

MicroRNA192-5p demethylation inhibits glucose metabolism and angiogenesis in non-small cell lung cancer by targeting EREG/PI3K/AKT

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Abstract

Background

MicroRNA192-5p (miR-192-5p) regulates glucose metabolism to alter cancer metabolism. However, the role and molecular mechanism of miR-192-5p on glucose metabolism proteins in non-small cell lung cancer (NSCLC) are still unclear. The purpose is to investigate the effects of miR-192-5p and miR-192-5p demethylation on glucose metabolism-related proteins and angiogenesis, as well as molecular mechanisms in NSCLC.

Methods

H&E staining was used to detect tissue morphology. IHC and WB were used to measure protein expression. qRT-PCR was used to measure the miR-192-5p level. CCK-8 was used to test cell proliferation. Wound healing and tube formation were used to measure cell migration and angiogenesis. Dual luciferase was used to detect the interaction between miR-192-5p and target genes.

Results

In vivo, the expression of glucose metabolism-related proteins (GLUT-1, HKII, PFKFB3, and LDH) increased in NSCLC, and the level of miR-192-5p decreased. In vitro, overexpression of miR-192-5p or miR-192-5p demethylation inhibited the expression of glucose metabolism-related proteins in A549 cells and the proliferation, migration, and angiogenesis of HUVECs. Mechanistically, miR-192-5p could bind to EREG, reducing the activation of the glucose metabolism regulation pathway PI3K/AKT. DNA demethylation promoted the expression of miR-192-5p and regulated its function.

Conclusion

MiR-192-5p and miR-192-5p demethylation could regulate glucose metabolism and angiogenesis in NSCLC through EREG/PI3K/AKT, providing a new target for targeting glucose metabolism in NSCLC and improving prognosis.

Introduction

The average survival of non-small cell lung cancer (NSCLC) has been improved through new therapeutic strategy, while the five-year survival rate is still only 19% for all stages(1–3). Continued research on relevant molecular mechanisms is needed to improve the prognosis of NSCLC(4, 5).

Unlimited proliferation is a hallmark of cancer(6). How metabolism is reprogrammed in cancer is a key question that drives cancer therapy(7). Evidence reveals that higher levels of glucose transporter-1 (GLUT1) and key glycolysis-related proteins, such as hexokinase2 (HKII), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), and lactate dehydrogenase (LDH), lead to increased glycolysis in cancer, forming an acidic tumor microenvironment, inducing vascular endothelial growth

factor (VEGF) secretion, promoting angiogenesis, and malignant growth of cancer(8–11). The potential of glycolysis in cancer treatment lies in interrupting or disrupting the glycolysis process in cancer, blocking their energy supply, and ultimately inhibiting cancer growth(12). Therefore, starting with the metabolism of NSCLC, targeting glucose metabolism proteins, and studying their mechanisms is a potential molecular therapy for NSCLC.

Research has confirmed that microRNAs (miRNAs) directly or indirectly participate in the regulation of glucose metabolism in cancer cells. MiRNAs alter the glucose metabolism process of cancer cells by positively or negatively regulating key enzymes, signaling pathways, and transcription factors, thereby affecting the biological characteristics of cancer cells(13). MicroRNA-192-5p (miR-192-5p) is a part of the microRNA192 family(14). It is in relation to the progression of many malignant cancers(15). Studies have confirmed that miR-192-5p can exert cancer-suppressive effects by guiding proliferation, migration, and apoptosis(16, 17). More and more evidence suggests that members of the miR-192 family may be involved in the occurrence and development of lung cancer and lung diseases(18–21). Although multiple studies have elucidated the mechanism of miR-192-5p in NSCLC, its mechanism in NSCLC is yet unclear, especially the effect of miR-192-5p on key proteins of glucose metabolism in NSCLC and its related molecular mechanism.

Epigenetic changes play an important role in regulating miRNAs in cancer cells. Abnormal changes in DNA methylation and demethylation are particularly important for regulating miRNA in different types of cancer(14, 22). Research has shown that the occurrence of cancer is characterized by a decrease in the overall methylation level of the genome and an abnormal increase in the local methylation level of CpG islands(21, 23–25). 5-aza-2'-deoxycytidine (5-aza-dC) is a DNA methyltransferase inhibitor with demethylation activity. Multiple studies have shown that the combination of DNA methyltransferase inhibitors and histone deacetylase inhibitors (HDACis) can have a dual impact on non-small cell lung cancer (NSCLC)(26). However, it is unclear whether DNA methyltransferase inhibitors can exert their effects on NSCLC by regulating miR-192-5p.

Therefore, controlling the expression of cancer-related miR-192-5p and studying its related molecular mechanisms is expected to become a new generation of drug models. The objective was to investigate the effects of miR-192-5p on glucose metabolism and angiogenesis in NSCLC, explore possible molecular mechanisms, and observe the regulatory effect of DNA demethylation on miR-192-5p.

Materials and Methods

Clinical samples

Six pairs of NSCLC tissues and adjacent normal tissues were obtained from patients who underwent surgical resection at the Department of Thoracic Surgery, Harbin Medical University Cancer Hospital. The study was approved by the Ethics Committee and Institutional Review Board of Harbin Medical

University Cancer Hospital (approval number: KY2023-19). Written informed consent was obtained from all participants..

H&E

Tissue sections are embedded in paraffin, stained with hematoxylin and eosin, dehydrated with ethanol and xylene, and then sealed with neutral glue. Scan images were taken by using the Nikon DS-F12.

Immunohistochemistry

Six NSCLC and adjacent tissues were fixed for immunohistochemistry. The primary antibodies GLUT-1 (Proteintech, China, 66290-1-Ig, RRID:AB_2881673), PFKFB3 (Proteintech, China, 66290-1-Ig, RRID:AB_2162854), HKII (Wanlei, China, WL02454), LDH (Proteintech, China, 66287-1-Ig, RRID:AB_2881670), as well as the secondary antibodies HRP-sheep anti-mouse IgG (Jackson ImmunoResearch, USA, 111-035-003, RRID:AB_10013389), HRP-sheep anti-rabbit IgG (Jackson ImmunoResearch, USA, 115-035-003, AB_2313599) were used to incubate the tissue sections. Photographs were taken using the Nikon Eclipse Ci-L microscope and Nikon DS-F12 camera system.

Cell Culture and Treatment

A549 (human NSCLC cell line) was acquired from Procell Life Science& Technology (Wuhan, China, SAc0023, RRID:CVCL_0023). Human umbilical vein endothelial cells (HUVECs) were obtained from BeNa Culture Collection (Beijing, China). All cell lines were authenticated by STR profiling and tested negative for mycoplasma contamination. Cells were cultured within 15 passages in a humidified atmosphere at 37°C with 5% CO₂.

Cell Transfection

After A549 cells had adhered, transfection was carried out, and the preparation was as follows: Solution 1: Opti-MEM (Invitrogen, USA, 31985070) and 125μl of Lipofectamine 3000 7.5μl (Invitrogen, USA, L3000) were mixed well. Solution 2: Opti-MEM 125μl + miRNA-192-5p mimic or mimic-NC (RIBOBIO, China) 75 pmol. The two solutions were then mixed. The above solution was slowly dropped into the cells, which were then gently shaken continuously. Cells were detected after 48 h.

qRT-PCR

A total RNA extraction kit was used to extract RNA (TRIpure, BioTeke, Beijing, RP1001). cDNA was obtained using the tailing method (Sangon, Shanghai, China, B532451). The miR-192-5p forward primer:5'-CTGACCTATGAATTGACAGCC – 3'; U6 (GenScript, China) was selected for an internal reference. Gene amplification was fulfilled using PCR. The level of RNA was calculated as $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$.

Western Blotting

Rabbit polyclonal antibodies to PFKFB3 (Proteintech, China, 13763-1-AP, RRID:AB_2162854), VEGFA (Wanlei, China, WL00009b, RRID: AB_3675426), HKII (Wanlei, China, WL02454, RRID: AB_3750793), β -actin (Wanlei, China, WL01372, RRID:AB_2847841), GAPDH (Proteintech, China, 10494-1-AP, RRID:AB_2263076), Phosphatidylinositol 3-kinase (PI3-kinase, Wanlei, China, WL03380, RRID: AB_3750798), AKT (Wanlei, China, WLP001a, RRID:AB_3665475), Epiregulin (EREG, Abmart, China, PK94578S, RRID: AB_3750800), mouse monoclonal antibody to GLUT1 (Proteintech, China, 66290-1-Ig, RRID:AB_2881673), LDH (Proteintech, China, 66287-1-Ig, RRID:AB_2881670) and the fluorescent secondary antibody (IRDye 800 CW goat anti-rabbit, 926-32211, RRID: AB_621843 or IRDye 680RD goat anti-mouse, 926-68070, 1:10000, LI-COR, RRID: AB_10956588) were sequentially incubated on PVDF membranes.

CCK-8

Cells were seeded on a 24-well plate and co-cultured with HUVEC and A549 for 48 hours. Replace the culture medium of HUVEC cells with a mixture of 500 μ l of medium and 50 μ l of CCK-8, and incubate in a 37 °C, 5% CO₂ incubator for 2 hours. Draw 110 μ l of mixed solution from a 24-well plate into a 96-well plate, with 5 wells in each group. For data analysis, measure its OD value at 450 nm on an enzyme-linked immunosorbent assay (ELISA) reader. All assays were performed in three independent biological replicates.

Wound Healing

After A549 and HUVECs were co-cultured for 48 h, the HUVECs culture medium in the lower chamber was superseded with serum-free DMEM. The cells were fostered for 24 hours after being scratched with a pipette tip. The migration distance of each experimental group was calculated. Experiments were performed in three biological replicates.

Tube Formation

Matrigel gels were thawed overnight at 4°C. Matrigel gels were pipetted into 96-well plates. A549 cells and HUVECs were co-cultured for 48 h. The HUVECs were harvested and added to 96-well cell culture plates for 6 h. An electronic microscope was applied for image recording.

Dual-Luciferase

Predict the target genes of miR-192-5p through the online database TargetScan. Construct wild-type and mutant reporter plasmids of the target gene separately, using pmirGLO as the vector. Co-transfection of miRNA mimics and reporter plasmids into cells. Dual-Luciferase Reporter Assay System (Promega company, USA, E1910) was used for sample Luciferase activity detection.

Statistical analysis

Statistical analysis: Data were analyzed using GraphPad Prism 8.0.1 and presented as mean±standard deviation (SD). Unpaired two-tailed Student's t-test was used for comparisons between two groups. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple group comparisons. Each experiment was performed in three independent biological replicates. $P < 0.05$ was considered statistically significant.

Results

Morphological comparison of NSCLC and adjacent tissues

We collected cancer and adjacent tissues of 6 in-patients from the Thoracic Surgery Department of Harbin Medical University Cancer Hospital. All the patients were pathologized as NSCLC. We first performed H&E staining on tissues to observe the morphological differences between non-small cell lung cancer and adjacent tissues. Compared with adjacent tissues, NSCLC tissues mainly show irregular cell arrangement, diverse cell morphology, reduced intercellular matrix, and forming cell-dense areas and cytoplasmic-free areas (shown in Fig. 1).

The expression of glycolysis-related proteins was increased in NSCLC tissues.

The expression of GLUT1, HKII, PFKFB3, and LDH was measured by IHC and WB. The results showed that GLUT1, a protein that regulates the flow of glucose into cells, was increased in NSCLC tissues than NC (shown in Fig. 2A, E, F). Glucose metabolism requires the participation of HKII, PFKFB3, and LDH. Results suggested that HKII was increased in NSCLC than NC (shown in Fig. 2B, E, G). We determined PFKFB3 and revealed that PFKFB3 expression level was elevated in NSCLC (shown in Fig. 2C, E, H). In addition, the LDH level in NSCLC was more than NC (shown in Fig. 2D, E, I).

The level of miR-192-5p in NSCLC was decreased compared with adjacent tissues.

To observe the condition of miR-192-5p in NSCLC, we evaluated miR-192-5p in NSCLC and adjacent tissues by RT-PCR. Results displayed that the miR-192-5p level was lower in NSCLC than in NC (shown in Fig. 2J).

MiR-192-5p overexpression inhibited glycolysis-related proteins in A549 cells.

To further observe the connection between miR-192-5p and glycolysis-related proteins, the following experiments were performed: First, A549 cells were transfected with the miR-192-5p mimic or mimic-NC, and qRT-qPCR was used to determine the effect of transfection. Results illustrated that the transfected A549 cells highly expressed miR-192-5p (shown in Fig. 3a). We then determined the expression levels of glycolysis-related proteins after miRNA-192-5p overexpression by WB. The results showed that compared to the control and mimic-NC groups, miR-192-5p inhibited the expression of GLUT1 (shown in Fig. 3B, C). And the expression levels of the three key proteins of glycolysis, HKII (shown in Fig. 3B, D),

PFKFB3 (shown in Fig. 3B, E), and LDH (shown in Fig. 3B, F), were decreased in A549 cells with high expression of miR-192-5p.

Demethylation promoted the expression of miR-192-5p and inhibited the expression of glycolysis-related proteins.

5-Aza-CdR was used to treat A549 cells, and the regulatory effect of demethylation on miR-192-5p and its impact on glucose metabolism proteins were observed. MiR-192-5p and glycolysis-related proteins were detected by using RT-PCR and WB. The results showed that 5-Aza-CdR could promote the level of miR-192-5p in cells (shown in Fig. 3L). At the same time, results presented that GLUT1 (shown in Fig. 3G, H), HKII (shown in Fig. 3G, I), PFKFB3 (shown in Fig. 3G, J), and LDH (shown in Fig. 3G, K) was reduced after the 5-Aza-CdR intervention compared to the normal group.

MiR-192-5p inhibited proliferation and VEGFA expression in HUVECs induced by A549, and demethylation played a similar role by up-regulating miR-192-5p.

We co-cultured A549 cells and HUVECs and measured the cell proliferation by CCK-8 and VEGFA by WB in HUVECs. Results demonstrated that compared with the control group, the proliferation ability and the expression of VEGFA in HUVECs increased after co-incubation of A549 and HUVECs, whereas the proliferation ability and the expression of VEGFA were reduced after co-incubation in overexpressing miR-192-5p A549 cells and HUVECs (shown in Fig. 4A, B, C). After 5-Aza-CdR intervention in A549 cells, the proliferation ability and the expression level of VEGFA in HUVECs decreased (shown in Fig. 4A, B, C).

MiR-192-5p inhibited migration and neovascularization of HUVECs induced by A549, and demethylation regulated the influence of miR-192-5p on migration and neovascularization.

We then used wound healing to observe the impact of miR-192-5p on the migration ability of HUVECs induced by A549 and the regulatory effect of 5-Aza-CdR (shown in Fig. 4D, E). Results manifested that, the migration ability of HUVECs increased after co-incubation with A549 cells. The migration ability of HUVECs decreased after miR-192-5p overexpression. To observe the regulatory effect of 5-Aza-CdR on miR-192-5p, we co-incubated A549 with HUVECs and intervened A549 with 5-Aza-CdR. The results conveyed that the migration ability of HUVECs decreased after the intervention of 5-Aza-CdR.

Tube formation was used to evaluate the impact of miR-192-5p on tube formation in HUVECs induced by A549 and the regulatory effect of 5-Aza-CdR (shown in Fig. 4F, G). Our results showed that A549 cells could promote tube formation in HUVECs compared to normal controls (shown in Fig. 4F, G). Tube formation of HUVECs was significantly reduced after the transfection of A549 cells with miR-192-5p overexpression. Additionally, the tube-forming ability of HUVECs was attenuated after the 5-Aza-CdR intervention.

MiR-192-5p targeted EREG and reduced the expression of EREG.

The target genes of miR-192-5p were predicted by TargetScan online database, and it was found that EREG might be a potential target of miR-192-5p; After determining the potential binding site between miR-192-5p and EREG, it was verified by dual-luciferase reporter assay experiment (Fig. 5A-C). The results showed that in A549 cells, miR-192-5p over-expressing decreased the luciferase activity of EREG 3'UTR reporter plasmid, while the luciferase activity was not significantly affected by miR-192-5p binding site mutation (Fig. 5D).

Over-expressed miR-192-5p reduced the activation of the canonical glucose metabolism regulatory pathway EREG/PI3K/AKT, and demethylation played the same role.

Then the effects of miR-192-5p and miR-192-5p demethylation on EREG/PI3K/AKT pathway were determined. The results showed that compared with the normal group, the expression of EREG/PI3K/AKT decreased after miR-192-5p overexpression (Fig. 6A-E). When cells were intervened with 5-Aza-CdR, the expression of EREG/PI3K/AKT was reduced compared with the normal group, which was consistent with the effect of miR-192-5p overexpression (Fig. 6F-J).

Discussion

Cancer cells activate or enhance metabolic pathways(27). A promising anticancer therapeutic strategy may be the inhibition of cancer metabolism(28, 29). Higher levels of GLUT1 and HKII, PFKFB3, and LDH, are linked to malignant growth(8–10). Our results confirmed that levels of GLUT1, HKII, PFKFB3, and LDH in NSCLC were greater than those in the adjacent tissues. These findings further illustrate that cancer cells meet their growth needs by increasing the expression of key proteins in glucose metabolism(30). However, the mechanism of abnormal glucose metabolism in NSCLC has not yet been elucidated.

MicroRNAs (miRNAs) directly or indirectly participate in the regulation of glucose metabolism in cancer. Controlling the expression of cancer-related miRNAs is expected to become a new generation of drug models. Evidence suggests that members of the miR-192 family may be involved in the occurrence and development of lung cancer and lung diseases(16–19). However, the role and related molecular mechanisms of miR192-5p in reprogramming glucose metabolism in NSCLC still need further exploration. In our research, the level of miR-192-5p was lessened in NSCLC. We then constructed an A549 cell model overexpressing miR-192-5p to observe the linkage between miR-192-5p and GLUT1, HKII, PFKFB3, and LDH. miR-192-5p negatively correlated with glycolysis-related proteins. MiRNA-192-5p not only affects the entry of glucose into cells but also participates in the process of glucose metabolism. This result reveals the regulatory role of miRNA-192-5p in glycolysis of NSCLC.

The expression of miRNA is affected by DNA demethylation(20, 21, 31). Abnormal DNA demethylation in cancer cells can lead to changes in miRNA expression(32). We treated A549 cells with 5-aza-dC to determine the regulatory effect of demethylation on miR-192-5p and its impact on glucose metabolism proteins. We have confirmed that miR-192-5p has a negative interaction with glucose metabolism proteins, and 5-aza-dC intervention in cells leads to an increase in miR-192-5p and a decrease in glucose metabolism protein expression. Therefore, demethylation, as a way to regulate miR-192-5p expression,

exerts a regulatory effect on glucose metabolism proteins by increasing the level of miR-192-5p. The lactic acid produced by aerobic glycolysis pathway can be released into the extracellular matrix through MCT transporters, thereby promoting endothelial cell proliferation, migration, and neovascularization. Therefore, we also measured the effects of miR-192-5p and miR-192-5p demethylation on HUVECs proliferation, migration, and angiogenesis after altering glucose metabolism in NSCLC. The results suggest that overexpression or demethylation of miR-192-5p inhibits the growth and neovascularization of HUVECs. MiR-192-5p and its demethylation are expected to become potential targets for altering glucose metabolism in NSCLC, thereby inhibiting HUVECs angiogenesis and proliferation.

TargetScan was applied to find potential genes targeted by miR-192-5p. EREG was found to be a downstream target of miR-192-5p involved in regulating glucose metabolism. In NSCLC cells, EREG acts as a ligand for ERBB, and as its quantity increases, it can bind to EGFR/ERBB through an autocrine loop mechanism and activate downstream signaling pathways, such as PI3K/AKT(33, 34). The phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway is a classic insulin signaling pathway, through which many miRNAs directly or indirectly affect cancer cells glucose metabolism(35–38). For example, AKT phosphorylates AS160 to enhance GLUT membrane transport, inhibit TXNIP and reduce its internalization, and increase GLUT1/4 membrane expression. In addition, AKT enhances the efficiency of glycolytic enzymes, including phosphorylation and activation of HK2, promoting PFKFB catalysis of fructose-6-phosphate to Fructose-2,6-bisphosphate, and promoting the expression of glycolytic components such as GLUT and LDH through HIF-1 α transcription factors, enhancing lactate secretion and aerobic glycolysis(39–41).

Based on the predicted downstream targets and related pathways, we further propose that miR-192-5p may affect the PI3K/AKT pathway and glucose metabolism proteins by binding to EREG, thereby altering the glucose metabolism process in NSCLC. As expected, the dual-luciferase results showed that miR-192-5p may directly bind to EREG. We detected the expression levels of EREG/PI3K/AKT in A549 cells co-transfected with miR-192-5p overexpression or demethylation miR-192-5p. As expected, the results showed a decrease in the levels of each protein. The same results can be obtained after demethylation of miR-192-5p. These findings suggest that miR-192-5p or demethylation miR-192-5p can regulate PI3K/ATK glucose metabolism-related signaling pathways by binding to EREG in NSCLC.

Overall, study discovered a negative link between miR-192-5p and glucose metabolism proteins in NSCLC. Overexpression of miR-192-5p reduced the expression of GLUT-1, HKII, PFKFB3, and LDH and inhibited the proliferation and neovascularization of HUVECs. In terms of molecular mechanism, miR-192-5p inhibits the activation of PI3K/AKT by binding to EREG, thereby affecting glucose metabolism in NSCLC. DNA methyltransferase inhibitors(5-Aza-CdR) promoted miR-192-5p level and regulated the effects caused by miR-192-5p (Fig. 7). Our experiment has identified a new target that can regulate glucose metabolism and improve prognosis in NSCLC.

Abbreviations

miR-192-5p, microRNA192-5p; NSCLC, non-small cell lung cancer ; H&E, hematoxylin and eosin; IHC, immunohistochemistry; WB, Western Blotting; qRT-PCR, Quantitative real time polymerase chain reaction; CCK-8, Cell Counting Kit-8; GLUT-1, glucose transporter-1; HKII, hexokinase2; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; LDH, lactate dehydrogenase; HUVECs, Human umbilical vein endothelial cells; EREG, Epiregulin; PI3K, Phosphatidylinositol 3-kinase ; AKT, Ak strain Transforming ; VEGF, vascular endothelial growth factor, miRNAs , microRNAs; 5-aza-dC, 5-aza-2'-deoxycytidine.

Declarations

Ethics approval and consent to participate

The program was investigated and in favor of the Ethical Committee and Institutional Review Board of Harbin Medical University Cancer Hospital. And the ethical approval number is KY2023-19. Written informed consent was obtained from all patients.

Availability of data and materials

All data supporting the findings of this study are available within the article and supplementary materials. Original western blot images and raw experimental data can be obtained from the corresponding authors upon reasonable request

Competing interests

The authors do not have a conflict.

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Authors' contributions

PJL, YXH, FJW, XLK, NJF, and WL formulated hypotheses and devised this study. PJL, YXH, FJW, and XLK fulfilled experimental procedures. PJL, YXH, XQW, WL, and NJF analyzed the data. PJL prepared the manuscript for this study. All of the authors listed above viewed this manuscript and authorized this manuscript.

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Reporting standards

This study followed ICMJE guidelines for clinical and laboratory research reporting.

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Figures

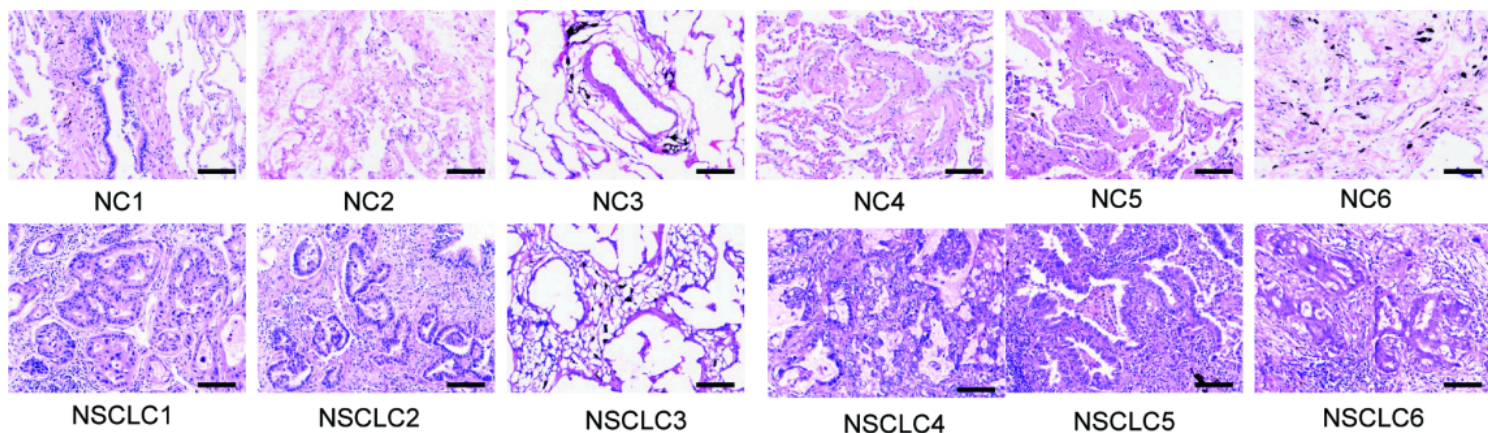


Figure 1

The morphological structure of non-small cell lung cancer and adjacent tissues by H&E. (NC: adjacent tissues, NSCLC: non-small cell lung cancer tissues)

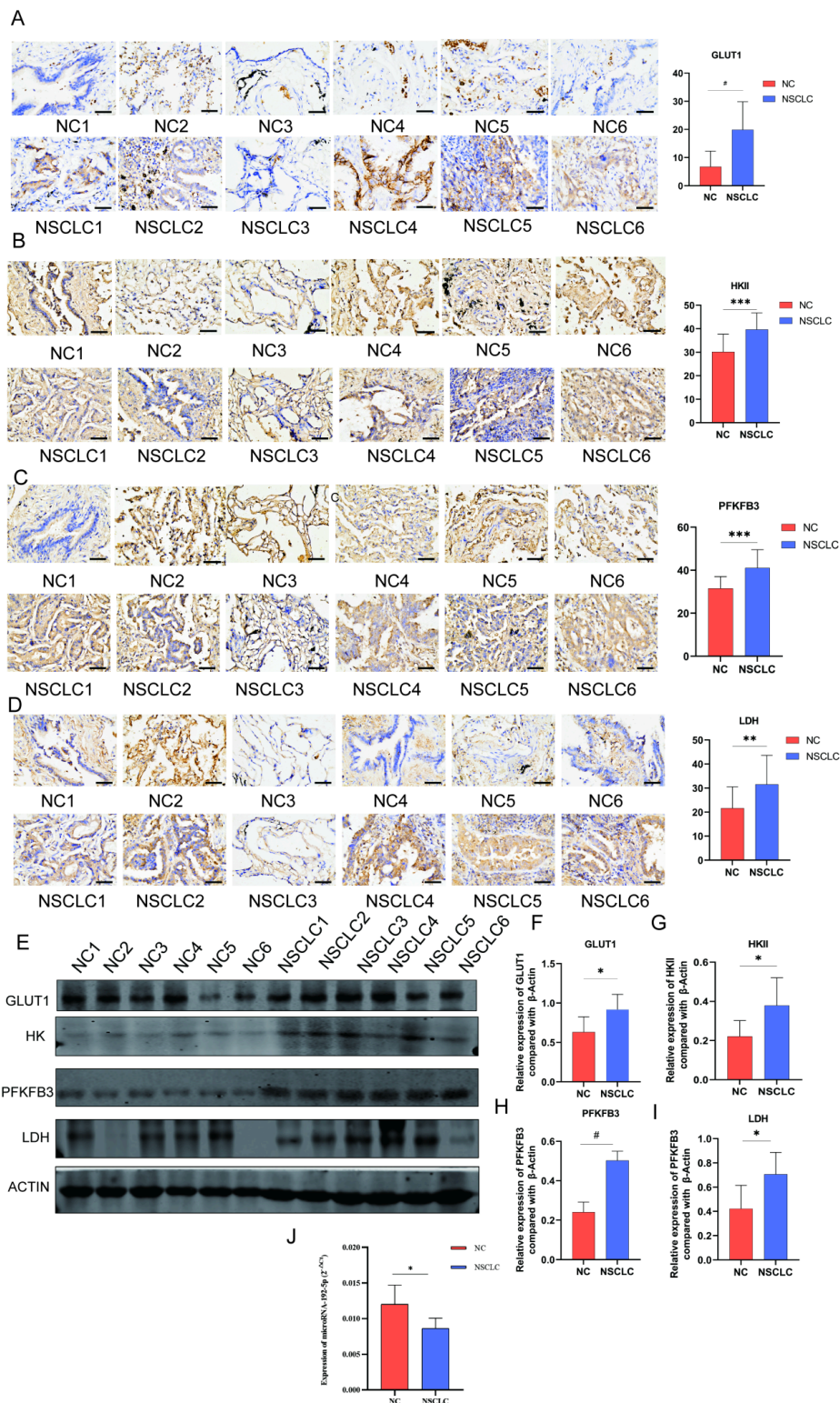


Figure 2

The determination of glycolysis-related proteins in NSCLC and NC by IHC and WB, and the level of miR-192-5p in NSCLC and NC by RT-qPCR. **(A, E, F)** The determination of GLUT1 by IHC and WB. **(B, E, G)** The determination of HKII by IHC and WB. **(C, E, H)** The determination of PFKFB3 by IHC and WB. **(D, E, I)** The determination of LDH by IHC and WB. **(J)** The level of miR-192-5p in NSCLC and NC by RT-qPCR. (NC: adjacent tissues, NSCLC: non-small cell lung cancer. ns= no significance, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,

#p<0.0001. Original gels/blots were presented in Supplementary Figure 2. And the samples derive from the same experiment and that gels/blots were processed in parallel in the figure legends.).

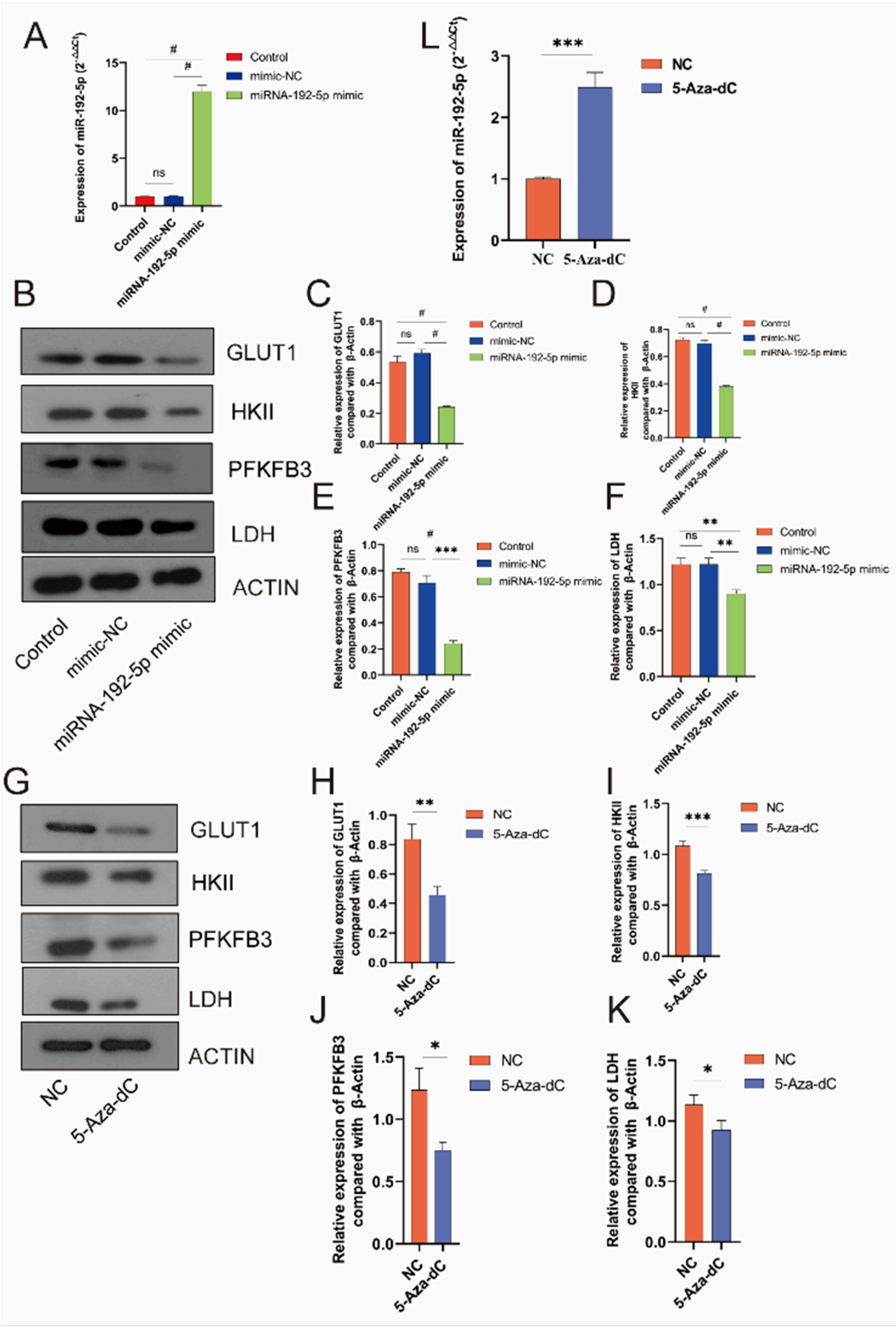


Figure 3

Effects of miR-192-5p and demethylated miR-192-5p on glucose metabolism proteins. **(A)** The level of miR-192-5p in A549 after overexpression. **(B, C)** Expression level of GLUT1 in A549 after miRNA-192-5p

transfection by WB. The expression level of (B, D) HKII, (B, E) PFKFB3, and (B, F) LDH in A549 after miRNA-192-5p transfection by WB. (G, H)The expression level of miR-192-5p in A549 after the 5-Aza-CdR intervention. Expression level of (G, H) GLUT1, (G, I) HKII, (G, J) PFKFB3, and (G, K) LDH in A549 after 5-Aza-CdR intervention. (ns= no significance, *p<0.05, **p<0.01, ***p<0.001, #p<0.0001. Original gels/blots were presented in Supplementary Figure 3. And the samples derive from the same experiment and that gels/blots were processed in parallel in the figure legends.)

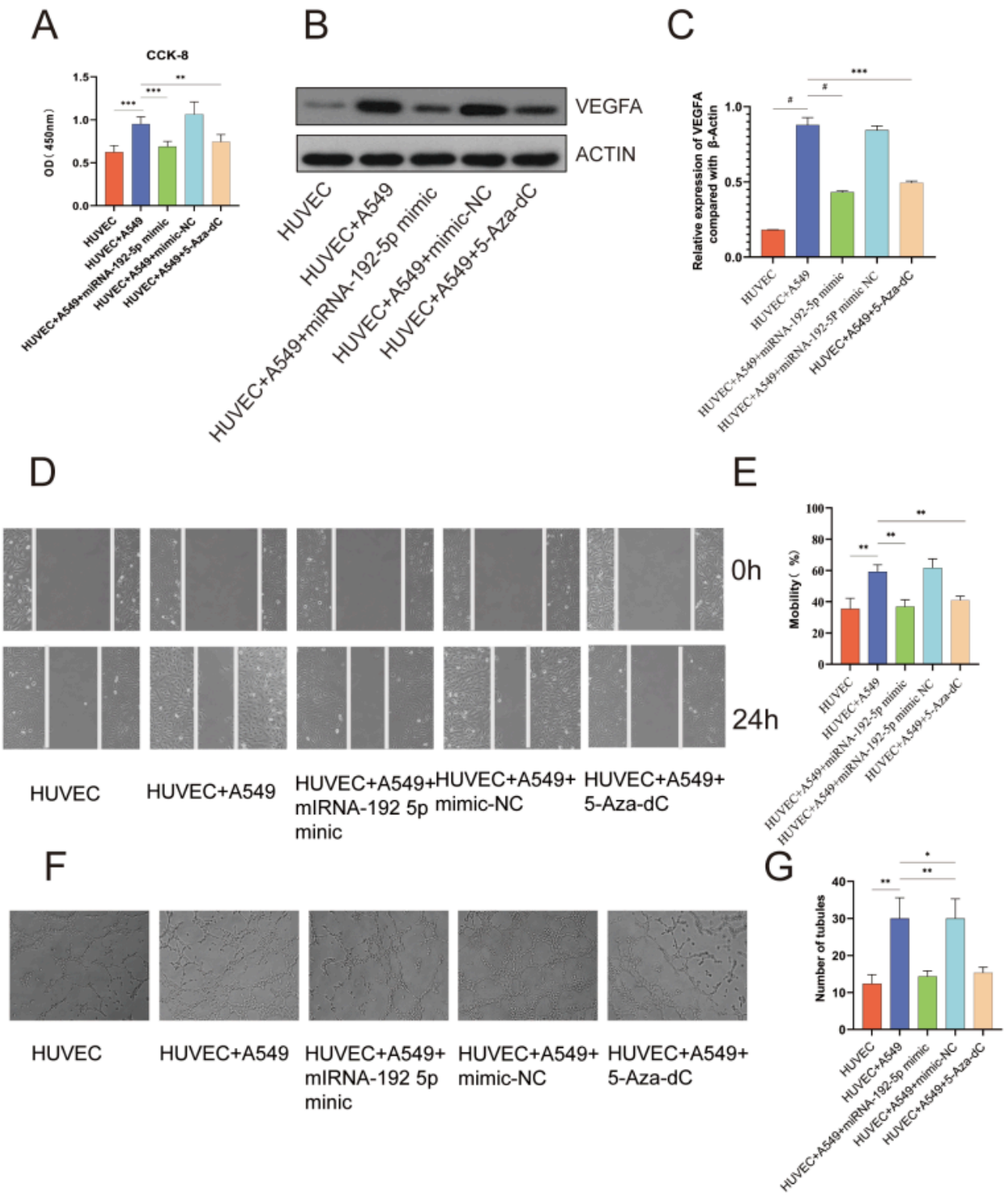


Figure 4

Effects of miR-192-5p and demethylated miR-192-5p on cell proliferation, migration, and angiogenesis. **(A)** Effects of miR-192-5p and demethylated miR-192-5p on cell proliferation. **(B, C)** VEGFA expression after miR-192-5p and 5-Aza-CdR intervention. **(D-G)** The migration and tube formation ability after miR-192-5p transfection and 5-Aza-CdR intervention. (ns= no significance, *p<0.05, **p<0.01, ***p<0.001. Original gels/blots were presented in Supplementary Figure 4. And the samples derive from the same experiment and that gels/blots were processed in parallel in the figure legends.)

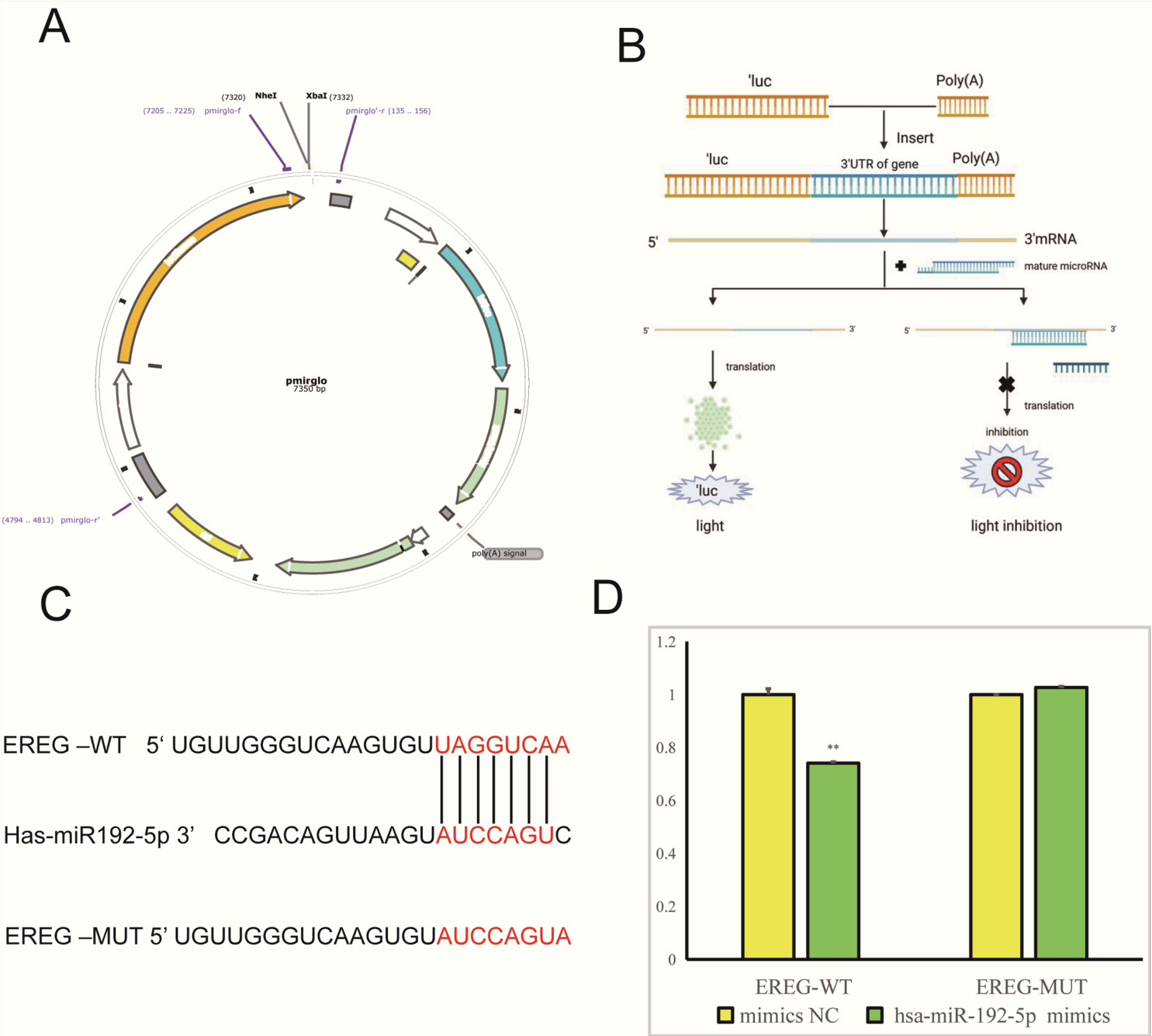


Figure 5

Interaction between miR-192-5p and EREG by dual-luciferase

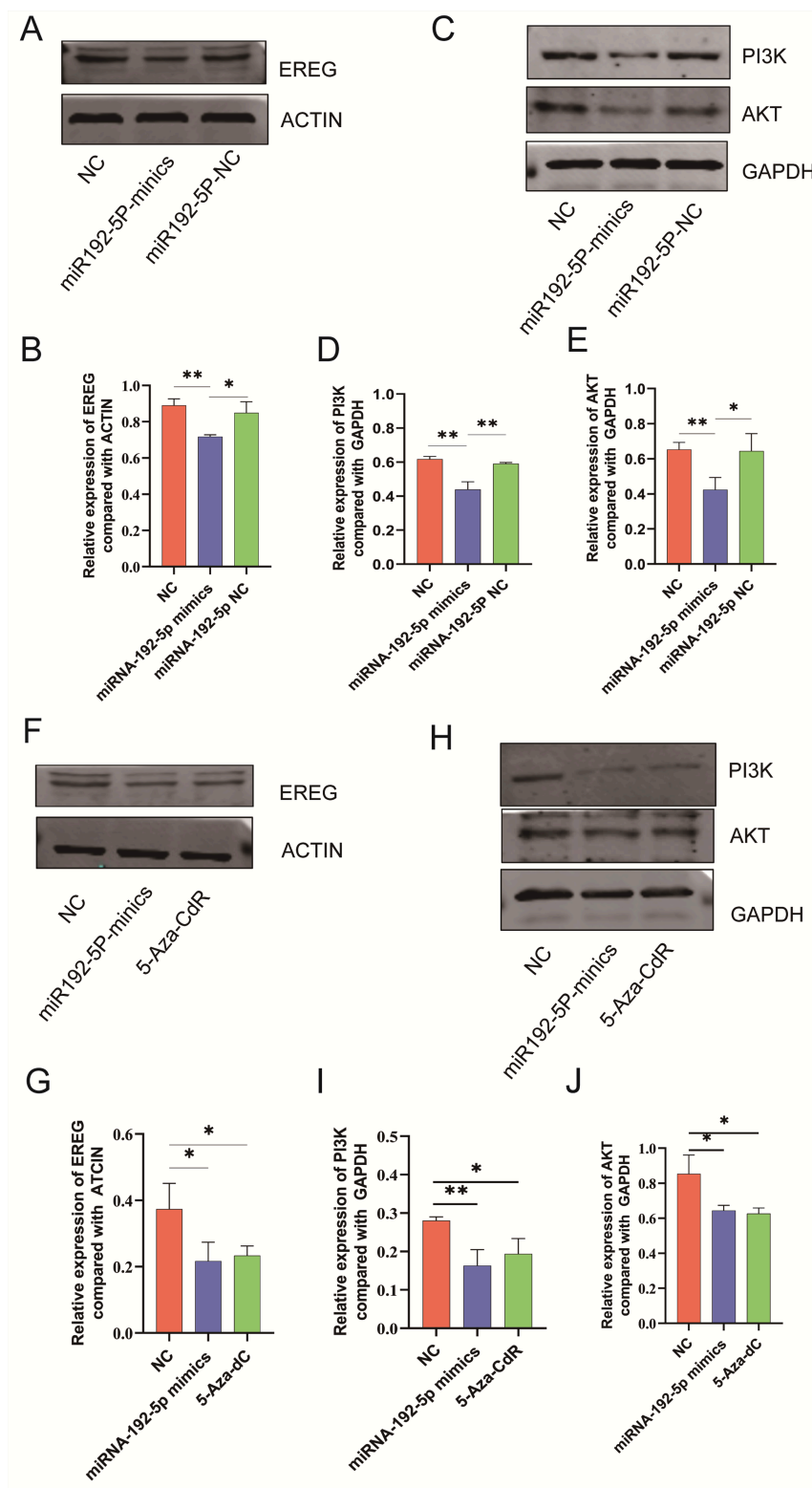


Figure 6

Effects of miR-192-5p and demethylated miR-192-5p on EREG/PI3K/AKT. **(A-E)** Effects of miR-192-5p on EREG/PI3K/AKT. **(F-J)** Effects of demethylated miR-192-5p on EREG/PI3K/AKT. (ns= no significance, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Original gels/blots were presented in Supplementary Figure 6. And the samples derive from the same experiment and that gels/blots were processed in parallel in the figure legends.)

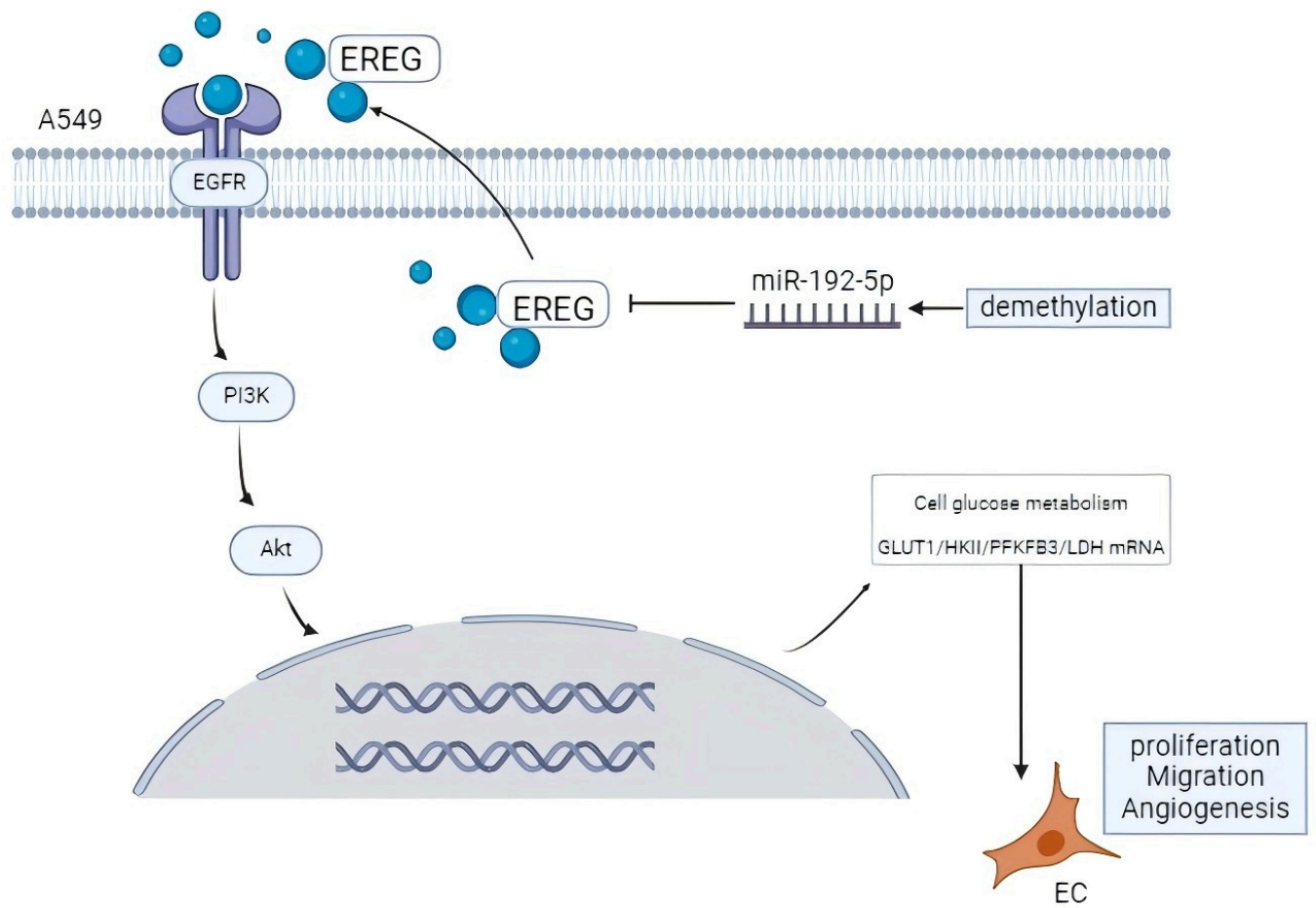


Figure 7

The effect and mechanism of miR-192-5p on glucose metabolism in NSCLC