, Yokoyama Y, Matsuo EI, Iwasa S, Mori M, Matsuura N, Monden M and Nishimura O: Clinical significance of circulating galectins as colorectal cancer markers. *Oncol Rep* 25: 1217-1226, 2011.
45. Zhou J, Belov L, Chapuis P, Chan C, Armstrong N, Kaufman KL, Solomon MJ, Clarke SJ and Christopherson RI: Surface profiles of live colorectal cancer cells and tumor infiltrating lymphocytes from surgical samples correspond to prognostic categories. *J Immunol Methods* 416: 59-68, 2015.
46. Passarella S, Schurr A and Portincasa P: Mitochondrial transport in glycolysis and gluconeogenesis: Achievements and perspectives. *Int J Mol Sci* 22: 12620, 2021.
47. Gupta R, Gupta N, Gupta R and Gupta N: Glycolysis and gluconeogenesis. Fundamentals of bacterial physiology and metabolism: 267-287, 2021.
48. Zhou L, Yu X, Li M, Gong G, Liu W, Li T, Zuo H, Li W, Gao F and Liu H: Cdh1-mediated Skp2 degradation by dioscin reprogrammes aerobic glycolysis and inhibits colorectal cancer cells growth. *EBioMedicine* 51: 102570, 2020.
49. Zhou CF, Li XB, Sun H, Zhang B, Han YS, Jiang Y, Zhuang QL, Fang J and Wu GH: Pyruvate kinase type M2 is upregulated in colorectal cancer and promotes proliferation and migration of colon cancer cells. *IUBMB Life* 64: 775-782, 2012.
50. Zhao J, Li J, Hassan W, Xu D, Wang X and Huang Z: Sam68 promotes aerobic glycolysis in colorectal cancer by regulating PKM2 alternative splicing. *Ann Transl Med* 8: 459, 2020.
51. Sethy C and Kundu CN: 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. *Biomed Pharmacother* 137: 111285, 2021.
52. Zou X, Liang J, Sun J, Hu X, Lei L, Wu D and Liu L: Allicin sensitizes hepatocellular cancer cells to anti-tumor activity of 5-fluorouracil through ROS-mediated mitochondrial pathway. *J Pharmacol Sci* 131: 233-240, 2016.
53. Zou K, JU JH and Xie H: Pretreatment with insulin enhances anticancer functions of 5-fluorouracil in human esophageal and colonic cancer cells. *Acta Pharmacol Sin* 28: 721-730, 2007.
54. Zuo T, Zhang Z, Jiang P, Zhang R, Ni D, Yuan Y and Zhang S: Characterization of chikusetsusaponin IV and V induced apoptosis in HepG2 cancer cells. *Mol Biol Rep* 49: 4247-4255, 2022.
55. Raeisi E and Mir LM: 2-NBDG, a fluorescent analogue of glucose, as a marker for detecting cell electroporability in vitro. *J Membr Biol* 245: 633-642, 2012.
56. Lin HC, Chen YJ, Wei YH, Lin HA, Chen CC, Liu TF, Hsieh YL, Huang KY, Lin KH, Wang HH and Chen LC: Lactic acid fermentation is required for NLRP3 inflammasome activation. *Front Immunol* 12: 630380, 2021.
57. Akram M: Mini-review on glycolysis and cancer. *J Cancer Educ* 28: 454-457, 2013.
58. Mondal P, Tiwary N, Sengupta A, Dhang S, Roy S and Das C: Epigenetic reprogramming of the glucose metabolic pathways by the chromatin effectors during cancer. *Subcell Biochem* 100: 269-336, 2022.
59. Zhou Q, Li J, Pang J, Fan F, Li S and Liu H: Gefitinib inhibits glycolysis and induces programmed cell death in non-small cell lung cancer cells. *Nan Fang Yi Ke Da Xue Xue Bao* 40: 884-892, 2020 (In Chinese).
60. Zu XY, Zhang QH, Liu JH, Cao RX, Zhong J, Yi GH and Pizzorno G: ATP citrate lyase inhibitors as novel cancer therapeutic agents. *Recent Pat Anticancer Drug Discov* 7: 154-167, 2012.
61. Zuo Xm, Sun Hw, Fang H, Wu Y, Shi Q and Yu YF: miR-4443 targets TRIM14 to suppress metastasis and energy metabolism of papillary thyroid carcinoma (PTC) in vitro. *Cell Biol Int* 45: 1917-1925, 2021.
62. Shah K and Kazi JU: Phosphorylation-dependent regulation of WNT/Beta-catenin signaling. *Front Oncol* 12: 858782, 2022.
63. Jung YS and Park JI: Wnt signaling in cancer: Therapeutic targeting of Wnt signaling beyond β -catenin and the destruction complex. *Exp Mol Med* 52: 183-191, 2020.
64. van Zuylen WJ, Rawlinson WD and Ford CE: The Wnt pathway: A key network in cell signalling dysregulated by viruses. *Rev Med Virol* 26: 340-355, 2016.
65. Zou Y, Liu L, Meng J and Dai M: Circular RNA circ_0068464 combined with microRNA-383 regulates Wnt/ β -catenin pathway to promote the progression of colorectal cancer. *Bioengineered* 13: 5113-5125, 2022.
66. Zou J, Huang Y, Chen Y, Wu Z, Xie H, Zhou H and Xing C: FOXC2-induced circCASK aggravates colorectal cancer progression by upregulating SIX1 expression. *IUBMB Life* 75: 659-672, 2023.
67. Zhou Y, Gu H, Shao B, Zhang S, Pall H, Peixoto RD, Mok SRS and Zhu G: Glycolysis-related gene dihydrolipoamide acetyltransferase promotes poor prognosis in hepatocellular carcinoma through the Wnt/ β -catenin and PI3K/Akt signaling pathways. *Ann Transl Med* 10: 1240, 2022.
68. Zhou J, Su CM, Chen HA, Du S, Li CW, Wu H, Tsai SH and Yeh YT: Cryptanshinone inhibits the glycolysis and inhibits cell migration through PKM2/ β -catenin axis in breast cancer. *Oncotargets Ther* 13: 8629-8639, 2020.
69. Wenxuan L, Liu L, Zhang L, Qiu Z, Wu Z and Deng W: Role of gonadally synthesized steroid hormones in the colorectal cancer microenvironment. *Front Oncol* 13: 1323826, 2023.



Copyright © 2025 Li et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.