Original Article

Scutellarin Protects against Myocardial Ischemia–reperfusion Injury by Enhancing Aerobic Glycolysis through miR-34c-5p/ALDOA Axis

Yijia Xiang, Zhongjiao Xu, Renyi Qian, Daying Wu, Li Lin, Jiayi Shen, Pengchong Zhu, Fenghui Chen, Chong Liu

Department of Cardiology, The Fifth Affiliated Hospital of Wenzhou Medical University, Lishui Central Hospital, Lishui, China

Abstract

Background: Aerobic glycolysis has recently demonstrated promising potential in mitigating the effects of ischemia–reperfusion (IR) injury. Scutellarin (Scu) possesses various cardioprotective properties that warrant investigation. To mimic IR injury *in vitro*, this study employed hypoxia/reoxygenation (H/R) injury. **Methods and Results:** First, we conducted an assessment of the protective properties of Scu against HR in H9c2 cells, encompassing inflammation damage, apoptosis injury, and oxidative stress. Then, we verified the effects of Scu on the Warburg effect in H9c2 cells during HR injury. The findings indicated that Scu augmented aerobic glycolysis by upregulating p-PKM2/PKM2 levels. Following, we built a panel of six long noncoding RNAs and seventeen microRNAs that were reported to mediate the Warburg effect. Based on the results, miR-34c-5p was selected for further experiments. Then, we observed Scu could mitigate the HR-induced elevation of miR-34c-5p. Upregulation of miR-34c-5p could weaken the beneficial impacts of Scu in cellular viability, inflammatory damage, oxidative stress, and the facilitation of the Warburg effect. Subsequently, our investigation revealed a decrease in both ALDOA mRNA and protein levels following HR injury, which could be restored by Scu administration. Downregulation of ALDOA or Mimic of miR-34c-5p could reduce these effects induced by Scu. **Conclusions:** Scu provides cardioprotective effects against IR injury by upregulating the Warburg effect via miR-34c-5p/ALDOA.

Keywords: *Aerobic glycolysis, ischemia–reperfusion injury, miR-34c-5p/ALDOA axis, myocardial, scutellarin*

Introduction

Coronary heart disease, alternatively referred to as ischemic heart disease (IHD), constitutes a subset of heart vascular diseases and stands as a primary contributor to global morbidity, mortality, and health-care costs. Percutaneous coronary intervention or thrombolysis represents a well-established and indispensable therapeutic approach for managing IHD.[1] Although these treatments have the potential to restore blood flow and oxygenation to ischemic myocardium, thereby preventing necrosis of myocardial tissue, it is important to acknowledge their associated adverse effects. Reperfusion therapy, for instance, can lead to ischemia–reperfusion (IR) injury, which not only worsens the existing ischemic injury but also restricts the extent of myocardial salvage.[2] IR injury occurs when the sudden return of blood flow induces the death or apoptosis of ischemic myocardial cells through complex intracellular events, including oxidative stress, imbalanced calcium homeostasis, and exacerbated inflammatory cell infiltration.[3] Unfortunately, the molecular mechanism of IR injury still remains ambiguous.

Mitochondrial energy metabolism, particularly aerobic glycolysis (known as the "Warburg effect"), has been a crucial area of research in cardiovascular disease for decades.[4-7] The results of previous studies indicate that an alteration in metabolism, specifically an increase in glucose oxidation, has advantageous implications in the context of myocardial IR injury models. In the case of H9c2 rat heart cells, an elevation in aerobic glycolysis has been observed to be associated with a reduction in reactive oxygen species levels, leading to cellular defense against simulated IR injury.[5] Moreover, the transition to aerobic glycolysis has the potential to augment the levels of mitochondrial spare capacity and cell respiratory control ratio in H9c2 cells,

How to cite this article: Xiang Y, Xu Z, Qian R, Wu D, Lin L, Shen J, *et al*. Scutellarin protects against myocardial ischemia–reperfusion injury by enhancing aerobic glycolysis through miR-34c-5p/ALDOA axis. Int J App Basic Med Res 2024;14:85-93.

Submitted: 12-Sep-2023
Revised: 10-Jan-2024
Accepted: 22-Feb-2024
Published: 24-May-2024

Address for correspondence:
Dr. Chong Liu, Department of Cardiology, The Fifth Affiliated Hospital of Wenzhou Medical University, Lishui Central Hospital, Lishui, China.
E-mail: liuchong_ls@163.com

Access this article online

Website: https://journals.lww.com/IJAB

DOI: 10.4103/ijabmr.ijabmr_415_23

Quick Response Code:

thereby indicating an elevated ability to endure stressful conditions.[7] Hence, triggering the metabolic shift toward aerobic glycolysis may be considered as one of the potential therapeutic strategies against myocardial IR injury.

Erigeron breviscapus, a traditional Chinese medicinal plant, has been utilized for over 1000 years in China to treat cardiovascular diseases.[8] Scutellarin (4′, 0, 5, 6-hydroxyl-flavone-7-glucuronide, Scu), the primary active flavonoid extracted from Erigeron breviscapus, is responsible for its pharmacological effects.[9] *In vivo* studies have shown that Scu can alleviate cardiac structure abnormality, improve diastolic dysfunction, promote autophagy, and inhibit inflammatory response and myocyte apoptosis in rat hearts of IR injury.[10] The *in vitro* study demonstrated that the administration of Scu exhibited protective effects in a dose-dependent manner, enhancing the proliferation of H9c2 cells, increasing antioxidant capacity, and improving the potential of the mitochondrial membrane. These effects were achieved by suppressing inflammation and oxidative damage induced by IR injury.[11] Therefore, Scu has multiple potentially cardioprotective properties.

A variety of biological processes are mediated by noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs).[12] miRNAs and lncRNAs have been extensively studied in the field of aerobic glycolysis regulation. For example, miR-206 repressed hexokinase 2 in non small cell lung cancer (NSCLC) cells to reduce glucose uptake, lactate production, and ATP generation.[13] It indicated that miR-142-3p could suppress tumor growth by reducing the Warburg effect in hepatocellular carcinoma.[14] In addition, several miRNAs and lncRNAs regulate aerobic glycolysis, such as lncRNA-Ftx,[15] miR-124,[16] LINC00152,[17] miR-489, and miR-186.[18]

In the present study, we first evaluated whether Scu protected against IR injury through enhance Warburg effect in H9c2 cell of IR vitro model. Then, we built a panel of 17 miRNAs[10,13,14,16-29] and 6 lncRNAs[15,17,23,25,28,30] that were reported to regulate the Warburg effect. After a series of tests, we identified miR-34c-5p/ALDOA as the key mediators in the protective effects of Scu.

Methods

Cell culture and treatment

Cell Bank/Stem Cell Bank (Shanghai Chinese Academy of Sciences) provided us with the rat cardiomyocyte cell line H9c2. Dulbecco's modified Eagle medium with 10% fetal bovine serum (Sigma, St Louis, MO, USA) and 1% penicillin/streptomycin solution (Gibco-BRL, Grand Island, NY, USA) was used for cell culture. Incubation conditions were 37°C, 5% CO_2, and 95% air.

Hypoxia/reoxygenation (H/R) injury was used to mimic IR injury *in vitro*. The protocol was as follows. An air-saturated anoxic solution was built by the glucose and serum-deprived medium with the mixed air of 1% O_2, 94% nitrogen, and 5% CO_2 for at least 2 h. Then, cells were washed three times with phosphate-buffered saline and reoxygenated for 24 h in a medium containing 4500 mg/L glucose. Cells in the control group were incubated in the condition of 37°C, 95% air, and 5% CO_2 for 26 h.

The cells were randomly divided into five groups: control group (con), HR group, H/R+25 μmol/L Scu group (HR + S25, H/R-treated cells with 25 μmol/L Scu), H/R+50 μmol/L Scu group (HR + S50, H/R-treated cells with 50 μmol/L Scu), and H/R + 100 μmol/L Scu group (HR+S100, H/R-treated cells with 100 μmol/L Scu).

Cell transfection

Transfecting the oligonucleotides into cells was performed according to the instructions provided by the manufacturer and previous study.[31] Briefly, to ensure 70%–80% cell density, cells were seeded 24 h before transfection. Then, the oligonucleotides (ALDOA siRNA, 100nM miR-34c-5p mimic) and lipofectamine 3000 transfection reagent (Invitrogen, USA) were diluted and mixed by Opti-MEM medium without antibiotics and serum. After 48 h of incubation in the condition of 5% CO_2 and 37°C, the transfection medium was replaced with fresh penicillin/streptomycin-free medium for 24 h before subsequent experiments.

Cell counting kit-8 assay

The cells were plated with 5 × 103 cells per well and starved for 24 h after adherence. Then, cells were incubated in 10 μL Cell Counting Kit-8 (Dojindo, Japan) for 1 h. A microplate reader (BioTek, USA) was used to determine the optical density at 450 nm.

Phosphofructokinase activity assay and pH measurement

The activity of phosphofructokinase (PFK) was tested by PFK Assay Kit (Sigma-Aldrich, USA). According to the manufacturer's instruction, treated cells were added with PFK Assay Buffer and Reaction Mix. Each mixture was tested with a microplate reader every 30 s for its OD value. The PFK activity was calculated as mU/mg of protein after normalization to the protein concentration. Extracellular pH was detected by pH indicator paper and pH meter.

Measurements of inflammation and oxidative stress markers

The commercially available enzyme-linked immunosorbent assay kits were used to quantify levels of inflammation (tumor necrosis factor-α [TNF-α] and Interleukin [IL]-1β) and oxidative stress (methylene dioxyamphetamine (MDA) and superoxide dismutase (SOD)) in culture supernatants, according to the manufacturer's instructions.[32]

Annexin V-APC/7-AAD double staining

Treated H9c2 cells were stained with Annexin-V APC/7-AAD cell apoptosis assay kit (Bjbalb Beijing, HR8285). There were four subpopulations identified: normal cells (Annexin V-APC-/7-AAD-), necrotic cells (Annexin V-APC-/7-AAD+), early apoptotic cells (Annexin V-APC-/7-AAD-), and late apoptotic cells (Annexin V-APC+/7-AAD+). The apoptosis index was the total rates of early apoptotic and late apoptotic cells.

Reverse transcription-quantitative polymerase chain reaction

TRIzol reagent (Invitrogen, CA) was used to extract total RNA. SYBR Green polymerase chain reaction (PCR) kit (Thermo) was used for PCR amplification. Three holes were provided for each sample. The internal reference of GAPDH was adjusted, and mRNA expression data were calculated with the $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in Table 1.

Western blot

H9c2 cells were lysed with RIPA to extract their total protein. Moreover, the protein was quantified by the BCA method. After SDS-PAGE electrophoresis separation, the membrane was transferred to polyvinylidene fluoride (PVDF) membranes, then blocked with 5% fat-free milk, and incubated with anti-PKM2 (1:1000, Boaosen, bs-0102M), anti-p-PKM2 (1:1000, CST, 3827), and anti-ALDOA (1:1000, Boaosen, bs-2406R), respectively. The relative expression was equal to the gray value ratio of the target protein to GAPDH.

Statistical analysis

The results were presented as the mean ± standard deviation. Using SPSS (Statistical Package for the Social Science) SPSS 22 version (SPSS Inc., Chicago, IL, USA), one-way ANOVA following *post hoc* tests was used to analyze the differences between groups. $P < 0.05$ was considered significantly different.

Results

Scutellarin-protected H9c2 cells from hypoxia/reoxygenation-induced injury

In the initial phase, we conducted an assessment to determine the potential of Scu in mitigating cytotoxicity induced by HR, focusing on its effects on cell viability, inflammation damage, apoptosis, and oxidative stress. As presented in Figure 1, different doses of Scu (25 μM, 50 μM, and 100 μM) could significantly increase H9c2 cell viability [Figure 1a], decrease inflammation factors (TNF-α and IL-1β) [Figure 1b and c], reduce apoptotic cells numbers [Figure 1d], and alleviate levels

Table 1: Primer sequences

	Forward	Reverse
miR-765	5’-GUAGCCAAGGAATCCGAAGGA-3’	5’-GCGAGGAAGGAGGAGGAAGGT-3’
miR-186-3p	5’-CGCGCAAAGAATTCTCCTTT-3’	5’-AGTGCAGGGTCCGAGGTATT-3’
miR-489	5’-CCCCGCCGTGACATCACATAT-3’	5’-CCAGTCGGTGGCTGCCGTATA-3’
miR-142-3p	5’-GGCCCATAAAGTAGAAAGC-3’	5’-TTTGGCACTAGCACATT-3’
miR-139-5p	5’-TCTACAGTGCACGTGTC-3’	5’-GAATACCTCGGACCCTGC-3’
miR-206	5-GCGTCTGGAATGTAAGGAAGTG-3’	5’-GTGCAGGGTCCGAGGT-3’
miR-124-3p	5’-CGGCAAGTTGTCGGAGACG-3’	5’-CCTGGAGGTTGGGATGCTCT-3’
miR-34c-5p	5’-GCG CAT CCC TTG CAT GGT-3’	5’-AGT GCA GGGTCCGAG GTATT-3’
miR-515-5p	5’-TTCTCCAAAAGAAAGCACTTTCTG-3’	5’-CTCGCTTCGGCAGCACA-3’
miR-12116	5’-GCCTTTGGTTCTTCTTAG-3’	5’-GCTCTGGGTTCTTCTTAG-3’
miR-30a-5p	5’-AACGAGACGACGACAGAC-3’	5’-TGTAAACATCCTCGACTGGAAG-3’
miR-101-3p	5’-GCCGCCACCATGGTGAGCAAGG-3’	5’-AATTGAAAAAAGTGATTTAATTT-3’
miR-455	5’-TAAGACGTCCATGGGCAT-3’	5’-GTGCAGGGTCCGAGGT-3’
miR-361-5p	5’-GCCGAGTTATCAGAATCTCCA-3’	5’-CTCAACTGGTGTCGTGGA-3’
miR-138-5p	5’-GCTTAAGGCACGCGG-3’	5’-GTGCAGGGTCCGAGG-3’
miR-199a-5p	5’-TCAAGAGCAATAACGAAAAATGT-3’	5’-GCTGTCAACGATACGCTACGT-3’
miR-383	5’-GACAGACCTTGTGAAGGTGACTCTG-3’	5’-GACCAGCTTCCAGAGGACAAGATCTC-3’
LINC01123	5’-ACAGTGGCCGCACGCATAGCTG-3’	5’-CTGACGACCGAGGTGACAACGATGA-3’
Lnc-SNHG9	5’-CCCGAAGAGTGGCTATAAACG-3’	5’-GGAGGACCAGTGTCCTAAGTGAA-3’
Lnc-MAFG/ASI	5’-ATGACGACCCCCAATAAAGGA-3’	5’-CACCGACATGGTTACCAGC-3’
LINC01391	5’-TGGCACCCGCTATGTCCA-3’	5’-GTAGCAGGGATTCTGTCTG-3’
Linc-00152	5’-CTCCAGCACCTCTACCTGTTG-3’	5’-GGACAAGGGATTAAGACACACA-3’
LncRNA KCNQ1OT1	5’-TTGGTAGGATTTTGTTGAGG-3’	5’-CAACCTTCCCCTACTACC-3’
Lnc-Ftx	5’-GAATGTCCTTGTGAGGCAGTTG-3’	5’-TGGTCACTCACATGGATGATCTG-3’
ALDOA	5’-ATGCCCTACCAATATCCAGCA-3’	5’-GCTCCCAGTGGACTCATCTG-3’
GAPDH	5’-GCACCGTCAAGGCTGAGAAC-3’	5’-GCCTTCTCCATGGTGGTGAA-3’

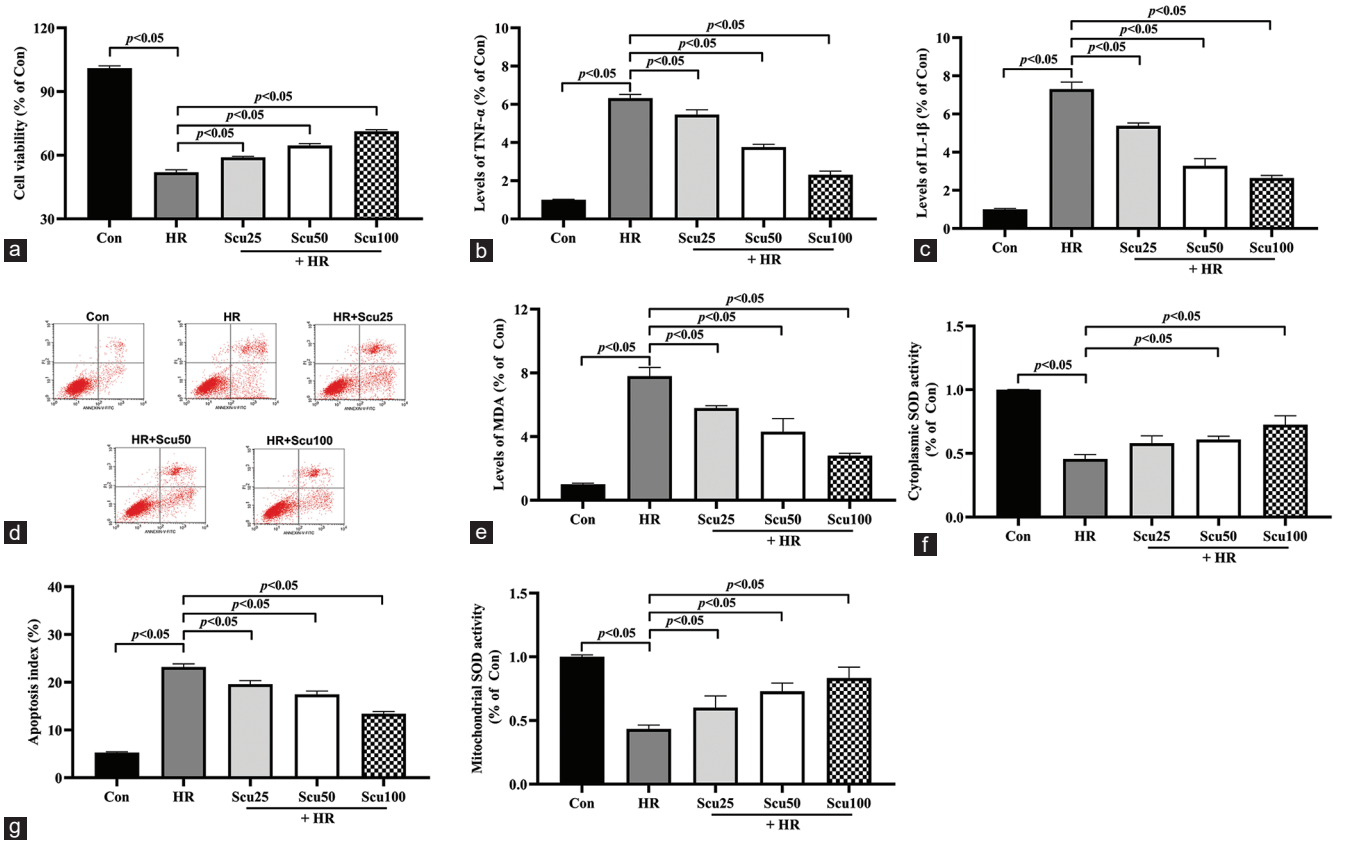


Figure 1: Scutellarin-protected H9c2 cells from hypoxia/reoxygenation-induced injury. (a) Viability of H9c2 cells was tested by cell counting kit-8. Inflammation levels of tumor necrosis factor-α (b) and interleukin-1β (c). (d) Apoptosis index was detected by flow cytometry. Levels of oxidative stress marker, including MDA (e), mitochondrial SOD (f), and cytoplasmic SOD (g). The error bar reflects the SE of at least three independent experiments. HR: Hypoxia/reoxygenation, TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin, MDA: Methylene dioxyamphetamine, SOD: Superoxide dismutase, SE: Standard error

of oxidative stress (MDA and SOD) [Figure 1e-g] under HR injury. Totally, these results indicated that HR-induced inflammation damage, apoptosis injury, and oxidative stress in H9c2 cells, whereas Scu could alleviate this HR-induced cytotoxicity in a dose-dependent manner.

Scutellarin enhanced aerobic glycolysis under hypoxia/reoxygenation injury in H9c2 cells

Then, we aimed to verify whether Scu could impact the Warburg effect in H9c2 cells under HR condition. As shown in Figure 2, Scu could remarkably trigger cellular energy metabolite switching to aerobic glycolysis under HR injury, including reduction of pH [Figure 2a] and elevation of lac in culture medium [Figure 2b]. In glycometabolism, PFK serves as the rate-limiting enzyme to catalyze fructose-6-phosphate to fructose-1, 6-diphosphate. Our data presented that Scu administration could enhance PFK activity [Figure 2c]. PKM2 is regarded as the final rate-limiting enzyme in aerobic glycolysis. Western blot data suggested that Scu treatment could obviously increase the ratio of p-PKM2/PKM2 [Figure 2d]. Our data showed that 2-deoxyglucose, the inhibitor of the glycolytic pathway, could reverse the enhancements of aerobic glycolysis induced by Scu. In general, these results indicated that Scu intensified aerobic glycolysis in H9c2 cells under the condition of HR injury in a dose-dependent manner.

Effects of scutellarin on noncoding RNAs linked with Warburg effect

In the next step, a panel of 6 lncRNAs and 17 miRNAs was built, which were reported to mediate the Warburg effect. The tool of miEAA (https://ccb-compute2.cs.uni-saarland.de/mieaa2/) was used to perform the KEGG enrichment analysis. As shown in Figure 3 and Supplementary Figure 1, reverse transcription-quantitative PCR (RT-qPCR) data presented that HR increased miR-34c-5p levels in H9c2 cells and 50 μM Scu could significantly reverse this upregulation. Hence, miR-34c-5p was selected for further experiments.

Scutellarin enhanced Warburg effect by regulating miR-34c-5p in H9c2 cells

Then, we aimed to explore whether miR-34c-5p participated in the enhancement of the Warburg effect induced by Scu. First, our data found that Scu could reverse HR-triggered elevation of miR-34c-5p in a dose-dependent manner [Figure 4a]. Then, we found that miR-34c-5p mimic could weaken the protective effects of Scu in terms of cell

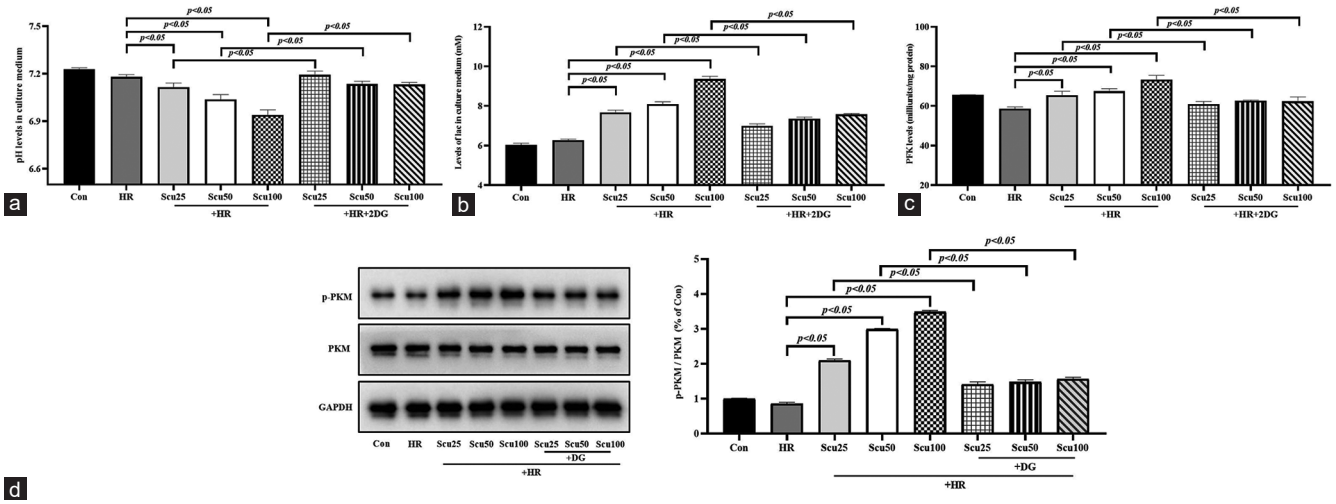


Figure 2: Scutellarin enhanced aerobic glycolysis under hypoxia/reoxygenation injury in H9c2 cells. (a) Levels of pH in each group. (b) Levels of lac in each group. (c) Activity of phosphofructokinase tested by Colorimetric assay. (d) Western blot analysis showing phosphorylation levels of PKM2 in each group. The error bar reflects the SE of at least three independent experiments. HR: Hypoxia/reoxygenation, PFK: Phosphofructokinase, SE: Standard error

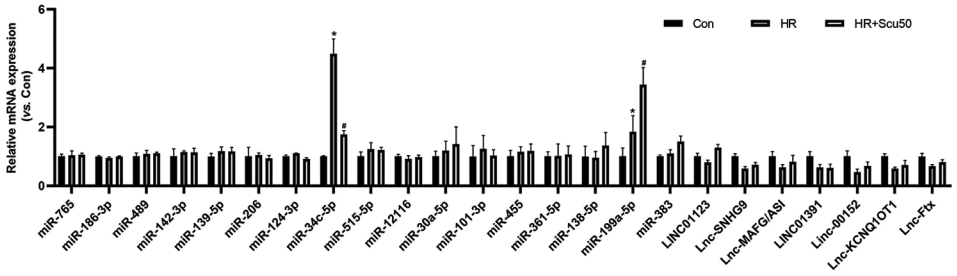


Figure 3: Effects of Scutellarin on different noncoding RNAs related to the Warburg effect. The error bar reflects the SE of at least three independent experiments. *vs. Con, *p*<0.05. #vs.HR, *p*<0.05, SE: Standard error

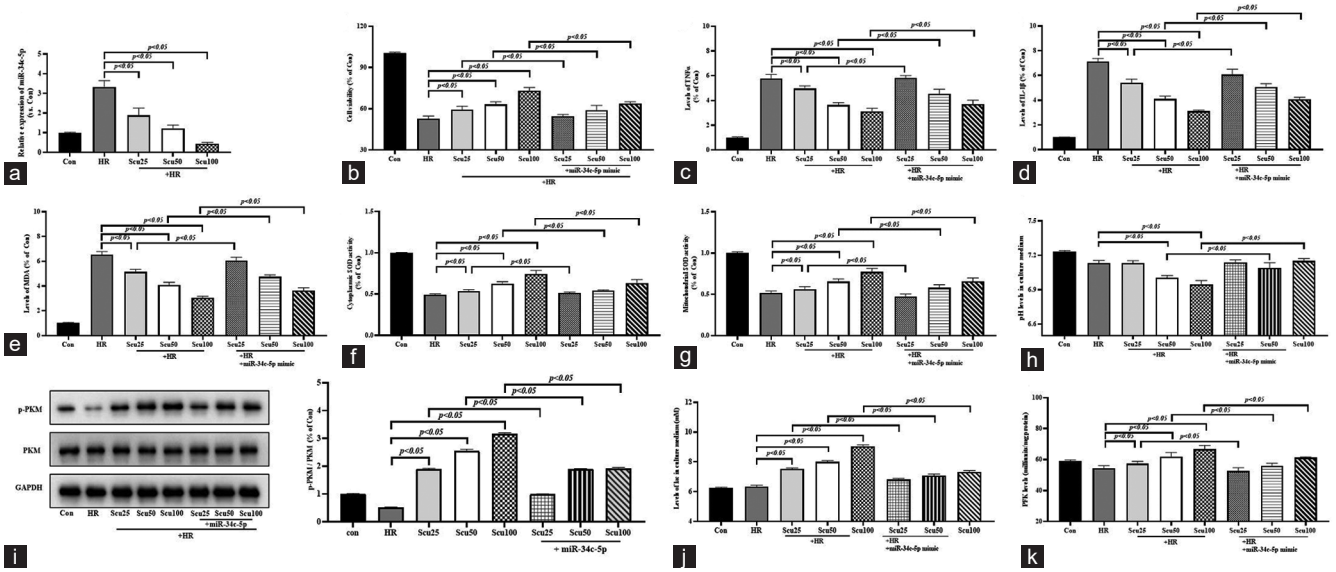


Figure 4: Scutellarin (Scu) enhanced the Warburg effect by regulating miR-34c-5p in H9c2 cells. (a) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) indicated that Scu could alleviate hypoxia/reoxygenation-induced elevation of miR-34c-5p in a dose-dependent manner. (b) Viability of H9c2 cells was tested by Cell Counting Kit-8. Levels of inflammation marker, including tumor necrosis factor-α (c) and interleukin-1β (d). Levels of oxidative stress index, including MDA (e), mitochondrial SOD (f), and cytoplasmic SOD (g). Levels of aerobic glycolysis in each group, including pH (h), phosphorylation levels of PKM2 (i), lac (j), and phosphofructokinase (k). The error bar reflects the SE of at least three independent experiments. HR: Hypoxia/reoxygenation, Scu: Scutellarin, PFK: Phosphofructokinase, SE: Standard error

viability [Figure 4b], inflammation injury [Figure 4c and d], and oxidative stress [Figure 4e-g]. The facilitation of the Warburg effect by Scu treatment was also reversed by miR-34c-5p mimic [Figure 4h-k] in H9c2 cells. Totally,

these data presented that the enhancement of Scu in the Warburg effect by regulating miR-34c-5p in H9c2 cells.

Scutellarin protected against hypoxia/reoxygenation injury by enhancing Warburg effect through miR-34c-5p/ALDOA axis in H9c2 cells

Then, we choose miR-34c-5p/ALDOA axis for further investigation. First, our results indicated that the mRNA expression [Figure 5a] and protein [Figure 5d] of ALDOA were reduced after HR injury, and Scu could reverse these in a dose-dependent manner. Next, a specific siRNA to silence ALDOA was applied [Figure 5b]. The effects of Scu to maintain the content of ALDOA could be weakened by ALDOA siRNA or miR-34c-5p mimic [Figure 5c and d]. The protective effects of Scu in improving cell viability [Figure 5e], relieving inflammation injury [Figure 5f and g], oxidative stress [Figure 5 h-j], and increasing aerobic glycolysis switch [Figure 5k-n] could be diminished by ALDOA siRNA. Hence, these data suggested that Scu prevented against HR damage through miR-34c-5p/ALDOA axis in H9c2 cells.

Discussion

IR injury is regarded as the major pathological process in IHD. In the last decades, tremendous advances have been made in IHD. However, there is still no available and effective method for IR injury prevention at the present time. Erigeron breviscapus is one of a traditional Chinese medicinal plant and widely used to treat cardiovascular diseases for over 1000 years. Scu is a major active flavonoid from Erigeron breviscapus. In this study, we found Scu treatment presented an increased aerobic glycolysis rate in H9c2 cell model of HR injury. This metabolic shift is associated with the alleviation of inflammation damage and oxidative stress triggered by IR.

The structure of Scu was identified a century ago. However, the modern pharmacology study of Scu started in the late 1970s, when China launched a large program to identify and modernize therapeutics from traditional Chinese medicine.[33] In our study, we found HR-induced inflammation damage, apoptosis injury, and oxidative stress in H9c2 cells, whereas Scu could alleviate this HR-triggered cytotoxicity in a dose-dependent manner. This finding is consistent with previous studies.[34,35] A meta-analysis, involving 16 randomized and controlled trials from 2001 to 2012 containing 1505 IHD patients, indicated that Scu enhanced the therapeutic effects of normal anti-IHD drugs and presented greater improvement of IHD symptoms, such as angina pectoris.[34] It has been reported that Scu attenuated inflammation damage and IR damage by inhibiting inflammation (TNF-α and IL-6) in the myocardial IR rat model.[35] Scu also served as an antioxidant property against IR injury.[36] Thus, in terms of mechanism, anti-IR effects of Scu are mostly due to its ability to suppress inflammation and oxidative stress.

To further explore the cardiac protective mechanism of Scu, we selected the Warburg effect as cut point. First, we found that Scu could intensify aerobic glycolysis in H9c2 cells under HR condition with a dose-dependent manner. The first evidence of aerobic glycolysis was discovered in tumor cells, which restrict their energy metabolism to glycolysis even under aerobic conditions.[37] In recent years, it has been demonstrated that a metabolic transition toward heightened glycolysis yields advantageous outcomes in the context of cardiac IR injury, as it diminishes oxidative stress levels and enhances stress tolerance.[5] The molecular mechanism still remains unclear. In our study, we built a panel of 6 lncRNAs and 17 miRNAs that were reported to mediate the Warburg effect. After RT-qPCR screening, we confirmed that HR increased miR-34c-5p levels in H9c2 cell model of HR injury, and this upregulation could be reversed by Scu. We further confirmed that miR-34c-5p mimic could weaken the protective effects of Scu against HR-induced inflammation and oxidative stress. MiR-34c-5p has been reported to be linked with the Warburg effect.[25] It is also a vital mediator in IR injury. Tu and Hu found miR-34c-5p played a crucial role in cerebral IR injury through inflammatory and apoptotic signaling pathways.[38,39] According to reports, miR-34c-5p participates in cardiac remodeling and repair during postischemic heart injury.[40] Our research supplemented the above results. In addition, we observed that HR condition increased the level of miR-138-5p and Scu treatment enhanced this effect, which indicated that Scu might aggravated HR injury trough upregulate miR-138-5p. According to our phenotypic analysis of HR, Scu has provided significant protective properties against HR injury in H9c2 cells. Thus, the mechanism of miR-138-5p in Scu administration needs further investigation.

In our study, we choose miR-34c-5p/ALDOA axis for further investigation. Our data indicated that Scu prevented against HR injury by upregulation of aerobic glycolysis through miR-34c-5p/ALDOA axis in H9c2 cells. MiR-34c-5p/ALDOA axis is a new explanation for the Warburg effect.[25] There has been a new explanation for the Warburg effect based on this axis.[25] ALDOA has been considered a promising target for the treatment of myocardial infarction and heart failure regarding its cardiac protective effects against oxidative stress induced by HR.[41,42] ALDOA is predominantly found in the muscle tissue and erythrocytes. It is a glycolytic enzyme and plays a vital role in glycolysis. Serum ALDOA shows lower levels in myocardial infarction patients and H/R-induced H9C2 cardiomyocytes. Upregulation of ALDOA inhibited H/R-triggered cell apoptosis and oxidative stress.[41] We believe that miR-34c-5p/ALDOA axis contributes to the point of junction in the Scu protective effect on enhancing the Warburg effect.

There are some limitations in our study. First, experimental validation *in vitro* is missing in our study to demonstrate the effectiveness of the drug. This will

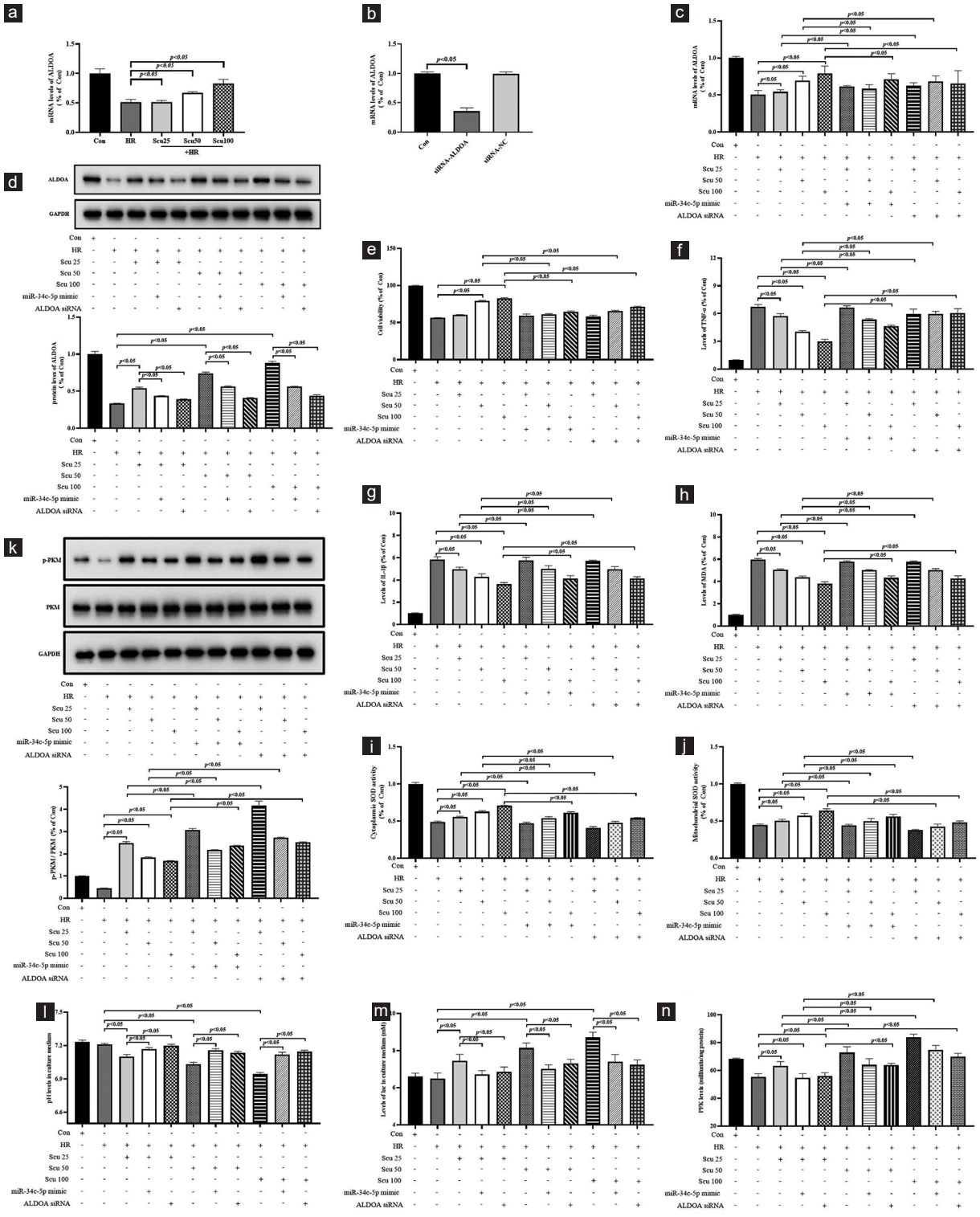


Figure 5: Scutellarin protected against hypoxia/reoxygenation injury by enhancing Warburg effect through miR-34c-5p/ALDOA axis in H9c2 cells. (a) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) results of ALDOS mRNA. (b) RT-qPCR results of ALDOS mRNA. mRNA (c) and protein (d) levels of ALDOA. (e) Viability of H9c2 cells was tested by Cell Counting Kit-8. Levels of inflammation marker, including tumor necrosis factor-α (f) and interleukin-1β (g). Levels of oxidative stress index, including MDA (h), mitochondrial SOD (i), and cytoplasmic SOD (j). Levels of aerobic glycolysis in each group, including phosphorylation levels of PKM2 (k), pH (l), lac (m), and phosphofructokinase (n). The error bar reflects the SE of at least three independent experiments. HR: Hypoxia/reoxygenation, Scu: Scutellarin, SE: Standard error

be performed in the follow study. Second, our research primarily concentrated on investigating the antioxidative and anti-inflammatory properties of Scu through the enhancement of aerobic glycolysis. The examination of Scu's impact on apoptosis will be pursued in forthcoming studies.

Conclusion

Summarily, this study demonstrated that Scu provides cardioprotective effects against IR-induced myocardial cell injury by upregulating the Warburg effect through miR-34c-5p/ALDOA pathway in H9c2 cell model.

Ethical statement

This is a vitro cell experiment.

Acknowledgments

The authors would like to thank all of the participants for their time and effort.

Financial support and sponsorship

This study was supported by the Project of Zhejiang Basic Public Welfare Research (BY21H020007).

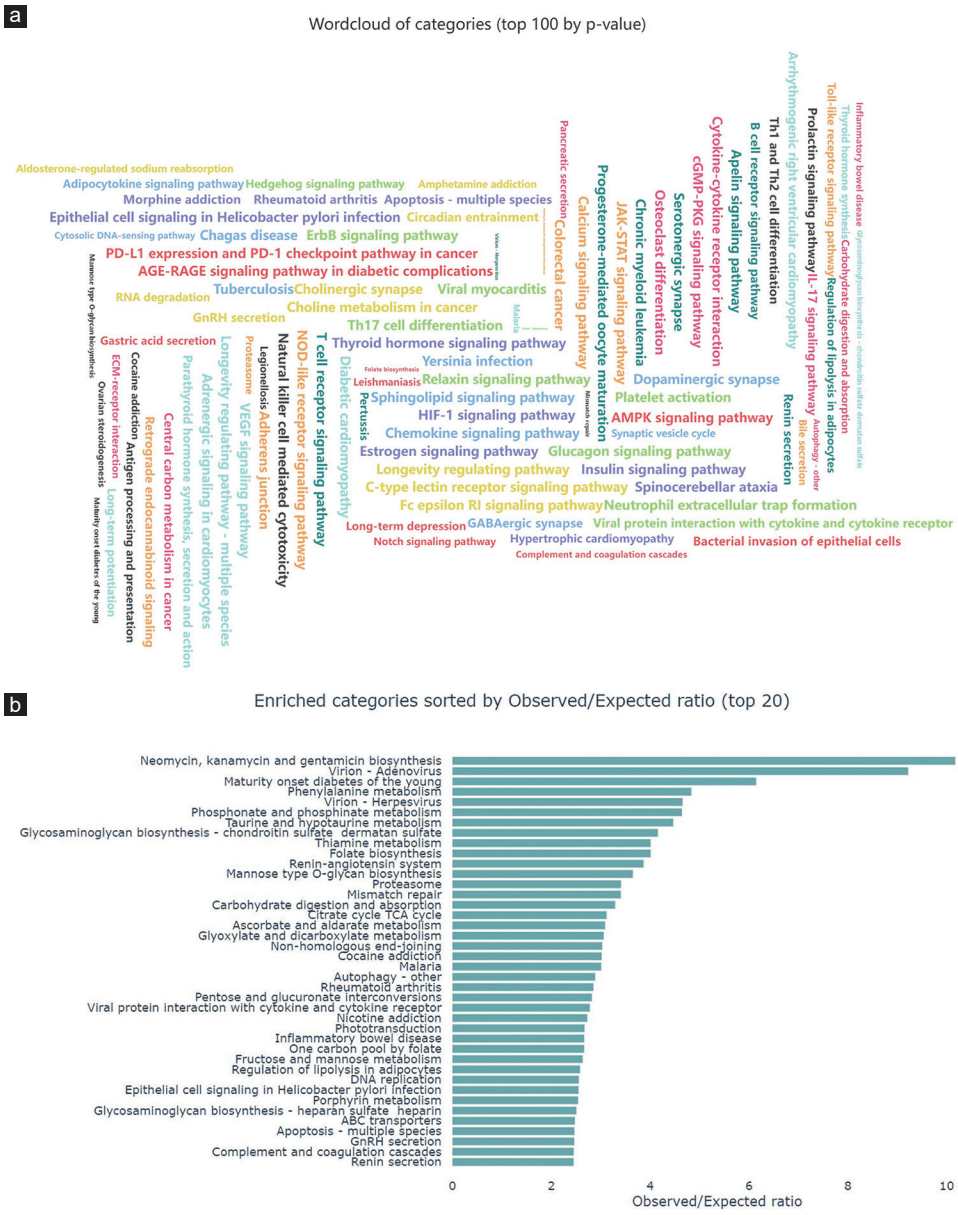
Conflicts of interest

There are no conflicts of interest.

References

1. Wang C, Wang C, Liu M, Chen Z, Liu S. Temporal and spatial trends of ischemic heart disease burden in Chinese and subgroup populations from 1990 to 2016: Socio-economical data from the 2016 global burden of disease study. BMC Cardiovasc Disord 2020;20:243.
2. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: A neglected therapeutic target. J Clin Invest 2013;123:92-100.
3. Pei YH, Chen J, Xie L, Cai XM, Yang RH, Wang X, *et al.* Hydroxytyrosol Protects against myocardial ischemia/reperfusion injury through a PI3K/Akt-dependent mechanism. Mediators Inflamm 2016;2016:1232103.
4. Wu Y, Chen S, Wen P, Wu M, Wu Y, Mai M, *et al.* PGAM1 deficiency ameliorates myocardial infarction remodeling by targeting TGF-β via the suppression of inflammation, apoptosis and fibrosis. Biochem Biophys Res Commun 2021;534:933-40.
5. Beltran C, Pardo R, Bou-Teen D, Ruiz-Meana M, Villena JA, Ferreira-González I, *et al.* Enhancing glycolysis protects against ischemia-reperfusion injury by reducing ROS production. Metabolites 2020;10:132.
6. Ong SG, Lee WH, Theodorou L, Kodo K, Lim SY, Shukla DH, *et al.* HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. Cardiovasc Res 2014;104:24-36.
7. Wanka H, Lutze P, Staar D, Grunow B, Peters BS, Peters J. An alternative renin isoform is cardioprotective by modulating mitochondrial metabolism. J Cell Mol Med 2018;22:5991-6001.
8. Liu X, Cheng J, Zhang G, Ding W, Duan L, Yang J, *et al.* Engineering yeast for the production of breviscapine by genomic analysis and synthetic biology approaches. Nat Commun 2018;9:448.
9. Yang G, Li Z, Wu F, Chen M, Wang R, Zhu H, *et al.* Improving solubility and bioavailability of breviscapine with mesoporous silica nanoparticles prepared using ultrasound-assisted solution-enhanced dispersion by supercritical fluids method. Int J Nanomedicine 2020;15:1661-75.
10. Ding C, Xi G, Wang G, Cui D, Zhang B, Wang H, *et al.* Exosomal Circ-MEMO1 promotes the progression and aerobic glycolysis of non-small cell lung cancer through targeting MiR-101-3p/KRAS axis. Front Genet 2020;11:962.
11. Wang Z, Yu J, Wu J, Qi F, Wang H, Wang Z, *et al.* Scutellarin protects cardiomyocyte ischemia-reperfusion injury by reducing apoptosis and oxidative stress. Life Sci 2016;157:200-7.
12. Wang J, Pei Y, Zhong Y, Jiang S, Shao J, Gong J. Altered serum microRNAs as novel diagnostic biomarkers for atypical coronary artery disease. PLoS One 2014;9:e107012.
13. Jia KG, Feng G, Tong YS, Tao GZ, Xu L. miR-206 regulates non-small-cell lung cancer cell aerobic glycolysis by targeting hexokinase 2. J Biochem 2020;167:365-70.
14. Hua S, Liu C, Liu L, Wu D. miR-142-3p inhibits aerobic glycolysis and cell proliferation in hepatocellular carcinoma via targeting LDHA. Biochem Biophys Res Commun 2018;496:947-54.
15. Li X, Zhao Q, Qi J, Wang W, Zhang D, Li Z, *et al.* lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPARγ pathway in hepatocellular carcinoma. Int J Oncol 2018;53:551-66.
16. Caruso P, Dunmore BJ, Schlosser K, Schoors S, Dos Santos C, Perez-Iratxeta C, *et al.* Identification of MicroRNA-124 as a major regulator of enhanced endothelial cell glycolysis in pulmonary arterial hypertension via PTBP1 (polypyrimidine tract binding protein) and pyruvate kinase M2. Circulation 2017;136:2451-67.
17. Sun K, Hu P, Xu F. LINC00152/miR-139-5p regulates gastric cancer cell aerobic glycolysis by targeting PRKAA1. Biomed Pharmacother 2018;97:1296-302.
18. He M, Jin Q, Chen C, Liu Y, Ye X, Jiang Y, *et al.* The miR-186-3p/EREG axis orchestrates tamoxifen resistance and aerobic glycolysis in breast cancer cells. Oncogene 2019;38:5551-65.
19. Zhang Y, Shi Z, Li Z, Wang X, Zheng P, Li H. Circ_0057553/miR-515-5p regulates prostate cancer cell proliferation, apoptosis, migration, invasion and Aerobic glycolysis by targeting yes1. Onco Targets Ther 2020;13:11289-99.
20. Qian C, Xu Z, Chen L, Wang Y, Yao J. Long noncoding RNA LINC01391 restrained gastric cancer aerobic glycolysis and tumorigenesis via targeting miR-12116/CMTM2 axis. J Cancer 2020;11:6264-76.
21. Zhang Z, Tan X, Luo J, Yao H, Si Z, Tong JS. The miR-30a-5p/CLCF1 axis regulates sorafenib resistance and aerobic glycolysis in hepatocellular carcinoma. Cell Death Dis 2020;11:902.
22. Zhao H, Wei H, He J, Wang D, Li W, Wang Y, *et al.* Propofol disrupts cell carcinogenesis and aerobic glycolysis by regulating circTADA2A/miR-455-3p/FOXM1 axis in lung cancer. Cell Cycle 2020;19:2538-52.
23. Qian CJ, Xu ZR, Chen LY, Wang YC, Yao J. LncRNA MAFG-AS1 accelerates cell migration, invasion and aerobic glycolysis of esophageal squamous cell carcinoma cells via miR-765/PDX1 Axis. Cancer Manag Res 2020;12:6895-908.
24. Long N, Chu L, Jia J, Peng S, Gao Y, Yang H, *et al.* CircPOSTN/miR-361-5p/TPX2 axis regulates cell growth, apoptosis and aerobic glycolysis in glioma cells. Cancer Cell Int 2020;20:374.
25. Shen Y, Xu J, Pan X, Zhang Y, Weng Y, Zhou D, *et al.* LncRNA KCNQ1OT1 sponges miR-34c-5p to promote osteosarcoma growth via ALDOA enhanced aerobic glycolysis. Cell Death Dis 2020;11:278.
26. Du J, Zhang L, Ma H, Wang Y, Wang P. Lidocaine suppresses cell proliferation and aerobic glycolysis by regulating circHOMER1/miR-138-5p/HEY1 axis in colorectal cancer. Cancer Manag Res

2020;12:5009-22.
27. Wu H, Wang X, Wu T, Yang S. miR-489 suppresses multiple myeloma cells growth through inhibition of LDHA-mediated aerobic glycolysis. Genes Genomics 2020;42:291-7.
28. Zhang H, Qin D, Jiang Z, Zhang J. SNHG9/miR-199a-5p/Wnt2 Axis regulates cell growth and aerobic glycolysis in glioblastoma. J Neuropathol Exp Neurol 2019;78:939-48.
29. Han RL, Wang FP, Zhang PA, Zhou XY, Li Y. miR-383 inhibits ovarian cancer cell proliferation, invasion and aerobic glycolysis by targeting LDHA. Neoplasma 2017;64:244-52.
30. Hua Q, Jin M, Mi B, Xu F, Li T, Zhao L, *et al.* LINC01123, a c-Myc-activated long non-coding RNA, promotes proliferation and aerobic glycolysis of non-small cell lung cancer through miR-199a-5p/c-Myc axis. J Hematol Oncol 2019;12:91.
31. Xu WL, Liu S, Li N, Ye LF, Zha M, Li CY, *et al.* Quercetin antagonizes glucose fluctuation induced renal injury by inhibiting aerobic glycolysis via HIF-1α/miR-210/ISCU/FeS pathway. Front Med (Lausanne) 2021;8:656086.
32. Pei YH, Chen J, Wu X, He Y, Qin W, He SY, *et al.* LncRNA peamir inhibits apoptosis and inflammatory response in PM2.5 exposure aggravated myocardial ischemia/reperfusion injury as a competing endogenous RNA of miR-29b-3p. Nanotoxicology 2020;14:638-53.
33. Wang L, Ma Q. Clinical benefits and pharmacology of scutellarin: A comprehensive review. Pharmacol Ther 2018;190:105-27.
34. Wang C, Li Y, Gao S, Cheng D, Zhao S, Liu E. Breviscapine injection improves the therapeutic effect of Western medicine on angina pectoris patients. PLoS One 2015;10:e0129969.
35. Chen ZQ, Zhou Y, Chen F, Huang JW, Zheng J, Li HL, *et al.* Breviscapine pretreatment inhibits myocardial inflammation and apoptosis in rats after coronary microembolization by activating the PI3K/Akt/GSK-3β signaling pathway. Drug Des Devel Ther 2021;15:843-55.
36. Li Y, Li S, Li D. Breviscapine alleviates cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti-inflammatory and anti-oxidant properties in a rat model. ACS Chem Neurosci 2020;11:4489-98.
37. Lunt SY, Vander Heiden MG. Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 2011;27:441-64.
38. Tu Y, Hu Y. MiRNA-34c-5p protects against cerebral ischemia/reperfusion injury: Involvement of anti-apoptotic and anti-inflammatory activities. Metab Brain Dis 2021;36:1341-51.
39. Wu W, Liu J, Yang C, Xu Z, Huang J, Lin J. Astrocyte-derived exosome-transported microRNA-34c is neuroprotective against cerebral ischemia/reperfusion injury via TLR7 and the NF-κB/MAPK pathways. Brain Res Bull 2020;163:84-94.
40. Giricz Z, Makkos A, Schreckenberg R, Pöling J, Lörchner H, Kiss K, *et al.* Swiprosin-1/EFhD-2 expression in cardiac remodeling and post-infarct repair: Effect of ischemic conditioning. Int J Mol Sci 2020;21:3359.
41. Luo G, Wang R, Zhou H, Liu X. ALDOA protects cardiomyocytes against H/R-induced apoptosis and oxidative stress by regulating the VEGF/Notch 1/Jagged 1 pathway. Mol Cell Biochem 2021;476:775-83.
42. Li Y, Zhang D, Kong L, Shi H, Tian X, Gao L, *et al.* Aldolase promotes the development of cardiac hypertrophy by targeting AMPK signaling. Exp Cell Res 2018;370:78-86.



Supplementary Figure 1: Word-cloud term scaling and bar chart. (a) Enrichment of KEGG channel word cloud map, the more prominent KEGG channel, and the font is larger. (b) Top 20 of enriched categories sorted by observed/expected ratio