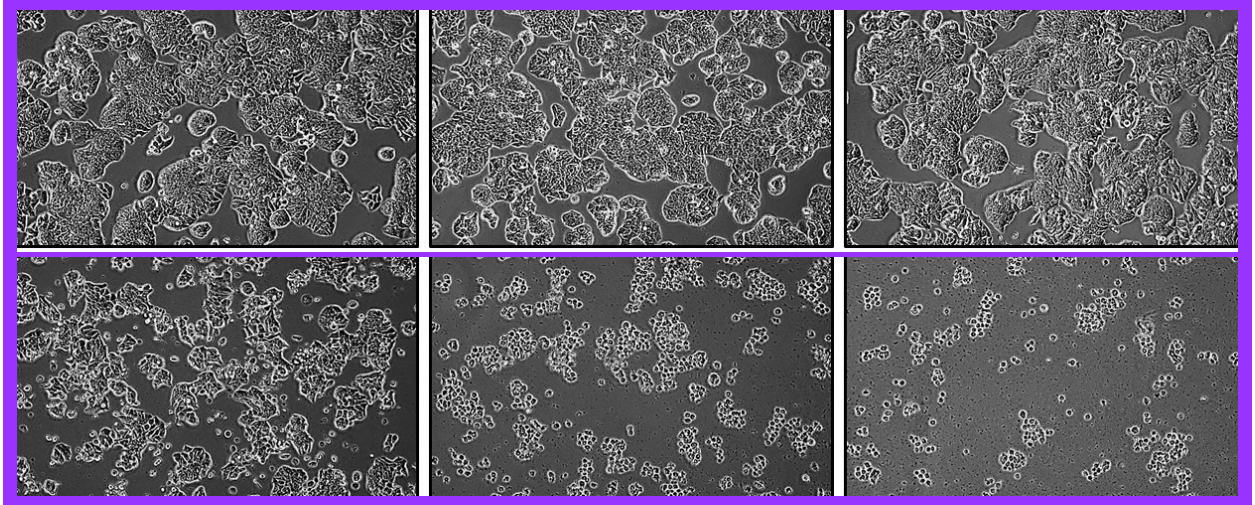

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LINC02582 Sequesters MiR-375 to Facilitate Lung Metastasis in Colorectal Cancer Through Lactate Dehydrogenase B-Mediated Glycolysis

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Abstract

Glycolysis plays a pivotal role in reprogramming the metastatic tumor microenvironment. Numerous long non-coding RNAs (lncRNAs) have been identified as oncogenic, orchestrating glycolytic pathways. In our study, we observed a progressive increase in LINC02582 expression from normal adjacent tissues to colorectal cancer (CRC) specimens, with a marked elevation in Stage IV tumors. Importantly, elevated levels of LINC02582 were associated with diminished survival rates, particularly in patients with pulmonary metastases. Functionally, LINC02582 was found to enhance glycolysis and the invasive potential of CRC cells in vitro, as well as augment their propensity for lung metastasis in vivo. Mechanistically, LINC02582 acts as a competitive endogenous RNA (ceRNA), modulating the expression of lactate dehydrogenase B (LDHB) through sequestration of miR-375. This interaction precipitates increased glycolytic activity in CRC cells, facilitating their invasion and subsequent metastasis to the lungs. Clinically, the concomitant overexpression of LINC02582 and LDHB serves as a prognostic indicator of poor survival outcomes in CRC patients. In summary, our findings designate LINC02582 as an oncogenic lncRNA in CRC, proposing the LINC02582/miR-375/LDHB axis as a novel prognostic biomarker for CRC patients with lung metastasis.

Introduction

Colorectal cancer (CRC) ranks as the second leading cause of cancer-related deaths worldwide, with approximately 30% of these fatalities attributed to lung metastases [1,2]. Notably, the lungs are identified as the secondary most frequent site for CRC metastasis following the liver [3]. Tumor metastasis encompasses a multifaceted process characterized by intricate gene interactions. Beyond the epithelial-mesenchymal transition, the metabolic reprogramming of tumor cells from oxidative glucose metabolism to glycolysis plays a critical role. This shift not only provides ample energy and substrates for cell proliferation but also promotes immune suppression and angiogenesis [4]. Known as aerobic glycolysis or the Warburg effect, this altered glucose metabolism hallmark in cancer cells [5], impacts the expression of genes and their associated signaling pathways, significantly influencing malignant traits including tumor invasion, metastasis, and immune evasion [6-8]. Aerobic glycolysis is particularly pronounced in metastatic cancer cells, which exhibit increased glucose uptake, lactate, and ATP production [9,10]. The mechanisms driving the metabolic reprogramming of cancer cells across different tumor regions vary, underlining the importance of investigating these processes to elucidate the underlying mechanisms of lung metastases in CRC.

Long non-coding RNAs (lncRNAs), defined as RNAs exceeding 200 nucleotides in length without the

capacity for protein encoding, have emerged as pivotal regulators in various biological processes. Recent studies increasingly highlight the involvement of lncRNAs in the metabolic reprogramming of glycolysis, a key event implicated in the distant metastasis of cancer cells [11]. For instance, the lncRNA-SOX2OT has been demonstrated to facilitate hepatocellular carcinoma invasion and metastasis through miR-122-5p-mediated activation of PKM2 [12]. Similarly, in gallbladder cancer, the lncRNA PVT1 is known to expedite tumor progression via the miR-143/HK2 axis [13]. Moreover, in non-small cell lung cancer, the c-Myc-activated lncRNA LINC01123 promotes cellular proliferation and aerobic glycolysis by modulating the miR-199a-5p/c-Myc pathway [14]. Further, lncRNAs associated with aerobic glycolysis have been identified as contributors to tumor recurrence and liver metastasis in colorectal cancer [15]. Therefore, the identification and mechanistic elucidation of novel aerobic glycolysis-related lncRNAs, especially those facilitating distant metastases such as lung metastasis, stand as crucial endeavors for enhancing the prognostic outcomes of patients with CRC.

In this study, we elucidated the critical roles of LINC02582 in colorectal cancer (CRC) progression and metastasis, demonstrating that: 1) LINC02582 is markedly overexpressed in CRC tissues, with the highest expression observed in Stage IV CRC cases; 2) Elevated LINC02582 levels correlate with decreased overall and recurrence-free survival in CRC patients, particularly in those with lung metastases; 3) LINC02582 functions by sequestering miR-375, thereby facilitating lung metastasis of CRC cells via the enhancement of lactate dehydrogenase B (LDHB)-mediated glycolysis; and 4) The regulatory cascade of LINC02582/miR-375/LDHB is indicative of poor survival outcomes in CRC patients. Collectively, our findings underscore the significance of the LINC02582/miR-375/LDHB axis in augmenting glycolysis and lung metastasis in CRC, positioning it as a potential prognostic marker for metastatic lung cancer.

Materials and Methods

Clinical specimens and ethical approval.

Human colorectal cancer (CRC) samples, along with their adjacent normal mucosa, were collected from 67 CRC patients who underwent surgical procedures at Shanghai Cancer Hospital within the period from January 2014 to September 2015. Prior to surgery, these patients had not been subjected to any form of anti-cancer therapy. The clinicopathological diagnoses were independently verified by a minimum of two experienced pathologists, adhering strictly to the guidelines set forth by the American Joint Committee on Cancer (AJCC). The conduct of this study received the green light from the Ethics Committee of Shanghai Cancer Hospital, ensuring adherence to ethical standards. In alignment with ethical protocols, informed consent was duly obtained from all participants prior to their inclusion in the study.

Cell culture

The human normal colon epithelial cell line NCM460, along with colorectal cancer (CRC) cell lines RKO, Caco-2, HCT-8, LoVo, SW480, and SW620, were acquired from the American Type Culture Collection (ATCC). These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, USA), supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, USA). The culturing process was conducted at a constant temperature of 37°C in an atmosphere containing 5% CO2 with humidity. This environment ensures optimal growth conditions for the cell lines involved in the study

Cell transfection

The plasmids designed to overexpress and knockdown LINC02582 were procured from GenPharma (Shanghai, China). Additionally, mimics and inhibitors for miR-375 overexpression were acquired from RiBoBio (Guangzhou, China). For the transfection assays, Lipofectamine 3000 (Invitrogen) was employed following the manufacturer's protocol.

Transwell assay

For the assessment of cell migration and invasion, Transwell chambers equipped with inserts featuring an 8-μm pore size were utilized. The migration assay was conducted using inserts without a Matrigel coating, whereas the invasion assay involved inserts coated with Matrigel. A total of 1×10^5 CRC cells were seeded into the upper chamber in FBS-free culture medium. The lower chamber was filled with culture medium containing 20% FBS to act as a chemoattractant. Following a 24-hour incubation period, cells that had migrated or invaded through the pores were fixed with

paraformaldehyde and subsequently stained with crystal violet for visualization and quantification.

Animal model

To evaluate the influence of LINC02582 on lung metastasis of colorectal cancer (CRC) in vivo, lung metastasis models were established using four-week-old male BALB/c nude mice, maintained under specific pathogen-free conditions. Mice were intravenously injected via the tail vein with 1×10^6 cells/mL (200 µL) of luciferase-tagged CRC cells. These cells were either overexpressing LINC02582, subjected to LINC02582 knockdown, or served as corresponding control groups. Six weeks post-injection, the mice were anesthetized and administered D-luciferin intraperitoneally at a dosage of 150 mg/kg. Imaging was performed 10 minutes after injection using the IVIS Illumina System (Caliper Life Sciences, USA) to evaluate metastatic dissemination.

Following a nine-week period, lung tissue samples were harvested for histopathological examination. Hematoxylin and eosin (HE) staining was utilized to identify and quantify metastatic nodules. The conduct of these animal studies received approval from the Ethics Committee of Fudan University Shanghai Cancer Center, ensuring compliance with ethical standards for animal research.

qPCR and western blot assay

Total RNA from cells was isolated using TRIzol reagent (Invitrogen, CA, USA) and subsequently reverse transcribed into cDNA utilizing the PrimeScript RT reagent kit (TaKaRa, Dalian, China). Real-time quantitative PCR (qPCR) analyses were conducted employing SYBR Premix Ex Taq II (TaKaRa), with U6 or GAPDH serving as internal controls for normalization. The short hairpin RNA (shRNA) sequences targeting LINC02582 are as follows:shLINC02582-1:5'-GAAAUCAAGUGCUGUUUAA-3';shLINC02582-2:5'-UAAAAUUGGUGAGAUGUUCCU-3'.

For protein analysis, cell lysates were prepared using RIPA buffer and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred onto polyvinylidene fluoride (PVDF) membranes, which were incubated with a primary antibody against LDHB (1:1000 dilution) followed by the appropriate secondary antibody. Detection of the proteins was achieved using a chemiluminescence system.

Measurement of glucose uptake, ATP and lactate production and LDH activity

To quantify intracellular glucose levels, the Glucose Assay Kit (BioVision, #K606-100) was utilized following the manufacturer's guidelines. Cellular ATP concentrations were assessed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega), with luminescence readings taken on a Promega luminometer. The results were normalized against the protein concentration in the cell lysates to account for cell number variations. The concentration of extracellular lactate in the culture medium was determined using the Lactate Assay Kit (BioVision, #K607-100), in accordance with the provided instructions.

Furthermore, lactate dehydrogenase B (LDHB) enzymatic activity was measured using the Lactate Dehydrogenase Assay Kit (Colorimetric) (ab102526). The optical density at 450 nm (OD450 nm), indicative of LDHB activity, was recorded using a microplate reader (TECAN, Switzerland). The values obtained were then calculated to determine LDHB activity levels.

RNA immunoprecipitation (RIP) assay

Colorectal cancer (CRC) cells were transfected with plasmids for LINC02582 overexpression or knockdown. Following transfection, RNA immunoprecipitation (RIP) assays using an AGO2 antibody were conducted with the Magna RIP™ Kit (Millipore). Quantitative PCR (qPCR) then quantified LINC02582 and LDHB enrichment, assessing their AGO2 association.

Luciferase reporter assay

The 3'-UTRs of LINC02582 and LDHB, harboring potential miR-375 binding sites, were amplified and cloned into pGL3 vectors. Mutations in these sites were introduced using the QuikChange Site-Directed Mutagenesis kit (Stratagene). CRC cells were co-transfected with either wild-type or mutant luciferase vectors along with miR-375 mimics or inhibitors. Luciferase activity was assessed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

Statistical analysis

Survival rates were evaluated using the Kaplan-Meier method alongside log-rank tests. The 'maxstat' R package facilitated the identification of optimal cut points for continuous variables using maximally selected rank statistics. The association between LINC02582 expression and clinicopathological variables in CRC patients was examined via Pearson χ^2 test or Fisher's exact test. Differences in continuous variables between two or multiple groups were assessed using Student's t-test or one-way ANOVA, respectively. Pearson's rank correlation test analyzed continuous variables' correlations. Data analyses were conducted using SPSS 21.0 and R 3.0 (SPSS Inc., Chicago, IL), considering $p < 0.05$ as statistically significant.

Results

High LINC02582 expression is positively correlated with lung metastasis and high glucose uptake in CRC

To uncover lncRNAs linked with CRC lung metastasis, we first analyzed dysregulated lncRNAs between CRC and normal tissues, and between early (stage I-II) and advanced (stage IV) CRC tumors using the TCGA colon adenocarcinoma lncRNASeq dataset. This analysis identified LINC02582 as significantly upregulated in both total CRCs and specifically in stage IV tumors, suggesting it as a key lncRNA candidate (Figures S1-S(a)-(d), 1(a)-(b), Tables S1-S2). Kaplan-Meier survival analysis further demonstrated that higher LINC02582 expression correlates with poorer overall survival (OS) and recurrence-free survival (RFS) (Figures 1(c), S2).

To validate LINC02582's expression pattern in CRC, qPCR was conducted on 67 CRC tumor and adjacent normal tissue pairs, confirming LINC02582's elevated expression in CRC, especially in advanced stages III-IV and in tumors with lung metastasis (Figures 1(d)-(f)). A detailed analysis of 67 CRC patients revealed LINC02582 expression positively associated with lymph node and lung metastases, and advanced AJCC stages (Table S3).

Positron emission tomography-computed tomography (PET/CT) using fluoro-2-D-deoxyglucose F18 [(18F)-FDG] revealed significantly higher glucose uptake in CRC patients with high LINC02582 expression compared to those with low expression, particularly in patients with lung metastasis (SUVmax values of 3.64 vs. 10.67; Figures 1(g)-(h)). Kaplan-Meier survival analysis further demonstrated that elevated LINC02582 levels were associated with poorer overall survival (OS) and recurrence-free survival (RFS) in a cohort of 67 CRC patients (Figures 1(i)-(j)). Notably, the prognostic significance of high LINC02582 expression for OS was specific to patients with lung metastasis and not observed in the non-lung metastatic group (Figures 1(k) and S3). Collectively, these findings suggest that LINC02582 may drive lung metastasis in CRC through the modulation of glucose metabolism.

LINC02582 promotes CRC cells' migration and invasion in vitro and lung metastasis in vivo

To investigate LINC02582's impact on CRC progression, its expression was assessed in six CRC cell lines and one normal colon epithelial cell via qPCR (Figure 2(a)). Caco-2 and SW480 cells underwent transfection with a LINC02582 overexpression plasmid, while HCT-8 and SW620 cells received a LINC02582 knockdown plasmid (Figures 2(b)-(c)). Transwell assays revealed that

LINC02582 upregulation significantly increased the migratory and invasive capacities of Caco-2 and SW480 cells in vitro (Figure 2(d)). Conversely, LINC02582 downregulation reduced migration and invasion in HCT-8 and SW620 cells (Figure 2(e)). Additionally, SW480 cells with LINC02582 overexpression and SW620 cells with silenced LINC02582, along with their respective controls, were injected into nude mice. Subsequent in vivo imaging and hematoxylin-eosin (HE) staining of lung tissues demonstrated that LINC02582 modulation significantly affected metastatic colony formation in the lungs, with overexpression or knockdown leading to increased or decreased metastasis, respectively (Figures 2(f)-(g)). These findings collectively indicate that elevated LINC02582 expression facilitates CRC cell migration, invasion in vitro, and enhances lung metastasis in vivo.

LINC02582 promotes the lung metastasis of CRC cells via LDHB dependent glycolysis

To investigate the role of glycolysis in colorectal cancer (CRC) progression, CRC cells were subjected to treatment with the glycolytic inhibitor 2-deoxy-D-glucose (2-DG). The treatment attenuated the enhancing effects of LINC02582 upregulation on the migration and invasion capabilities of SW480 cells (Figure 3(a)). Similarly, 2-DG mitigated the impact of LINC02582 downregulation on the migratory and invasive behaviors of SW620 cells (Figure 3(b)), indicating that LINC02582 mediates CRC cell progression through glycolysis.

Further analyses assessed LINC02582's influence on glycolytic activity in CRC cells. Assays related to glycolysis demonstrated that glucose uptake, cellular ATP, lactate levels, and lactate dehydrogenase (LDH) activity were either elevated in LINC02582-overexpressing SW480 cells or reduced in LINC02582-silenced SW620 cells, in comparison to their respective controls (Figures 3(c)-(f)). To decipher how LINC02582 modulates glycolysis, a correlation analysis between LINC02582 expression and glycolytic enzyme levels in CRC tissues from the TCGA dataset was conducted. This analysis revealed a positive correlation between LINC02582 and lactate dehydrogenase B (LDHB) expression in Stage IV CRC tissues (Figures 3(g), S4-S5). Similarly, Pearson's correlation analysis of LINC02582 and LDHB mRNA levels in 67 CRC tissue samples confirmed their positive association (Figure 3(h)). Moreover, LDHB expression levels were correspondingly increased or decreased following the upregulation or downregulation of LINC02582 (Figure 3(i)).

Collectively, these findings illustrate that LINC02582 facilitates CRC progression and lung metastasis by modulating LDHB-mediated glycolysis.

LINC02582 regulates LDHB expression though competitively binding to miR-375

Exploring the mechanism behind LINC02582's regulation of LDHB, we analyzed five miRNA datasets to predict potential miRNAs that might bind to LDHB, identifying miR-375, miR-330-3p, miR-590-3p, and miR-23a-3p as common candidates across three or more datasets (Figure 4(a)). Further analyses, leveraging the TCGA dataset, revealed miR-375's lower expression in CRC tissues compared to normal colon tissues, with its low expression correlating with poor survival outcomes (Figures S6(a)-(h), Table S4). The Targetscan database identified potential miR-375 binding sites (GAACAA) within the 3'UTR of LDHB (Figure 4(b)). Interestingly, the "GAACA" sequence was also found in LINC02582, suggesting its competitive binding to miR-375, potentially regulating LDHB expression. Pearson's correlation analysis in 67 CRC tissues showed a negative association between miR-375 and LINC02582 expression (Figure 4(c)). Western blot analysis confirmed that the effects of LINC02582 overexpression or knockdown on LDHB expression were moderated by transfecting miR-375 mimics or inhibitors (Figure 4(d)).

To determine if LINC02582 regulates LDHB expression via miRNA sponge action, RNA immunoprecipitation (RIP) assays targeting AGO2, a core component of the RNA-induced silencing complex, were conducted. These assays revealed altered LDHB enrichment correlating with LINC02582 expression changes (Figures 4(e)-(f)), indicating LINC02582's competition with LDHB for Ago2-based miRNA-induced repression. Following validation with qPCR (Figure 4(g)), luciferase reporter assays with wild type or mutant miR-375 binding site LINC02582 constructs were performed. These assays showed differential luciferase activity when co-transfected with miR-375 mimics or inhibitors, suggesting miR-375's binding to LINC02582 in CRC cells (Figures 4(h)-(i)). Moreover, qPCR revealed LDHB mRNA levels were inversely affected by miR-375 expression modulation (Figure 4(j)). Luciferase assays of LDHB's 3'UTR with wild or mutant miR-375 binding sites further confirmed miR-375's direct interaction with LDHB mRNA (Figures 4(k)-(l)). Additionally, miR-375 mimics attenuated the upregulation effect of LINC02582 on LDHB 3'UTR luciferase activity, while miR-375 inhibitors reduced the downregulation effect (Figures 4(m)-(n)). Thus, we confirmed LINC02582 enhances LDHB expression by competitively binding to miR-375.

LINC02582 promoted CRC progression and glycolytic though competitively binding to miR-375

Investigating whether LINC02582 accelerates colorectal cancer (CRC) progression by acting as a competitive endogenous RNA (ceRNA) for miR-375, CRC cells were simultaneously treated with a LINC02582 overexpression plasmid and miR-375 mimics, or a LINC02582 knockdown plasmid and miR-375 inhibitors. Transwell assays revealed that miR-375 mimics mitigated LINC02582 overexpression-induced migration and invasion in SW480 cells (Figure.5(a)). Conversely, miR-375 inhibitors countered the reduction in migration and invasion triggered by LINC02582 knockdown in SW620 cells (Figure.5(b)). Glycolysis-related assays indicated that miR-375 mimics diminished the increases in glucose uptake, cellular ATP, lactate levels, and LDHB activity prompted by LINC02582 overexpression in SW480 cells (Figures 5(c)-(f)). Likewise, miR-375 inhibitors reversed the declines in glucose uptake, cellular ATP, lactate levels, and LDH activity resulting from LINC02582 downregulation in SW620 cells (Figures 5(c)-(f)). Furthermore, miR-375 mimics weakened the lung metastasis enhancement caused by LINC02582-overexpressing SW480 cells in nude mice (Figure.5(g)), whereas miR-375 inhibitors partially reversed the lung metastasis suppression observed with LINC02582 knockdown (Figure.5(h)). Collectively, these findings indicate that LINC02582 fosters CRC progression and glycolytic activity by competitively sponging miR-375.

Combination of high LINC02582 and LDHB predicts poor survival for CRC patients.

Kaplan-Meier analysis was employed to assess the prognostic impact of LINC02582 and LDHB expression on colorectal cancer (CRC) patients' outcomes. The analysis revealed that patients exhibiting high mRNA levels of both LINC02582 and LDHB experienced significantly worse overall survival (OS) and recurrence-free survival (RFS) compared to other patients (Fig.6(a)-(b)). Therefore, the concurrent elevation of LINC02582 and LDHB mRNA levels serves as a predictive marker for increased relapse risk and poorer survival in CRC patients.

Discussion

Lung metastasis ranks as the second most frequent distant metastasis and serves as a principal cause of mortality in colorectal cancer (CRC) patients. The current lack of understanding regarding the mechanisms underlying lung

metastasis restricts treatment effectiveness, leading to poor patient survival. Aerobic glycolysis, known as metabolic reprogramming, is a crucial cancer hallmark, recognized for its role in facilitating tumor progression and metastasis through complex intercellular communications [17]. In our research, we found that LINC02582, a lncRNA associated with glycolysis, is upregulated in CRC tissues. Notably, high levels of LINC02582 correlate with a worse prognosis in CRC patients, particularly those with lung metastases. Furthermore, our findings indicate that LINC02582 overexpression enhances CRC cell invasion in vitro, promotes lung metastasis in vivo, and stimulates aerobic glycolysis. Mechanistically, LINC02582 acts as a competitive endogenous RNA (ceRNA) for miR-375, significantly driving aerobic glycolysis and lung metastasis in a LDHB-dependent manner. This study underscores the pivotal role of LINC02582 in CRC lung metastasis, suggesting the need for further investigation and clinical studies to confirm its prognostic value for lung metastasis in CRC.

The complexity of lung metastasis in colorectal cancer (CRC) is underscored by intricate molecular interactions and the pivotal role of key genes [18]. Research focusing on the regulatory mechanisms of these genes offers a promising avenue for refining treatment strategies for affected patients. The application of genomic methodologies, including non-coding RNA and mRNA microarrays, alongside bioinformatic analyses, has become increasingly prevalent in cancer mechanism studies. Leveraging bioinformatics analyses of The Cancer Genome Atlas (TCGA) dataset and corroborating these findings with our own data, we identified LINC02582 as a metastasis-associated gene within CRC, where its elevated expression correlates with diminished survival prospects, particularly for individuals with lung metastasis. This observation aligns with reports of LINC02582 functioning as an oncogene in breast cancer [19], suggesting its potential universality across different cancer types. Such findings emphasize the importance of further investigating LINC02582's roles and mechanisms in facilitating lung metastasis in CRC, thereby enhancing our understanding and approach to managing this aggressive cancer progression.

Elevated invasion, metastasis, and aerobic glycolysis, commonly known as the 'Warburg effect,' are established hallmarks of cancer. As cancer progresses, glycolysis is rapidly harnessed to provide efficient energy for tumor cell growth and plays a crucial role in the formation of a pre-metastatic niche [20]. Our research reveals that LINC02582 overexpression markedly enhances CRC cell migration, invasion, and glycolytic activity both in vitro and manifests as lung metastasis in mouse models. Additionally, PET-CT scans have shown that CRC patients with high LINC02582 expression in tumor tissues consistently exhibit increased SUVmax values in primary tumors, suggesting a significant correlation with the Warburg effect. This observation aligns with the notion that the metabolic reprogramming of cancer cells offers a viable target for optimizing cancer therapy [21]. Our findings further illuminate LINC02582's role in driving glycolytic metabolic reprogramming during CRC cell lung metastasis, presenting it as a promising biomarker for predicting lung metastasis risk.

Long non-coding RNAs (lncRNAs) are well-documented for their ability to act as "molecular sponges," binding microRNAs (miRNAs) through incomplete pairing and thus sequestering them from the 3’ untranslated regions (UTRs) of their target mRNAs. This interaction prevents miRNA-mediated gene silencing, establishing complex networks of competitive endogenous RNA (ceRNA) regulation involving lncRNA-miRNA-mRNA interactions [22, 23]. In this context, we hypothesized that LINC02582 may enhance lung metastasis and glycolysis in colorectal cancer (CRC) through a ceRNA mechanism. Our

bioinformatic analyses and luciferase assays revealed that LINC02582 upregulates lactate dehydrogenase B (LDHB) expression and augments CRC cell invasion and glycolysis in vitro, as well as lung metastasis in vivo, by sponging miR-375. Moreover, an inverse correlation between LINC02582 and miR-375 expression was observed in CRC tissue samples. These results underscore the potential of simultaneously assessing LINC02582, miR-375, and LDHB levels as effective prognostic indicators for CRC patients at risk of lung metastasis.

In summary, our research has uncovered that the glycolysis-associated lncRNA LINC02582 acts as a competitive endogenous RNA (ceRNA), facilitating glycolysis and lung metastasis in colorectal cancer (CRC) cells. Significantly, the synergistic relationship between LINC02582, miR-375, and LDHB emerges as a potential prognostic biomarker for CRC, offering predictive value for the risk of lung metastasis. Moreover, targeting the LINC02582-driven glycolytic pathway presents innovative therapeutic avenues for managing CRC with lung metastasis. Thus, our findings not only propose new methods for assessing lung metastasis risk in CRC patients but also contribute to a deeper understanding of the reprogramming of glucose metabolism associated with tumor metastasis in CRC.

Declarations

Ethics Approval

This research received approval from the Ethics Committee of Shanghai Cancer Hospital (reference number 050432-4-1911D), with all participating patients providing signed informed consent prior to their inclusion in the study. Similarly, the animal experiments conducted as part of this investigation were authorized by the Ethics Committee of Fudan University Shanghai Cancer Center, ensuring adherence to ethical standards for both human and animal research.

Competing Interests

The authors declare no competing interests.

Authors' Contributions

Formal analysis: Wang Song, Fangqi Liu, Bin Quan and Ye Xu; Methodology and Resources: Wang Song, Congcong Zhu, Zhonglin Zhu, Cong Li, Sanjun Cai; Writing – original draft: Wang Song; Writing – review & editing: Wang Song, Ye Xu; Conceptualization: Ye Xu, Bin Quan. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this article and its supplementary information files.

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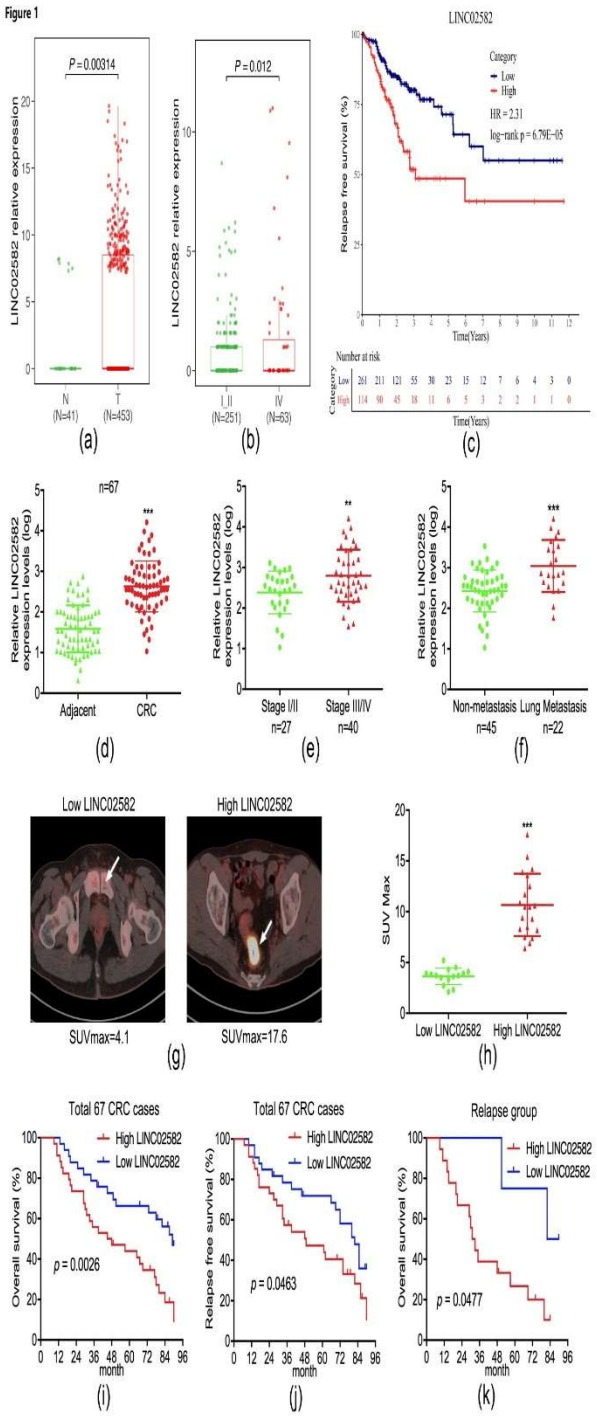

Figure 1: LINC02582 is high expression in CRC tissues and is positively correlated with tumor progression and high metabolism level. (a) Relative expression of LINC002582 in 453 CRC and 41 normal colon tissues form TCGA COAD dataset. (b)Relative expression of LINC002582 in 251 stage I-II and 63 stage IV CRC tissues form TCGA COAD dataset. (c) Kaplan-Meier survival analysis with log-rank test was used to determine the association of LINC02582 and RFS in 375 CRC patients form TCGA COAD dataset. (d) qPCR analyses of LINC002582 expression in 67 CRC cases and paired normal tissues. (e)Levels of LINC002582 expression in stage I-II and stage III-IV CRC tissues. (f) Levels of LINC02582 expression in the primary tumor tissues from lung metastasis and non-metastasis groups. (g-h) Representative 18F-FDG PET/CT images of colorectal cancer patients with low or high LINC002582 expression; Difference analysis of SUVmax in the LINC002582-low and LINC002582-high groups. (i-j) Overall survival (OS) and recurrence free survival (RFS) of 67 CRC patients was analyzed with Kaplan–Meier analysis and a log-rank test according to LINC02582 expression. (k) OS in lung metastasis groups was analyzed with Kaplan–Meier analysis and a log-rank test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

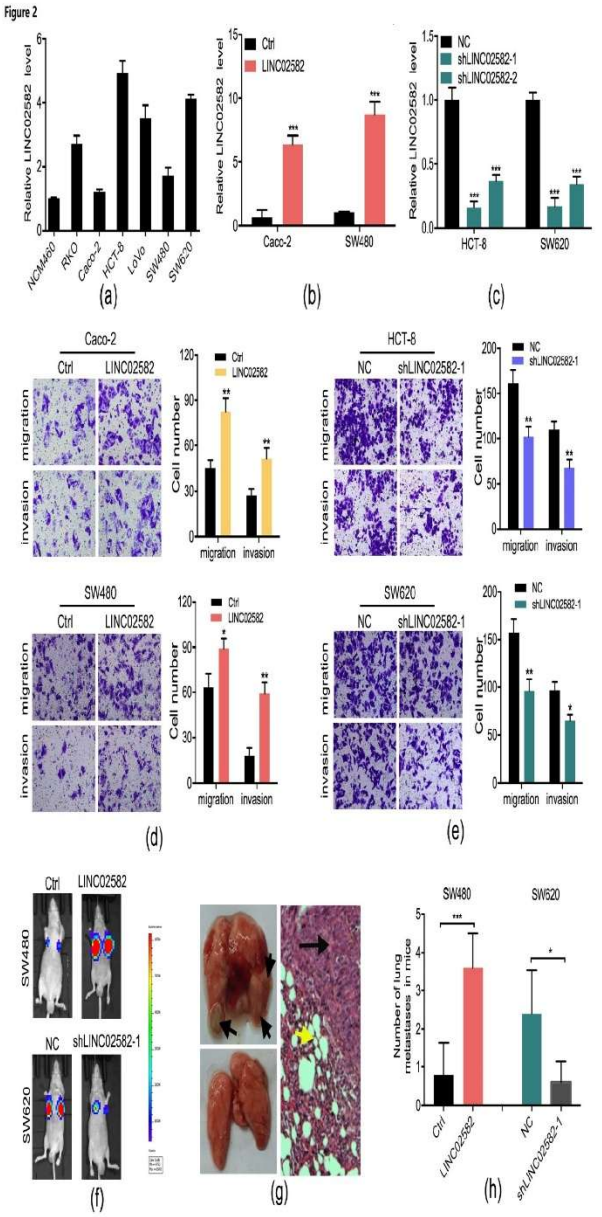

Figure 2: LINC02582 promotes CRC cells migration and invasion *in vitro* and *in vivo*. (a) Relative expression of LINC02582 in 6 CRC cell lines and one normal intestinal epithelial cell. (b) qPCR analyses of LINC02582 expression in Caco-2 and SW480 cells after transfected with LINC02582 overexpression plasmids. (c) Changes of LINC02582 expression in HCT-8 and SW620 cells transfected with LINC02582 knockdown plasmids. (d) The effects of LINC02582 overexpression on migratory and invasive abilities in Caco-2 and SW480 cells. (e) The effects of LINC02582 knock down on migratory and invasive capabilities in HCT-8 and SW620 cells. (f-h) Nude mice were injected with LINC02582 overexpressed SW480 cells or LINC02582 silenced SW620 cells or their control cells via tail vein (n=5). Then Animal in-Vivo Imaging System and HE staining was performed to analyze lung metastasis in nude mice after seven weeks. (f) Representative images of Animal in-Vivo Imaging System analyses. (g) Representative images of mice lung and hematoxylineosin (HE) staining, black arrow indicates tumor nodes. (h) The number of metastatic colony in the HE staining images of lung was counted. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

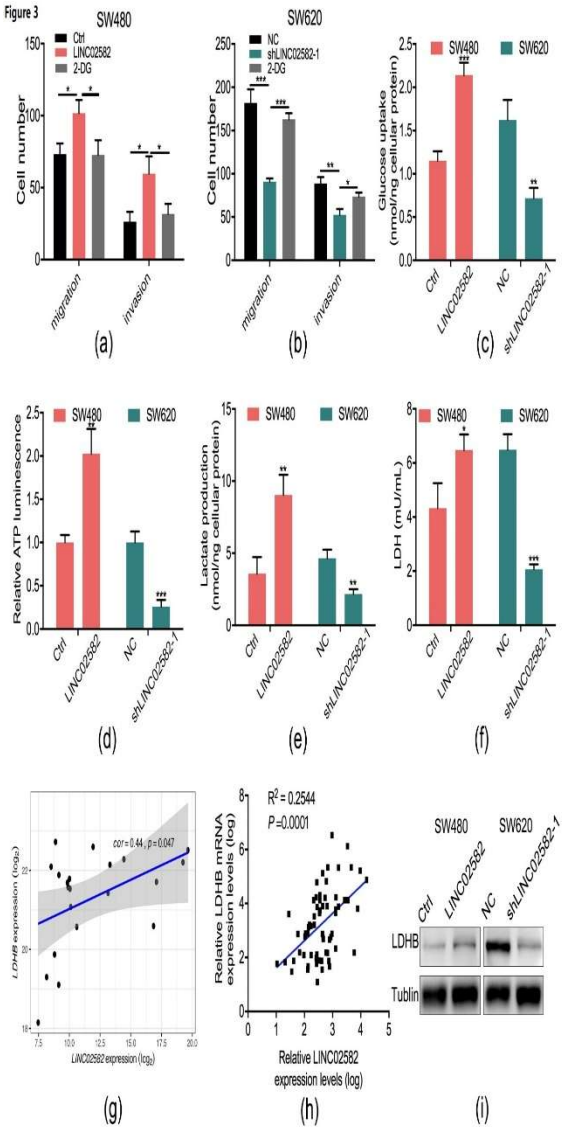

Figure 3: LINC02582 regulates CRC progression via glycolytic. (a-b) The effects of 2-DG on migratory and invasive capabilities in LINC02582 overexpressed SW480 cells and LIN02582 silenced SW620 cells. (c-f) Changes of glucose uptake (c), cellular ATP levels (d), lactate production (e) and LDH activity (f) in SW480 cells transfected with LIN02582 overexpression plasmid or SW620 cells transfected with LINC02582 knockdown plasmid. (g) Correlation analysis of LIN02582 and LDHB expression in stage IV CRC tissues from TCGA dataset. (h) Pearson's correlation analysis of LIN02582 and LDHB mRNA expression in 67 CRC tissues. (i) Western blot assay measured LDHB expression in LINC02582 overexpressed SW480 cells and LIN02582 silenced SW620 cells. * $P < 0.05$; ** $P < 0.01$; ***<0.001.

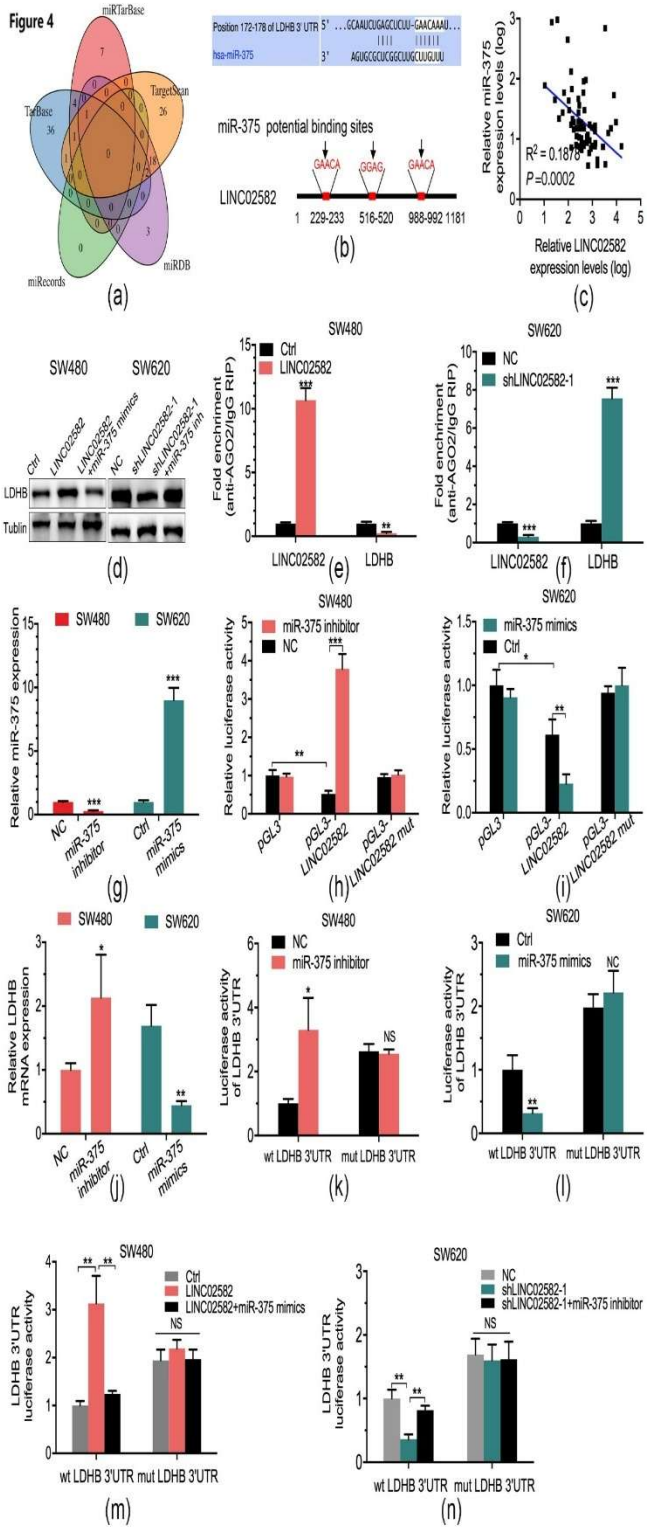

Figure 4: LINC02582 regulates LDHB expression though competitively binding to miR-375 (a) Potential binding miRNAs of LDHB 3'UTR were predicted with five miRNA target databases. (b) Potential binding bases of miR-375 on 3'UTR of LINC02582 and LDHB were predicted

bioinformatically. (c) Pearson's correlation analysis of LIN02582 and miR-375 expression in 67 CRC tissues. (d) Western blot analyzed the change of LDHB regulated by LINC02582 and miR-375. (e-f) AGO2-RIP followed by qPCR to evaluate LDHB level after LINC02582 knockdown or overexpression. (g) Changes of miR-375 after CRC cells transfected with miR-375 inhibitor or mimics (h-i) Effects of miR-375 knock down or overexpression on luciferase reporter activity with the wild-type and mutant LINC02582. (j) Analysis of LDHB mRNA expression after miR-375 knockdown or overexpression. (k-l) Effects of miR-375 knock down or overexpression on luciferase reporter activity with the wild-type and mutant LDHB 3'UTR. (m-n) Effects of miR-375 overexpression or knock down on LINC02582 regulated luciferase reporter activity with the wild-type and mutant LDHB 3'UTR. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS for no significance.

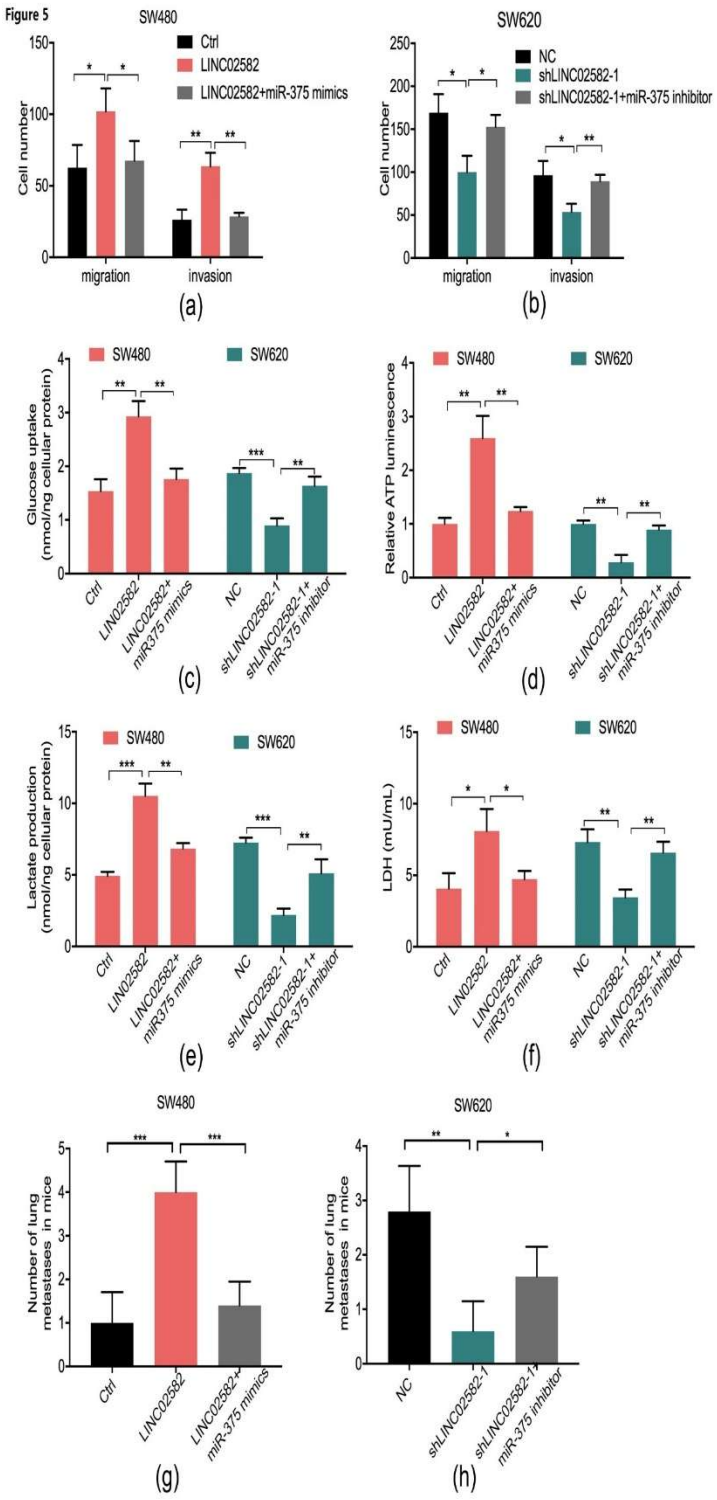

Figure 5: LINC02582 promoted CRC progression and glycolytic though competitively binding to miR-375. (a-b) The effects of miR-375 on migratory and invasive capabilities in LINC02582 overexpressed SW480 cells and LIN02582 silenced SW620 cells. (c-f) The effects of miR-375 on glucose uptake (c), cellular ATP levels (d), lactate production (e) and LDHB activity

(f) in SW480 cells overexpressed LINC02582 and SW620 cells knockdown of LIN02582. (g-h) The effects of miR-375 on the number of metastatic colonies in the lungs of the nude mice from LINC02582 overexpressed SW480 cells(g) and LIN02582 silenced SW620 cells(h). * P < 0.05; ** P < 0.01.

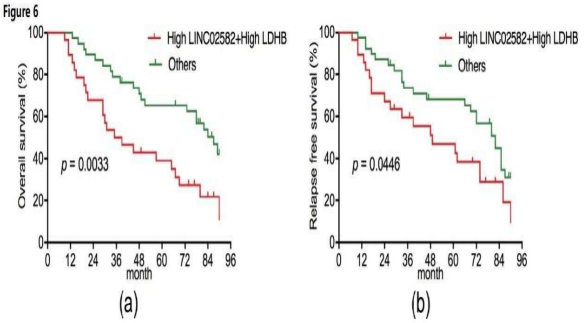

Figure 6: Combination analysis the effects of LINC02582 and LDHB expression on the prognosis of CRC patients. (a-b) Kaplan–Meier analysis with a log-rank test for OS (a) and DFS (b) in 67 CRC patients according to LINC02582 and LDHB mRNA expression.

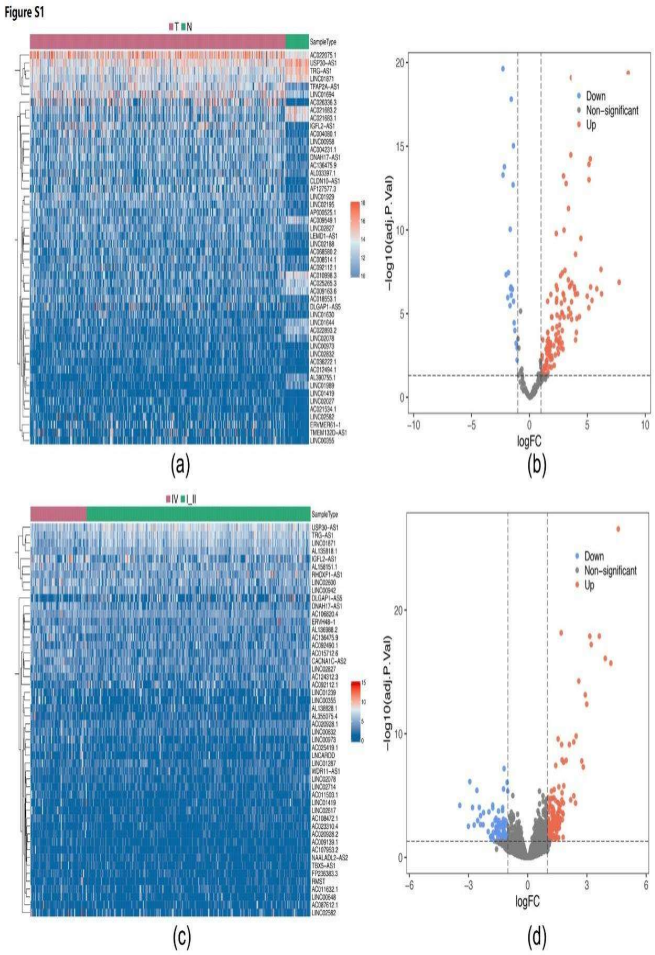

Figure S1. Analysis of lncRNA expression profiles according to the latest colon adenocarcinoma (COAD) lncRNASeq dataset from The Cancer Genome Atlas (TCGA) database. (A and B) Heatmap and volcano plot showing differentially expressed lncRNAs in CRC and normal colon tissues form TCGA COAD dataset. (C-D) Heatmap and volcano plot showing differentially expressed lncRNAs in stage IV and stage I-II CRC tissues form TCGA COAD dataset.

Figure S2

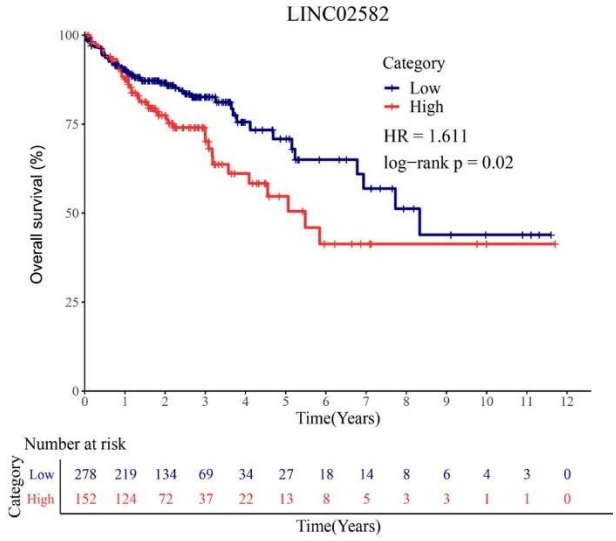

Figure S2. The effects of LINC02582 expression on overall survival of CRC patients. Kaplan-Meier survival analysis with log-rank test was used to determine the association of LINC02582 and OS in 430 CRC patients form TCGA COAD dataset.

Figure S3

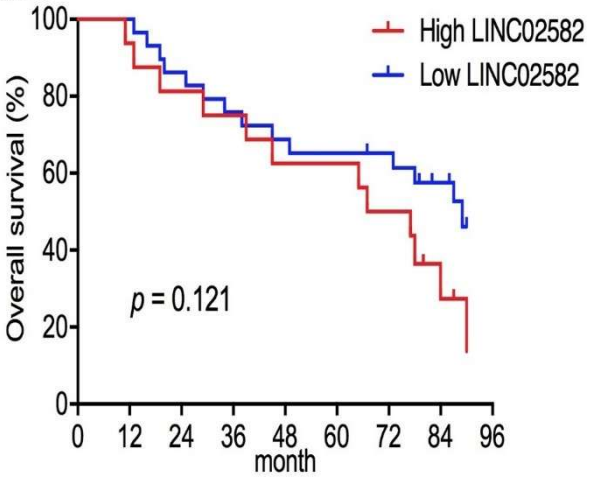

Figure S3. The effects of LINC02582 expression on overall survival of CRC patients without lung metastasis. Kaplan–Meier analysis with log-rank test was used to analyses the OS in non-lung metastasis group according LINC02582 expression.

Figure S4

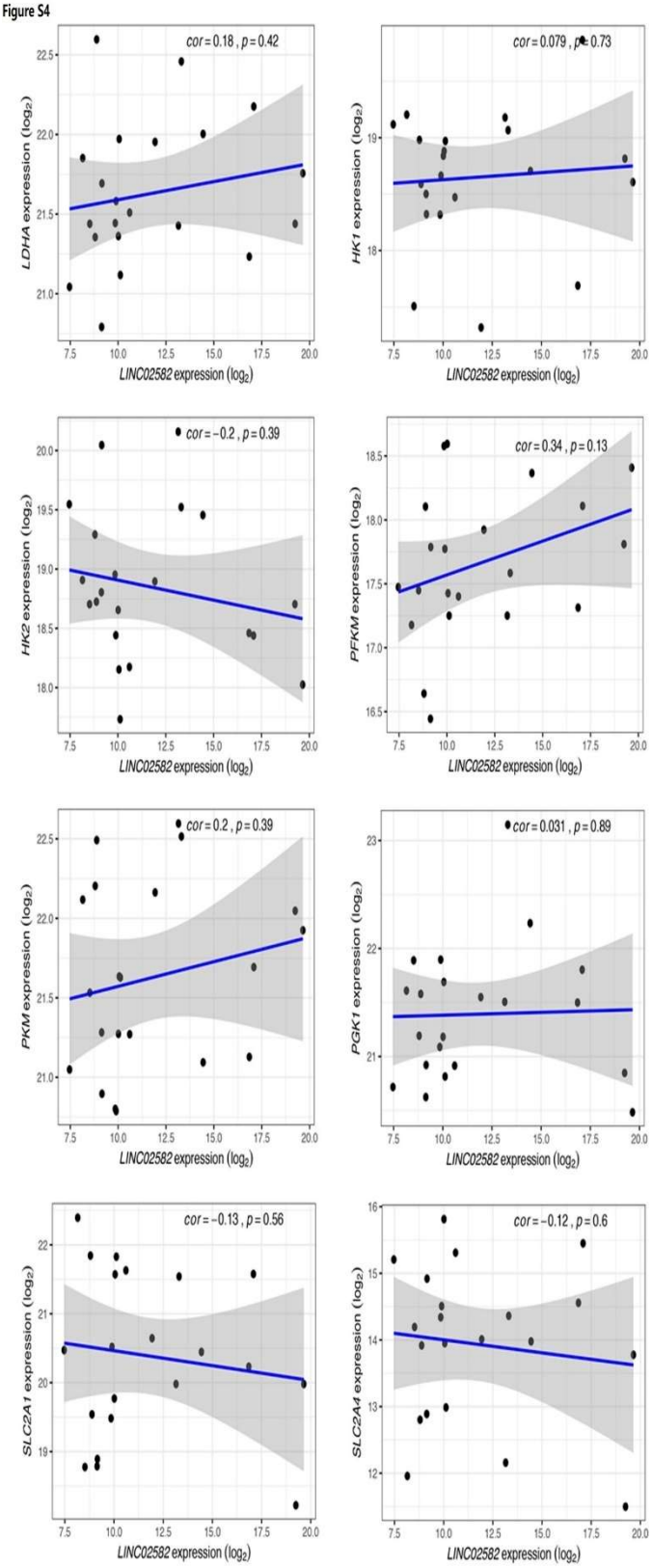

Figure S4. The correlation analysis of LINC02582 and glycolytic related proteins in stage IV CRC tissues from TCGA dataset. Correlation analysis of LIN02582 and LDHA , HK1, HK2, PFKM, PKM, PGK1, SLC2A1, SLC2A4 mRNA expression in stage IV CRC tissues from TCGA dataset.

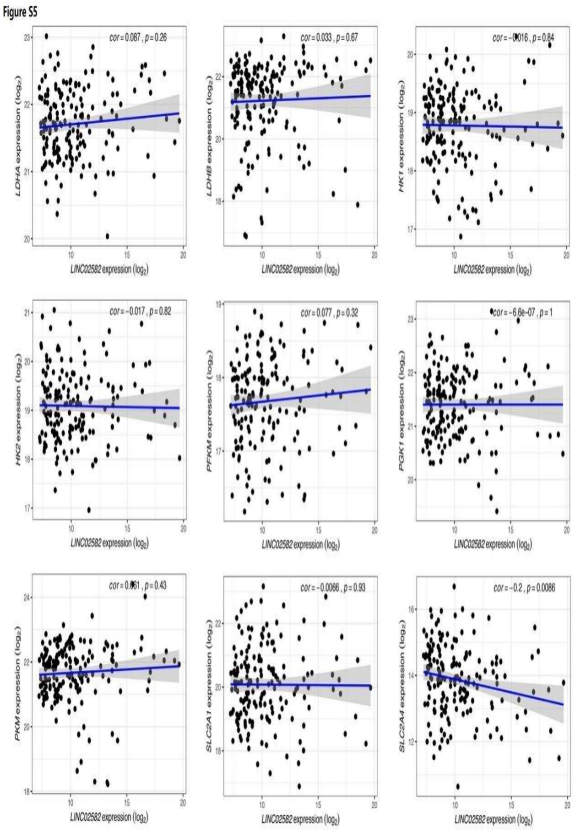

Figure S5. The correlation analysis of LINC02582 and glycolytic related proteins in stage CRC tissues from TCGA dataset. Correlation analysis of LIN02582 and LDHA, LDHB, HK1, HK2, PFKM, PKM,PGK1, SLC2A1, SLC2A4 mRNA expression level in total CRC group from TCGA dataset.

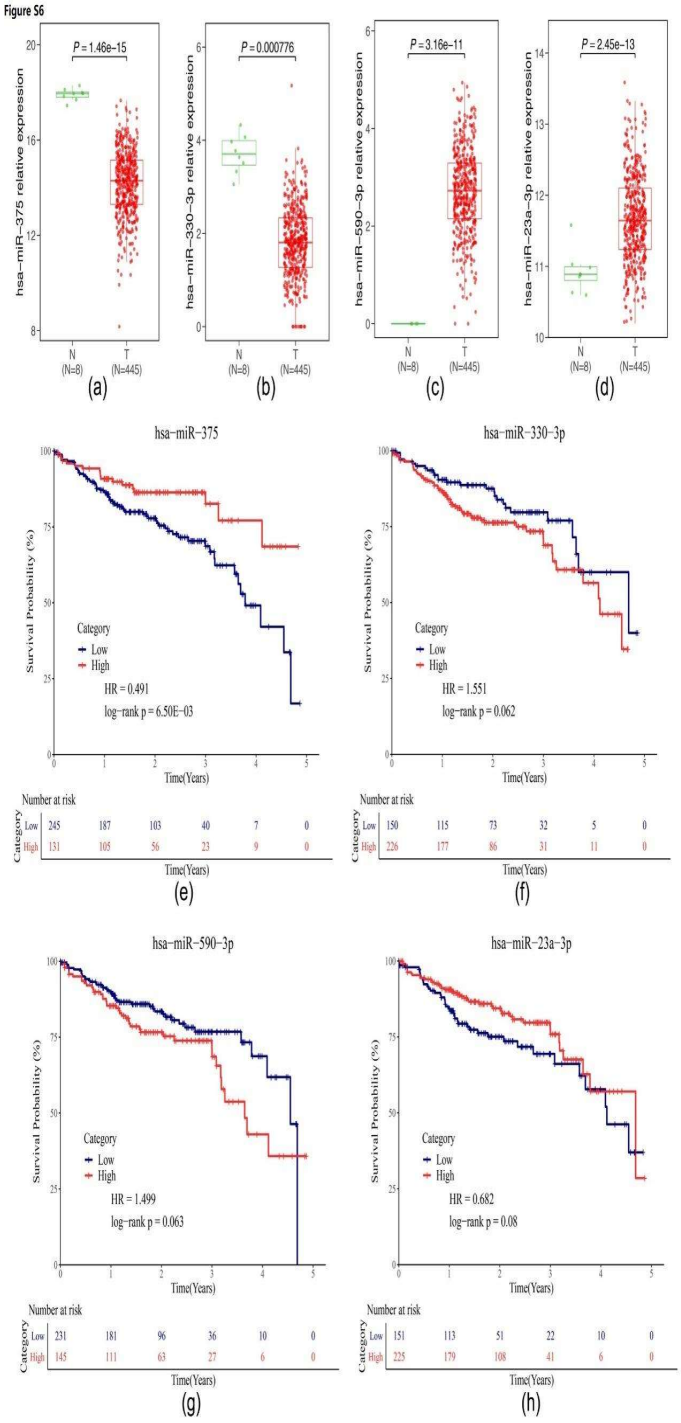

Figure S6 Differential expression analysis and survival analysis of miR-375, miR-330-3p, miR-590-3p, miR-23a-3p according to TCGA dataset. (A-D) Relative expression of miR-375, miR-330-3p, miR-590-3p, miR-23a-3p in 445 CRC and 8 normal colon tissues form TCGA COAD dataset. (E-H) Kaplan-Meier survival analysis with log-rank test for OS according to miR-375, miR-330-3p, miR-590-3p, miR-23a-3p expression in 376 CRC patients form TCGA COAD dataset