GLDC promotes colorectal cancer metastasis through epithelial-mesenchymal transition mediated by Hippo signaling pathway

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Research Article

Abstract

Metastasis remains the leading cause of death in cancer patients, and Epithelial-Mesenchymal Transition (EMT) plays a decisive role in cancer metastasis. Recently, abnormal expression of Glycine Decarboxylase (GLDC) has been shown in the development of tumors, and GLDC is up-regulated in cancers such as lung cancer, prostate cancer, bladder cancer, and cervical cancer. However, the exact role of GLDC in colorectal cancer (CRC) progression remains to be elucidated. The aim of our study was to explore the action of GLDC in CRC metastasis. The GSE75117 database was used to investigate GLDC expression in tumor center and invasive front tissues and found that GLDC expression level was higher in invasive front tissue. GLDC expression level was negatively correlated with CRC patient prognosis. In vitro studies showed that GLDC could promote invasion and migration of CRC cells by inhibiting the Hippo signaling pathway and modulating the EMT process. Blocking the Hippo signaling pathway with Verteporfin reduced the effect of GLDC on CRC metastasis. In vivo metastasis experiments further confirmed that tail vein injection of GLDC$^{+/+}$ cells promoted lung metastasis, compared with normal CRC cells. The results of this study suggested that GLDC promotes EMT through Hippo signaling pathway in CRC metastasis, providing a new therapeutic target for CRC metastasis.

Introduction

Colorectal cancer (CRC) has the third highest incidence and the second highest mortality among cancers worldwide[1]. It is estimated that by 2030, there will be approximately 2.2 million new cases of CRC and 1.1 million deaths from CRC worldwide[2]. Surgery is the treatment of choice for CRC, but about 50% of patients with CRC develop metastasis within 2 years after radical resection, and the survival rate of patients with CRC metastasis is only 10%[3].

Glycine Decarboxylase (GLDC) gene is located on the short arm of chromosome 9 (9p24.1). It was first discovered that its main effect was to decompose glycine into S-aminomethyl dihydrofatty acyl protein, etc. Abnormal expression of GLDC has recently been implicated in the development of multiple types of tumors[4, 5, 6]. Zhang WC *et al* reported that GLDC was up-regulated in lung cancer, prostate cancer, bladder cancer, cervical cancer and other tumors, and lung cancer patients with higher GLDC expression had worse prognosis[7]. In addition, Li X *et al* reported that GLDC was able to promote the proliferation of p53-mutated B lymphoma cells[6]. We found that the level of GLDC in CRC metastases was higher than that in early-stage patients, compared to non-tumor patients, and the expression level of GLDC in CRC metastases was significantly higher than that in carcinoma in situ tissues, and CRC patients with higher GLDC had worse prognosis and a lower survival rate. We further investigated that GLDC could promote invasion and metastasis of CRC cells.

GLDC can regulate biological processes through its metabolites or affecting the expression of other genes or proteins[4, 8]. Go MK *et al* reported that GLDC promotes tumor formation by synthesizing serine through decarboxylation of glycine[8]. Woo CC *et al* found that GLDC can promote pyruvate and lactate

metabolism, thereby promoting the development of liver cancer, prostate cancer, cervical cancer, lung cancer and other swollen tumors[9]. In addition, GLDC is also able to participate in disease formation by regulating gene expression. Wei HY *et al* found that GLDC was able to up-regulate DNA methyltransferases (DNMTs) and promote lung cancer metastasis[4]. We further found that GLDC reduced phosphorylation levels of STE20-Like Kinase 1 (MST1) and Yes Associated Protein 1 (YAP) in CRC cells. MST1 and YAP are core components of the Hippo signaling pathway. The study of Hippo signaling pathway began with the discovery of the tumor suppressor gene- "Wts" in fruit fly in 1995. In mammals, the core components of the Hippo signaling pathway include MST1/2, Salvador family WW domain protein 1 (SAV1), LATS1/2 (Wts in Drosophila), and MOB kinase activator 1 (MOB1), of which SAV1 and MOB1 are adapter proteins that respectively bind to MST1/2 and LATS1/2, and enhance their phosphorylation. YAP is a major effector molecule downstream of this pathway and can be directly phosphorylated by LATS1/2. P-YAP binds to protein in the cytoplasm and is subsequently ubiquitinated and degraded, thereby inhibiting the growth promoting, anti-apoptotic functions of YAP. The Hippo signaling pathway negatively regulates YAP activity through this cascade of phosphorylation reactions. In contrast, when the Hippo signaling pathway is inhibited, YAP can be transported into the nucleus to bind to transcription factors such as TEADs to promote expression of target genes. Hippo signaling pathway is mainly responsible for regulating biological processes such as cell proliferation, differentiation, aging, and organ growth[10, 11]. Recent studies have shown that Hippo signaling pathway plays an important role in the regulation of cancer development and stem cell function. Inhibition of Hippo signaling pathway promotes metastasis of breast cancer, lung cancer, liver cancer and other tumors[12, 13, 14]. Guo PD *et al* reported that inhibition of the Hippo signaling pathway was able to promote CRC metastasis[15]. Hippo signaling pathway is regulated by various of signals, and the expression of MST1 can be modulated by apoptotic and stress stimuli, such as Staurosporine, hydrogen peroxide, retinoic acid, and some anticancer drugs [16, 17, 18]. Guo Z *et al* reported that DNMT1 was able to up-regulate MST1 expression in glioma cells[19]. Kuser-Abali G *et al* reported that MYC and EZH2 could jointly regulate MST1 expression in prostate cancer cells[20]. Upstream regulatory mechanisms of Hippo signaling pathway in CRC have not been elucidated. The above studies suggest that Hippo signaling pathway is regulated by GLDC, and Hippo signaling pathway is a potential pathway for GLDC to promote CRC metastasis.

In this paper, we investigated the biological function of GLDC-mediated Hippo signaling pathway in EMT and metastasis of CRC to provide a potential therapeutic target for CRC progression.

Materials and methods

Data processing methods

GEO database was built by the National Center for Biotechnology Information (NCBI) and is a gene expression database and online genome resource that collects high-throughput gene expression data uploaded from research institutes around the world. "Colorectal cancer" was entered as search objects.

Gene expression microarray datasets GSE75117 was selected and downloaded, which based on the GPL16699 platform. GSE75117 includes 133 multiples spatially separated samples which were obtained from 8 patients with metastatic colorectal cancers and 8 with non-metastatic colorectal cancers, from the tumor center (TC), and invasive front (IF). The National Human Genome Research Institute and the National Cancer Institute first launched the Cancer Genome Atlas (TCGA) system in 2006 to map cancer genes, thereby understanding potential pathways of cancer, and improving the ability to inhibit cancer progression, accurately diagnose, and cure cancer. 380 CRC patient samples and 51 normal colorectal tissues genome sequences were downloaded from TCGA database for correlation analysis.

Plasmid construction and cell transfection

Chemically synthesized human GLDC (Gene ID: 2731) gene was used in this study. The GLDC$^{+/+}$ sequence was subcloned into vector (PGMLV-CMV-MCS-3×Flag-PGK-Puro, Genomeditech(Shanghai) Co). Control and GLDC$^{+/+}$ lentiviruses were transfected into SW620 and Lovo with relatively high GLDC expression. Before lentiviral transfection, SW620 and Lovo cells were inoculated into 6-well plates and placed in an incubator at 37°Covernight. After the cells adhered, control lentivirus and GLDC$^{+/+}$ lentivirus were added into wells, Virus Volume =(MOI×Cell Number)/ Virus Titer. 12h after transfection, the medium containing lentivirus was removed and fresh medium was added.

Cell wound healing assay

When the cell growth density reaches more than 90% and the growth status is good, scratch the bottom of the culture dish with a tip and gently wash the detached cells with PBS for 3 times. Verteporfin and 1% fetal bovine serum were added to the drug-treated group, and only 1% fetal bovine serum was added to the control cells and placed in an incubator for continued culture. Photographs were taken at the same location on the scratch at 0h, 24h, and 48h to observe cell wound healing. Each set of experiments was repeated three times. The scratch area was calculated and statistically analyzed with Image J software.

Cell migration assay

Take out cells in a logarithmic growth period, add 0.25% trypsin to digest the cells and prepare into single cell suspension. The cells were diluted with serum-free medium to make the final concentration of cell suspension 2×10^{5}cells/mL. Dilute Matrigel with medium and lay it on the bottom of the chamber. After polymerization, the basement membrane is hydrated. Transwell inserts were placed into 24-well plates, 600μL complete serum medium or complete serum medium containing Verteporfin was added to the lower chamber, and 200 μL cell suspension was added to the upper chamber to ensure uniform spread of cells in the upper chamber. Following incubation for 48h in an incubator, migrating cells were analyzed after paraformaldehyde fixation and crystal violet staining, five views were randomly selected, counted, and photographed with an inverted microscope at 300X magnification.

Western blot assay

Western blot assay
After 48h of cell culture in each group, the medium was discarded, the cells were rinsed three times with PBS. Total protein, cytoplasmatic protein and nuclear protein were extracted according to the procedures of NucleoProtein Extraction Kit (Solarbio, China). The extracted proteins were quantified with BCA and added into SDS-PAGE gel electrophoresis system, after which the proteins in the gel were transferred to PVDF membranes and blocked in 10% BSA. Next, PVDF membranes were incubated overnight at 4°C with primary antibodies, washed three times with TBST and incubated with HRP-labeled Goat Anti-Rabbit IgG for 2h at room temperature. They were visualized by enhanced chemiluminescence, photographed directly and quantified.

Quantitative real-time PCR

Total RNA was extracted with Trizol (Beyotime) according to the instructions. cDNA was synthesized with HiScript III RT SuperMix (Vazyme) using 500 ng of total RNA as template. ChamQ SYBR qPCR Master Mix (Vazyme) was used for real-time PCR analysis. GAPDH was used as a loading control to detect the relative mRNA expression of GLDC. Realtime PCR results were defined by the threshold cycle (Ct), and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. PCR was performed with ABI 7500 instrument (Applied Biosystems, USA). Primers involved were GAPDH, forward primer: 5-TCGGAGTCAACGGATTTGGT – 3, reverse primer: 5- TTCCCGTTCTCAGCCTTGAC – 3; GLDC, forward primer:5-GGCCCATCGGAGTGAAGAAA-3, reverse primer:5- TATCGCAGTTTCCGTGGCTT-3.

Immunofluorescence staining

Place the sterilized cell slides into a 24-well plate, and select cells in a logarithmic growth period to inoculate on the slides, 4×10^4 cells per well. After the cells adhered, the culture medium was discarded, fixed in methanol and permeabilized cells with 0.1% Triton-X-100 and blocked with 5% BSA. First bound with YAP mouse antibody, then stained with Alexa Fluor 488-conjugated goat anti-mouse IgG (Beyotime). Nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI) solution. Cell imaging was performed with Leica SP-8 laser scanning confocal microscope (Leica, Germany).

In vivo study

All animal experimental studies have been approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (PZSHUTCM210913009). Six-week male BALB/c nude mice were purchased from SHANGHAI SLAC LABORATORY ANIMAL CO, and all mice were housed in cages maintained at 24°C-25°C and 60%-65% humidity with 12h light/dark cycle and free access to water and food. After habituated feeding for 1 week, the cancer metastasis model in nude mice was established by tail vein injection of CRC cells in control groups and GLDC^{+/+}groups. Mice in the GLDC^{+/+}+VP group

received an intraperitoneal injection of verteporfin (100 mg/kg) every 3 days for 3 weeks. Nude mice were weighed every 4 days. Nude mice were euthanized 36 days after injection to detect the number of metastatic nodules and collect metastatic tumor tissues.

HE and Immunohistochemistry staining analysis

Tissues from nude mice were fixed in 4% paraformaldehyde for 48h, dehydrated in alcohol, and embedded in paraffin. Tissues were sectioned and stained with routine Hematoxylin and Eosin, and sections of tissues were air-dried and mounted scanned and photographed using Jiangfeng digital pathological section scanner. Following the HE staining procedure described above, tissues from nude mice were embedded, sectioned, and deparaffinized. Next, tissues were treated by adding 3% H_2O_2 to deparaffinized hydrated sections, incubating blocked at room temperature, and adding YAP antibody and corresponding secondary antibody. Sections were visualized using the DAB baseplate toolkit (Vector Laboratories, Burlingame, CA, USA). The sections were visualized with DAB staining solution, nuclei were stained with hematoxylin solution, and finally loaded into slides with neutral gel. The expression of YAP in tissues was observed under a light microscope.

Statistical Analysis

Statistical analysis was performed with SPSS software (version 26.0, USA) and GraphPad Prism 9 (GraphPad Software, USA). Data were expressed as Mean ± SD, One-way ANOVA was used for comparison between multiple groups, and Student's t test was used for difference between two groups. All experiments were independently repeated at least three times, with $P < 0.05$ indicating statistical difference.

Results

GLDC-mediated Hippo signaling pathway is a potential mechanism for CRC metastasis

To investigate the association of GLDC-mediated Hippo signaling pathway with CRC metastasis, we analyzed the expression of GLDC and genes which regulated by Hippo signaling pathway in CRC tissues with GSE75117 dataset. There were 27 samples in the dataset, including 15 invasive front samples and 12 tumor center samples. Data analysis showed that compared with central CRC tissues, the expression levels of GLDC, CTGF and CYR61 in invasive front tissues were higher (Fig. 1A). 380 CRC tissues and 51 normal colorectal tissues were downloaded from TCGA database to analyze the correlation between GLDC expression and the expression levels of ANKRD1, CTGF and CYR61, which regulated by Hippo signaling pathway. The statistical results showed that there was a linear relationship between GLDC expression and the expression levels of CTGF (R = 0.20, $P < 0.005$; Fig. 1B), ANKRD1 (R = 0.14 $P < 0.01$; Fig. 1C), CYR61 (R = 0.22, $P < 0.005$; Fig. 1D) in 380 CRC tissues. 380 CRC patient samples were

downloaded from the TCGA database to analyze the correlation between GLDC expression and mesenchymal cell marker gene expression. MUC1 was signature genes of epithelial cells, FN, CDH21 and VIM were signature genes of mesenchymal cells, while CTNNB1, MMP2 and CDH1 were important signature genes of Epithelial-Mesenchymal Transition (EMT). Analysis showed that the expression levels of GLDC, ANKRD1, CTGF and CYR61 were negatively correlated with MUC1 expression and positively correlated with FN, CDH21, VIM, CTNNB1, MMP2 and CDH1. All have indicated that GLDC may be involved in the EMT process of CRC cells through Hippo signaling pathway (Fig. 1E).

GLDC Promotes EMT in CRC Cells

Bioinformatics analysis have showed that GLDC was closely associated with CRC metastasis, we next observed the role of GLDC in CRC metastasis in vitro. First, we examined GLDC expression in NCM460 and 7 CRC cell lines, and GLDC was highly expressed in SW620 cells and Lovo cells compared with other cells (Fig. 2A). To elucidate the function of GLDC, we overexpressed the GLDC gene in CRC cells. As confirmed by RT-qPCR data, GLDC mRNA expression was markedly upregulated in GLDC$^{+/+}$ group, compared with Empty Vector group. Western blot assay was performed to detect the expression level of GLDC protein (Fig. 2B), and the results were consistent with the RT-qPCR results. We observe the effect of GLDC on CRC cell migration by transwell and wound healing assays. Transwell analysis showed that GLDC$^{+/+}$ group could significantly promote migration of SW620, and Lovo cells compared with Empty Vector group (Fig. 2C). The results of the wound healing assay were similar to those of the transwell assay. SW620 cells and Lovo cells migrating more strongly in GLDC$^{+/+}$ group than that in Empty Vector group (Fig. 2D). EMT is a critical step in the invasion and migration of CRC. When EMT occurs, in order to obtain the motility and invasion ability, many epithelial phenotypes discarded, the phenotype changed, cancer cells detached from the epithelial layer, and a series of significant changes will occur. During this process, expression of E-cadherin, which is epithelial cell marker, is suppressed, initiating the expression of Vimentin, a component of the mesenchymal cytoskeleton. Meanwhile, N-cadherin replaces E-cadherin protein. Activation of Vimentin regulates organelle and membrane-associated proteins and promotes cell motility. Thus, E-cadherin, N-cadherin, Vimentin, and Snail are widely recognized as characteristic proteins of EMT. Observed data showed a significant decrease in the expression of E-cadherin and increase in the protein expression of N-cadherin, Snail and Vimentin in SW620 or Lovo cells in GLDC$^{+/+}$ group compared to Empty Vector group (Fig. 2E). All the results showed that GLDC could promote EMT process, as well as invasion and metastasis of CRC cells.

GLDC Inhibits Hippo Signaling in CRC

A series of studies have shown that Hippo signaling pathway is involved in tumor invasion and metastasis. Activated Hippo signaling promotes the phosphorylation of YAP, and p-YAP binds to proteins in the cytoplasm and remains in the cytoplasm, which is subsequently ubiquitinated and degraded, thereby inhibiting YAP played the role of pro-transfer, anti-apoptosis and other functions. The Hippo

signaling pathway negatively regulates YAP activity through this cascade of phosphorylation reactions. In contrast, YAP can be transported into the nucleus when the Hippo signaling pathway is inhibited, where it binds transcription factors such as TEADs to promote expression of EMT-related genes. To explain the role of GLDC in YAP expression and localization, we examined YAP protein localization in CRC cells after overexpression of GLDC by immunofluorescence. The results showed that GLDC increased nuclear translocation of YAP in CRC cells (Fig. 3A). To further understand the mechanism of GLDC in the Hippo signaling pathway, this study investigated the regulatory effect of GLDC on the expression of Hippo-related proteins, such as p-MST1, MST1, YAP, p-YAP. As shown by the data, $GLDC^{+/+}$ group have higher level of the expression of YAP and less expression of p-MST1 and p-YAP in total protein of CRC cells, compare with Empty Vector group (Fig. 3B). Next, we examined YAP expression in the cytoplasm and nucleus. As shown in Fig. 3C, $GLDC^{+/+}$ was followed by decreased YAP expression in the cytoplasm, decreased p-YAP expression, and increased YAP expression in the nucleus. The above studies showed that GLDC could inhibit the Hippo signaling pathway and promote YAP protein entry in CRC cell nuclear.

GLDC inhibits EMT in CRC cells through the Hippo signaling pathway

To verify that GLDC promotes EMT progression through the Hippo signaling pathway, we observed the effect of Verteporfin on CRC cell migration by transwell and wound healing assays after intervening $GLDC^{+/+}$ CRC cells with 0.5µM Verteporfin. Transwell analysis showed that $GLDC^{+/+}$ significantly promoted the invasion of SW620 and Lovo cells, but the intervention of Verteporfin greatly reduced this effect (Fig. 4A). The results of the wound healing assay were similar, and migration was significantly attenuated after Verteporfin intervention compared with the $GLDC^{+/+}$ group (Fig. 4B). $GLDC^{+/+}$SW620 and Lovo cells were treated with or without Verteporfin for 48h. EMT-related protein levels were measured by Western blot assay. The results showed that $GLDC^{+/+}$ promoted the progression of EMT compared with Empty Vector group, but this effect was substantially attenuated after Verteporfin blocked the YAP-TEAD interaction (Fig. 4C). In summary, GLDC exerts an inhibitory effect on EMT progression by activating the Hippo signaling pathway in CRC cells.

GLDC Regulates Colorectal Cancer Metastasis in Vivo

To validate the role of GLDC in CRC metastasis in vivo, we established metastasis model of nude mice by caudal vein injection, then we divided them into three groups including Empty Vector group, $GLDC^{+/+}$group, and $GLDC^{+/+}$+VP group (Fig. 5A). There was a significant difference in the trend of weight change among the three groups, and weight was the lowest in $GLDC^{+/+}$ group compared with Empty Vector group (Fig. 5B). Assessing the amount of lung metastases in three groups, there were more sites of lung metastases observed in the lungs of $GLDC^{+/+}$ group and fewer lung metastases in $GLDC^{+/+}$+VP group than in $GLDC^{+/+}$ group (Fig. 5C). Metastatic lesions were then excised and examined by HE staining. According to the imaging results, there were more lung metastases and larger areas in the $GLDC^{+/+}$ group compared with Empty Vector group, but metastases were significantly improved in $GLDC^{+/+}$+VP group (Fig. 5D). The expression level of YAP was detected by immunohistochemistry. YAP

reached higher levels in the $GLDC^{+/+}$ group compared to Empty Vector group (Fig. 5E). In summary, the results of this study suggest that GLDC can promote lung metastasis of CRC by modulating the Hippo signaling pathway.

Discussion

In recent years, increasing studies have shown that GLDC plays an important role in the progression of cancer, but its specific mechanism in CRC is still unclear. In our study, data showed that GLDC inhibits the activation of the Hippo signaling pathway and promotes the transport of YAP, an effector protein of the Hippo signaling pathway, to the nucleus, which drives the development of EMT and leads to the metastasis of CRC.

Glycine is a non-essential amino acid and one of the important metabolites in many proteins. High levels of glycine are toxic by conversion to metabolites such as aminoacetone and methylglyoxal. Various studies have demonstrated that glycine metabolism is essential for tumorigenesis[21, 22, 23]. Glycine Cleavage System(GCS) controls glycine decarboxylation and deamination through a multistage reaction to generate CO_2, NH_3, NADH, and 5,10-methylene-THF [24, 25]. GCS is a multi-enzyme complex, consisting of glycine decarboxylase (GLDC, also called P protein), amino-methyltransferase (T protein), dihydrolipoamide dehydrogenase (L protein), and the hydrogen carrier protein (H protein) [26]. GLDC is an oxidoreductase, which is mainly involved in the metabolic process of intracellular amino acids and affects various metabolic pathways such as glycolysis and pyrimidine synthesis. It has also been shown that GLDC is highly active in different cancer cells and plays an important role in cancer metastasis. For example, Woo CC *et al* found that GLDC was able to promote pyruvate and lactate metabolism, thereby promoting tumor development such as liver cancer, prostate cancer, cervical cancer, and lung cancer.

To observe the role of GLDC in CRC, we first downloaded the GSE75117 dataset from GEO database, which included 15 invasive front samples and 12 tumor center tissues. Data analysis showed that the expression levels of GLDC, CTGF and CYR61 were higher in invasive front, compared with tumor center of CRC. Then the correlation between the expression of GLDC and the expression levels of ANKRD1, CTGF and CYR61 regulated by Hippo signaling pathway was analyzed by TCGA database. The results showed that the expression levels of GLDC and CTGF, ANKRD1 and CYR61 were linear in 380 cases of CRC. The expression of genes regulated by Hippo signaling pathway was negatively correlated with the expression of signature genes in epithelial cells, and positively correlated with signature genes in mesenchymal cells and important signature genes for EMT, indicating that GLDC may be involved in the EMT process of CRC through Hippo signaling pathway. To test this conjecture, the effect of GLDC on CRC cell invasion and migration was observed. The data showed that GLDC could promote the invasion and migration of CRC cells, and further studies indicated that GLDC could significantly promote the EMT process of CRC cells, which could be manifested by down-regulation of E-cadherin and up-regulation of N-cadherin, Vimentin and Snail expression in CRC cells. Therefore, the results of the above in vitro experiments showed that GLDC could promote CRC cell invasion and migration by promoting the EMT process, which was similar

to previous results investigating the important role of GLDC in lung cancer, prostate cancer, bladder cancer and cervical cancer.

We then considered the potential mechanism by which GLDC regulates EMT and metastasis in CRC. The Hippo signaling pathway effector protein YAP promotes the expression of target genes by entering the nucleus and binding to transcription factors such as TEADs, triggering the transcription of their downstream target genes and promoting the development and progression of a variety of cancers. The interaction between GLDC and the Hippo signaling pathway increases nuclear entry of YAP and promotes cell migration, invasion, and EMT. Therefore, we further investigated the effect of GLDC on the Hippo signaling pathway. The data suggests that GLDC inhibits the Hippo signaling pathway by decreasing the phosphorylation level of MST1, increases YAP accumulation in the nucleus, and leads to decreased YAP and p-YAP in the cytoplasm. We subsequently tested this mechanism by blocking the nuclear binding of YAP to TEADs and found that intervention of cells with Verteporfin significantly reduced the promotion of CRC metastasis and EMT by GLDC, suggesting an important role of GLDC in promoting EMT in CRC progression in part through the Hippo signaling pathway. Taken together, our findings suggest that GLDC has an important role in the regulation of EMT in CRC cells, and GLDC-mediated Hippo signaling pathway is an important mechanism of CRC metastasis.

Conclusion

In this study, we found that GLDC could inhibit the activation of Hippo signaling pathway, promote YAP protein to enter the nucleus, and then promote the EMT process of CRC and promote the metastasis of CRC. Therefore, GLDC is an important target for CRC metastasis, which provides theoretical support for the subsequent clinical prevention and treatment of CRC metastasis.

Declarations

Competing Interest: The authors declare that there are no conflicts of interest.

Ethics approval: All animal experimental studies have been approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (PZSHUTCM210913009).

Author contributions

Hao Yu and Xueqing Hu conceived and designed the study. Hao Yu and Jiajia Wang analysed the data. Hao Yu and Yingru Zhang drafted the paper. Xueqing Hu, Yan Wang and Huirong Zhu revised the manuscript. All authors read and approved the final manuscript.

Data availability

Data will be made available on request.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. 10.3322/caac.21492.

2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683–91. 10.1136/gutjnl-2015-310912.

3. Barugel ME, Vargas C, Krygier Waltier G. Metastatic colorectal cancer: recent advances in its clinical management. Expert Rev Anticancer Ther. 2009;9(12):1829–47. 10.1586/era.09.143.

4. Wei HY, Feng R, Shao H, Feng B, Liu HQ, Men JL, Zou W. Serum glycine dehydrogenase is associated with increased risk of lung cancer and promotes malignant transformation by regulating DNA methyltransferases expression. Mol Med Rep. 2018;18(2):2293–9. 10.3892/mmr.2018.9214.

5. Li N, Li H, Cao L, Zhan X. Quantitative analysis of the mitochondrial proteome in human ovarian carcinomas. Endocr Relat Cancer. 2018;25(10):909–31. 10.1530/ERC-18-0243.

6. Li X, Cui C, Guo Y, Yang G. Glycine Decarboxylase Expression Increased in p53-Mutated B Cell Lymphoma Mice. Oncol Res Treat. 2015;38(11):586–9. 10.1159/000441595.

7. Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, Ma S, Soh BS, Sun LL, Tai BC, Nga ME, Bhakoo KK, Jayapal SR, Nichane M, Yu Q, Ahmed DA, Tan C, Sing WP, Tam J, Thirugananam A, Noghabi MS, Pang YH, Ang HS, Mitchell W, Robson P, Kaldis P, Soo RA, Swarup S, Lim EH, Lim B. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell. 2012;148(1–2):259–72. 10.1016/j.cell.2011.11.050.

8. Go MK, Zhang WC, Lim B, Yew WS. Glycine decarboxylase is an unusual amino acid decarboxylase involved in tumorigenesis. Biochemistry. 2014;53(5):947–56. 10.1021/bi4014227.

9. Woo CC, Kaur K, Chan WX, Teo XQ, Lee THP. Inhibiting Glycine Decarboxylase Suppresses Pyruvate-to-Lactate Metabolism in Lung Cancer Cells. Front Oncol. 2018;8:196. 10.3389/fonc.2018.00196.

10. Ma S, Meng Z, Chen R, Guan KL. The Hippo Pathway: Biology and Pathophysiology. Annu Rev Biochem. 2019;88:577–604. 10.1146/annurev-biochem-013118-111829.

11. Yu FX, Zhao B, Guan KL. Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer. Cell. 2015;163(4):811–28. 10.1016/j.cell.2015.10.044.

12. Deng Q, Jiang G, Wu Y, Li J, Liang W, Chen L, Su Q, Li W, Du J, Wong CKC, Chen Z, Wang H. GPER/Hippo-YAP signal is involved in Bisphenol S induced migration of triple negative breast cancer (TNBC) cells. J Hazard Mater. 2018;355:1–9. 10.1016/j.jhazmat.2018.05.013.

13. Zhao W, Zhang LN, Wang XL, Zhang J, Yu HX. Long noncoding RNA NSCLCAT1 increases non-small cell lung cancer cell invasion and migration through the Hippo signaling pathway by interacting with CDH1. FASEB J. 2019;33(1):1151–66. 10.1096/fj.201800408R.

14. Ni W, Zhang Y, Zhan Z, Ye F, Liang Y, Huang J, Chen K, Chen L, Ding Y. A novel lncRNA uc.134 represses hepatocellular carcinoma progression by inhibiting CUL4A-mediated ubiquitination of LATS1. J Hematol Oncol. 2017;10(1):91. 10.1186/s13045-017-0449-4.

15. Guo PD, Lu XX, Gan WJ, Li XM, He XS, Zhang S, Ji QH, Zhou F, Cao Y, Wang JR, Li JM, Wu H. RARγ Downregulation Contributes to Colorectal Tumorigenesis and Metastasis by Derepressing the Hippo-Yap Pathway. Cancer Res. 2016;76(13):3813–25. 10.1158/0008-5472.CAN-15-2882.

16. Rawat SJ, Chernoff J. Regulation of mammalian Ste20 (Mst) kinases. Trends Biochem Sci. 2015;40(3):149–56. 10.1016/j.tibs.2015.01.001.

17. Kittur FS, Lin Y, Arthur E, Hung CY, Li PA, Sane DC, Xie J. Recombinant asialoerythropoetin protects HL-1 cardiomyocytes from injury via suppression of Mst1 activation. Biochem Biophys Rep. 2019;17:157–68. 10.1016/j.bbrep.2019.01.004.

18. Arthur E, Kittur FS, Lin Y, Hung CY, Sane DC, Xie J. Plant-Produced Asialo-Erythropoietin Restores Pancreatic Beta-Cell Function by Suppressing Mammalian Sterile-20-like Kinase (MST1) and Caspase-3 Activation. Front Pharmacol. 2017;8:208. 10.3389/fphar.2017.00208.

19. Guo Z, Li G, Bian E, Ma CC, Wan J, Zhao B. TGF-β-mediated repression of MST1 by DNMT1 promotes glioma malignancy. Biomed Pharmacother. 2017;94:774–80. 10.1016/j.biopha.2017.07.081.

20. Kuser-Abali G, Alptekin A, Cinar B. Overexpression of MYC and EZH2 cooperates to epigenetically silence MST1 expression. Epigenetics. 2014;9(4):634–43. 10.4161/epi.27957.

21. Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science. 2012;336(6084):1040–4. 10.1126/science.1218595.

22. Kim D, Fiske BP, Birsoy K, Freinkman E, Kami K, Possemato RL, Chudnovsky Y, Pacold ME, Chen WW, Cantor JR, Shelton LM, Gui DY, Kwon M, Ramkissoon SH, Ligon KL, Kang SW, Snuderl M, Vander Heiden MG, Sabatini DM. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. Nat 2015 Apr 16;520(7547):363–7. doi: 10.1038/nature14363.

23. Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K, Vousden KH. Modulating the therapeutic response of tumours to dietary serine and glycine starvation. Nature. 2017;544(7650):372–6. 10.1038/nature22056.

24. Nakai T, Nakagawa N, Maoka N, Masui R, Kuramitsu S, Kamiya N. Structure of P-protein of the glycine cleavage system: implications for nonketotic hyperglycinemia. EMBO J. 2005;24(8):1523–36. 10.1038/sj.emboj.7600632.

25. Kume A, Koyata H, Sakakibara T, Ishiguro Y, Kure S, Hiraga K. The glycine cleavage system. Molecular cloning of the chicken and human glycine decarboxylase cDNAs and some characteristics involved in the deduced protein structures. J Biol Chem. 1991;266(5):3323−9.

26. Hiraga K, Kikuchi G. The mitochondrial glycine cleavage system. Functional association of glycine decarboxylase and aminomethyl carrier protein. J Biol Chem. 1980;255(24):11671−6.
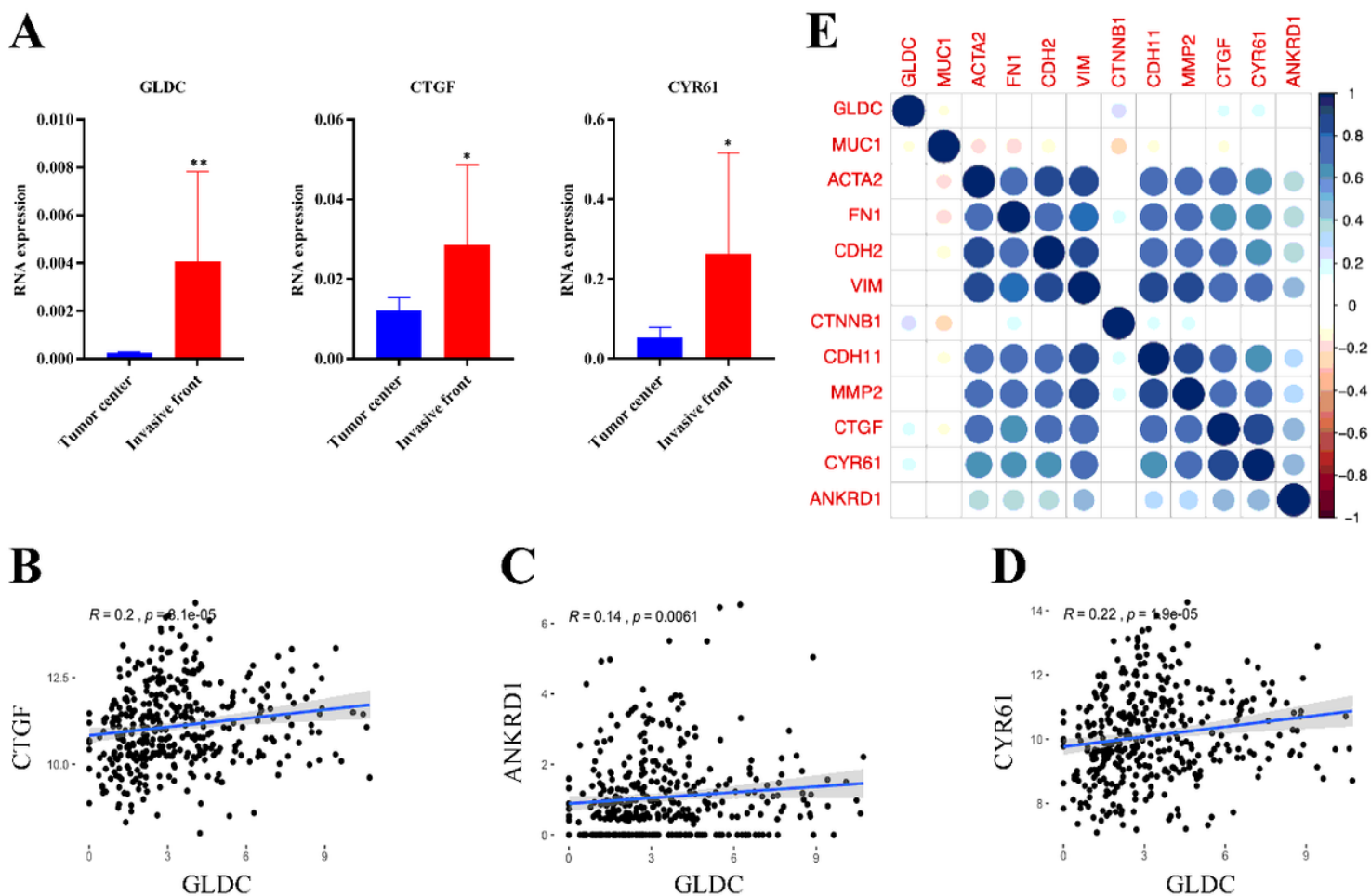
Figures

Fig 1: GLDC-mediated Hippo signaling pathway is a potential target for CRC metastasis. A. Using the results of genome-wide transcription sequencing of GEO database samples, the expression levels of GLDC and Hippo signaling pathway regulatory genes CTGF and CYR61 in tumor center and invasive front were detected. B-D. The correlation between GLDC expression and Hippo signaling pathway in 380 patients with CRC in situ was analyzed by TCGA database. E. The correlation between GLDC expression and mesenchymal stem cell marker gene expression in 380 colorectal cancer patient samples was analyzed using the TCGA database.

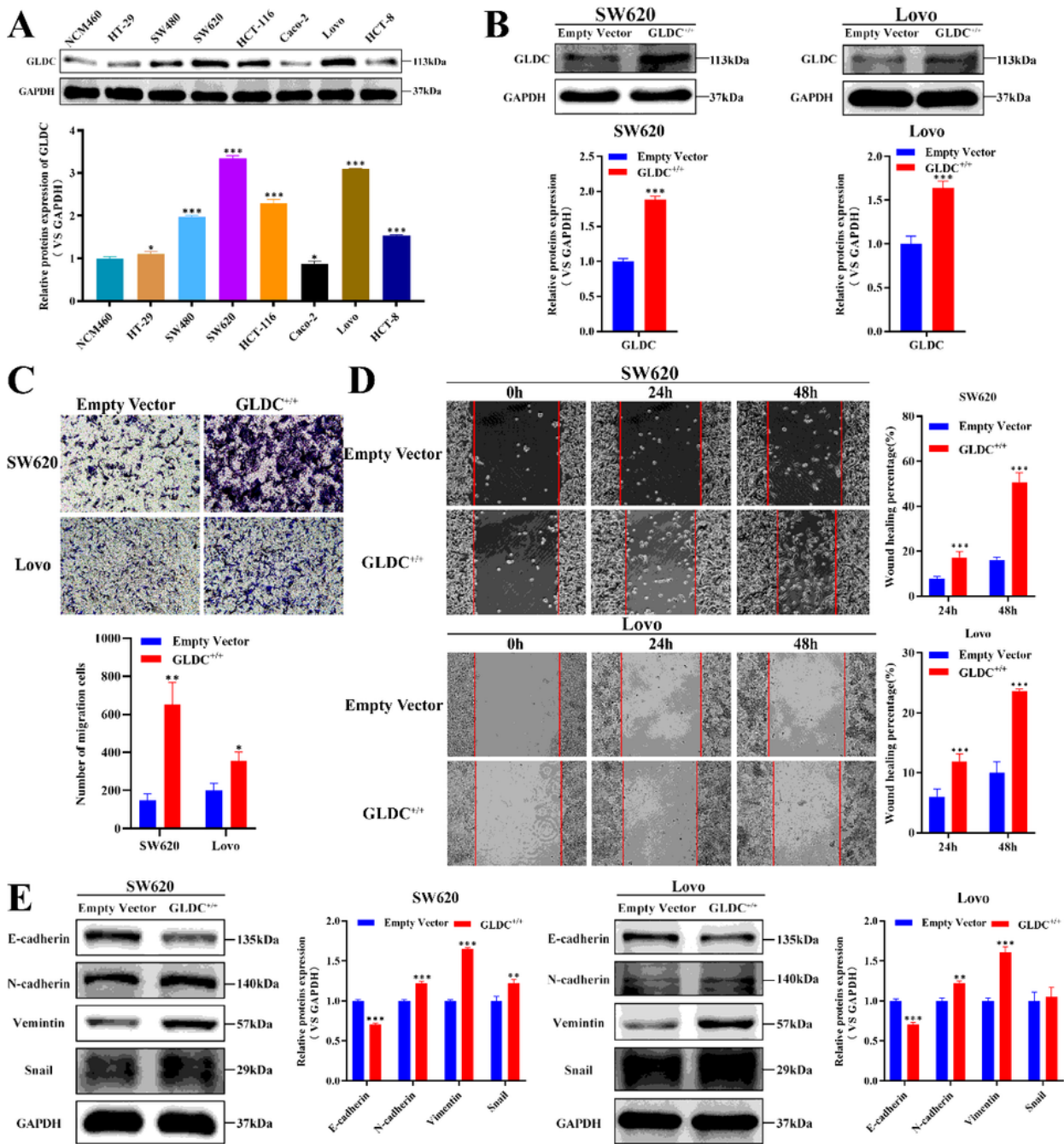## Figure 1

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Fig.2: GLDC promotes the EMT process of CRC cells in vitro. A.GLDC expression in NCM460 and 7 CRC cell lines; B.Western blot assay was used to detect the overexpression efficiency of GLDC protein, SW620 and Lovo cells were transfected with Empty Vector lentivirus or GLDC$^{+/+}$ lentivirus; C. Transwell assay was used to detect the invasion of SW620 and Lovo cells, and the number of invasive cells was expressed as mean ± SD, n = 3; D. Wound healing assay was used to detect the migration of CRC cells; E. Western blot was used to detect the expression of EMT-related proteins in CRC cells, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Figure 2
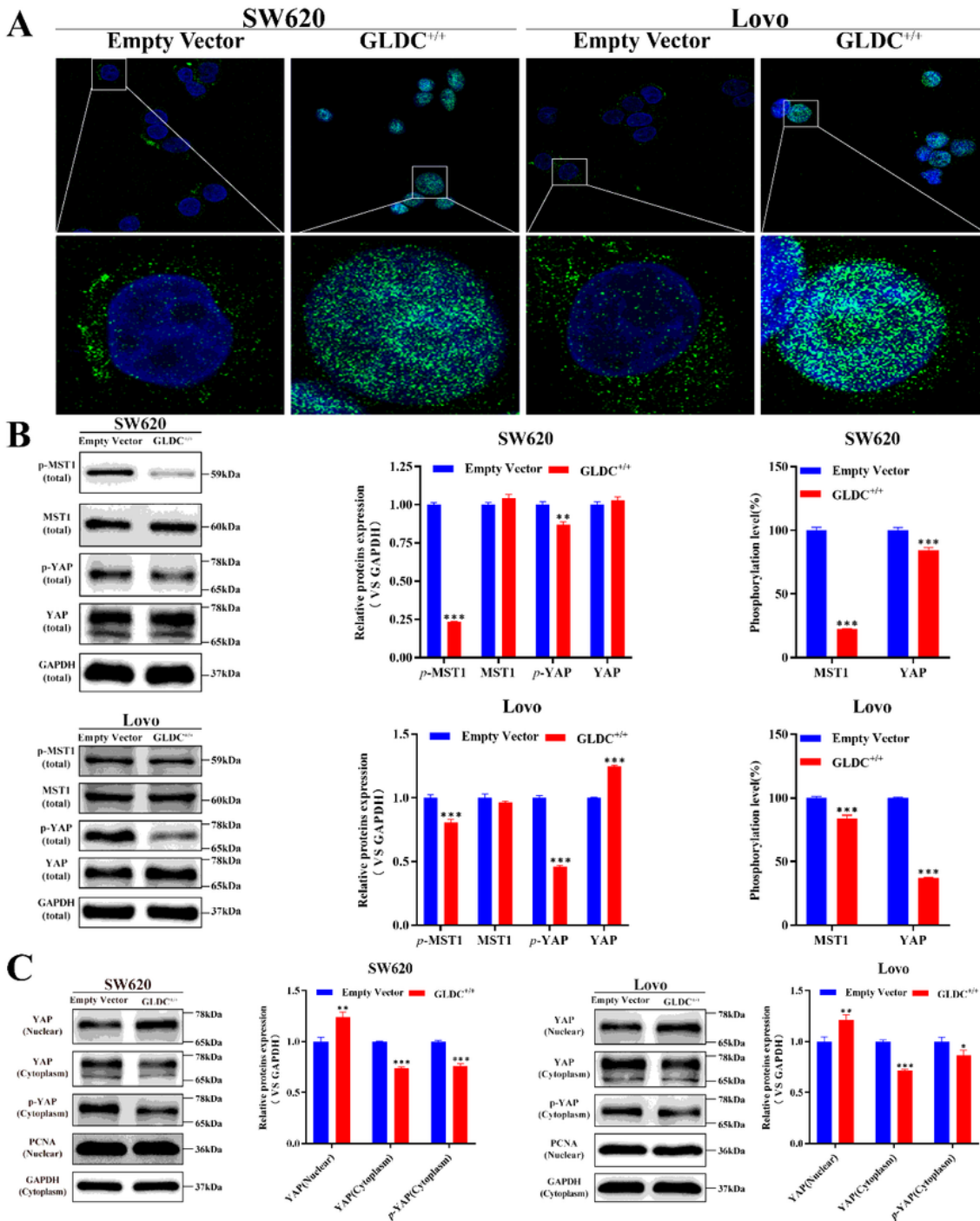
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Fig 3: GLDC inhibits Hippo signaling in CRC cells. A. The expression and localization of YAP in CRC cells after overexpression of GLDC were detected by immunofluorescence. B. Western blot was used to detect the expression of Hippo-related proteins in total proteins in CRC cells. C. Western blot was used to detect the expression of YAP and p-YAP proteins in cytoplasmatic protein and nuclear protein in CRC cells,*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Figure 3
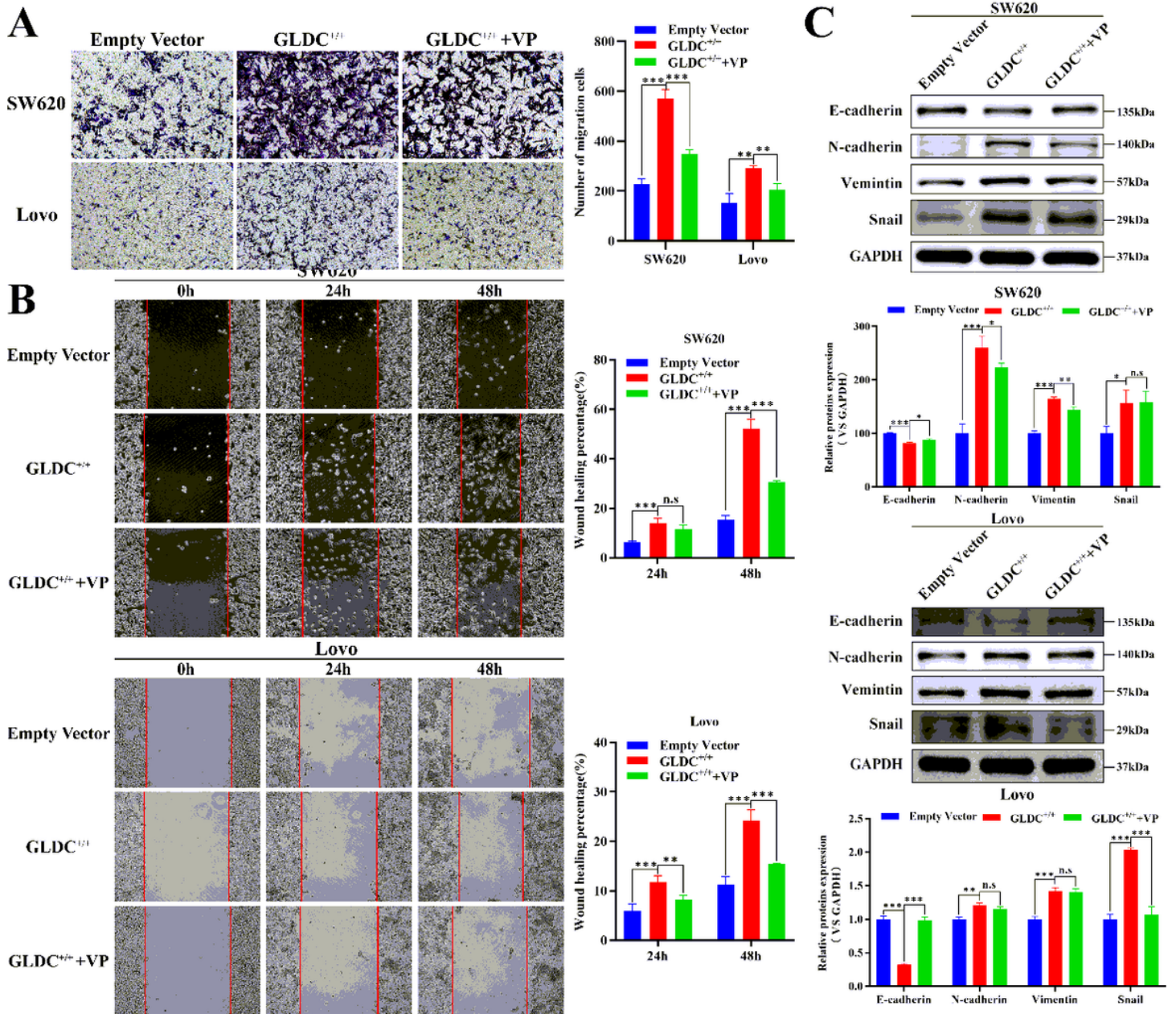
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Fig 4: GLDC inhibits CRC metastasis through the Hippo signaling pathway. A. Transwell assay was used to detect cell migration after intervention with Verteporfin, which could block the YAP-TEAD interaction. B. The effect of inhibiting the Hippo signaling pathway on the migration of CRC cells was examined with wound healing assay. C. Western blot was used to detect the effect of Hippo signaling pathway on the EMT process in CRC cells,*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Figure 4

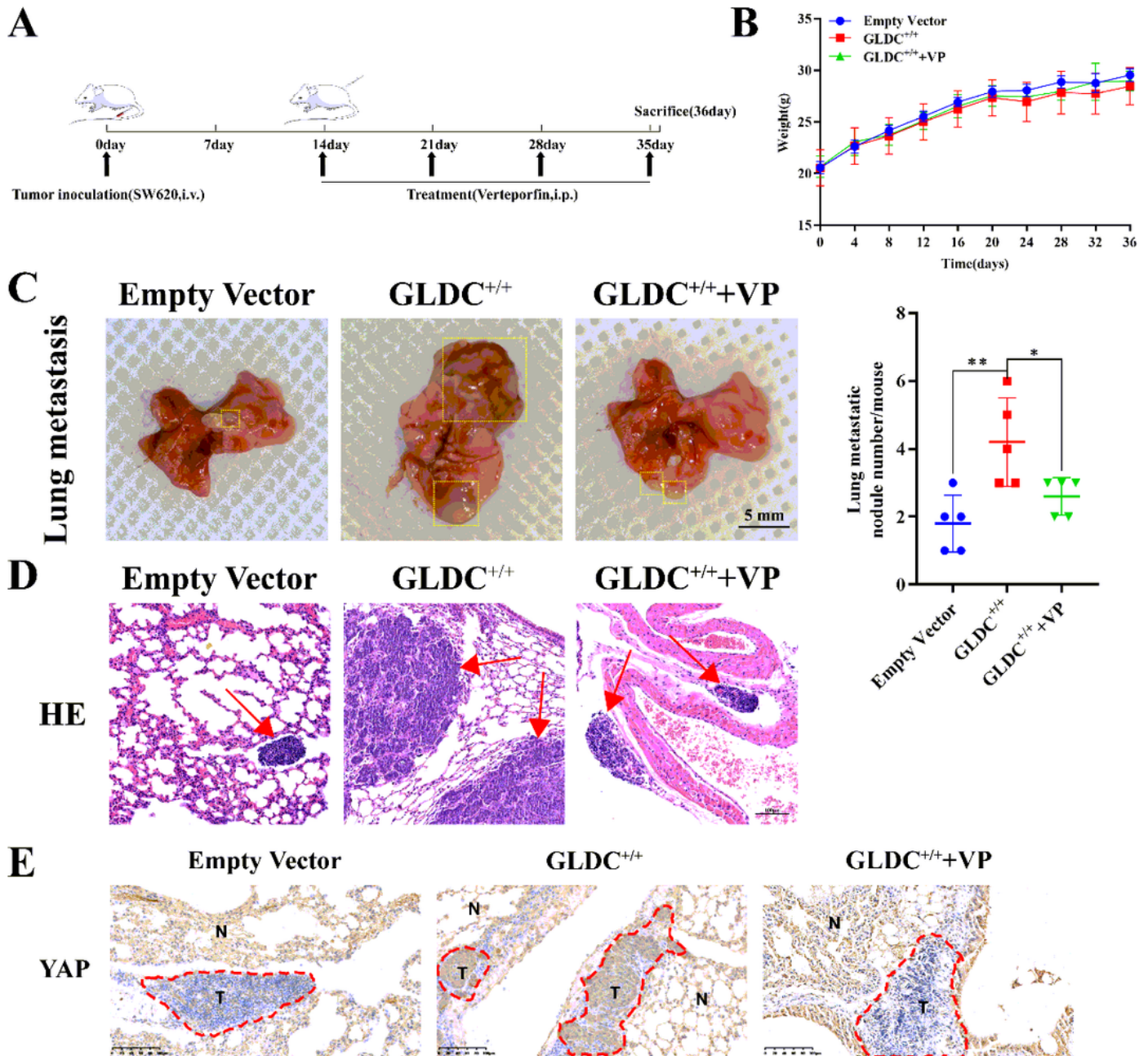Figure legend not available with this version.

Fig 5: GLDC-mediated Hippo signaling pathway promotes CRC lung metastasis in vivo. A. Tail vein injection of SW620 cells was divided into Empty Vector group, GLDC$^{+/-}$ group and GLDC$^{+/+}$+VP group, and intraperitoneal injection of Verteporfin (100mg/kg) was intervened for 3 weeks. B. Weigh nude mice every 4 days. C. Nude mice are sacrificed on day 36, representative images of lung tissue from each group as well as the number of lung metastatic nodules, yellow box, metastatic nodules. D. Representative image of lung hematoxylin-eosin staining; red arrow, metastatic nodule. E. IHC staining detects YAP expression and representative images are shown,T, metastatic tumor; N, normal lung tissue,*, $P < 0.05$, **, $P < 0.01$.

Figure 5

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