© IJHG 2022 Int J Hum Genet, 22(2): 107-116 (2022)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2022/22.02.808

LncRNA HOTAIR/MiR-217/GPD2 Axis Medicates Hypoxia Injury of Myocardial Cells

LiHua Dai[1], Zhen Zhou[2], LiLi Yin[1], Yong Wang[1], ZheQian Wu[1], QingYao Xv[1], ShiJie Lu[1] and Jie Zhao[3]

[1]Department of Emergency, Shidong Hospital of Yangpu District, Shanghai 200438, China
[2]East District Center of Shanghai Medical Emergency Center, Shanghai 200082, China
[3]Intensive Care Unit?Shanghai Jiahui International Hospital, Shanghai 200081, China

KEYWORDS Cardiomyocyte. Glycerol-3-phosphate Dehydrogenase 2. HOX Transcript Antisense RNA. Hypoxia Damage. MicroRNA-217

ABSTRACT This study examined impacts of lncRNA HOX Transcript Antisense RNA (HOTAIR), miR-217 and Glycerol-3-phosphate dehydrogenase 2 (GPD2) on progressions of cardiomyocytes after hypoxia damage. Hypoxia treatment induced low cell viability and increased apoptosis. RT-qPCR evaluated suppressed expressions of lncRNA HOTAIR. Overexpressed lncRNA HOTAIR accelerated AC16 cell viability but restrained cell apoptosis and pro-inflammatory protein expressions while the knockdown of HOTAIR caused opposite results. MiR-217 then was examined to be inhibited by HOTAIR overexpression, whose upregulation reduced AC16 cell viability but facilitated apoptosis and pro-inflammatory protein expressions. Luciferase reporter test then verified that GPD2 was bound and decreased by miR-217, which promoted AC16 cell viability but hampered cell apoptosis and pro-inflammatory protein expressions after overexpression. Moreover, PI3K/AKT signaling pathway was activated by overexpression of GPD2.

INTRODUCTION

Ischemia reperfusion injury (IRI) is the first element leading to coronary heart diseases (Hausenloy and Yellon 2013), which can cause injury and necrosis to myocardial cells by blood reperfusion after myocardial ischemia (Frank et al. 2012). According to the research about pathomechanism of IRI, it can be regulated by multiple factors, in which oxidative stress is an important mechanism for inducing reperfusion injury (Kurian et al. 2016). When myocardial cells are faced with reperfusion or reaeration after ischemia or hypoxia, mitochondrial transport chain of cells can't make full use of oxygen causing excessive oxygen free radicals and even lipid peroxidation, DNA damages as well as cell death (Ibanez et al. 2015; Martindale and Metzger 2014). Death of myocardial cells contains autophagy, necrosis and apoptosis and so on and the role of apoptosis has been paid more and more attention (Chiong et al. 2011; Whelan et al. 2010). Hypoxia can induce occurrences of injuries in myocardial cells that is related to oxidative stress and endoplasmic reticulum stress and so on (Li et al. 2017). Moreover, when myocardial cells are faced with hypoxia, ATP production is reduced, which is the main energy source of cell metabolism. After cells are treated with long-time hypoxia, cells necrosis occurs due to a sudden decrease in ATP resulting in insufficient energy for apoptosis, revealing that the ATP level is a main part to determine apoptosis or necrosis of cells (Mironova et al. 2019; Portman et al. 1996; Rozova et al. 2015).

Recently, studies have determined that noncoding RNAs take part in process of cardiovascular diseases through regulating differentiation, proliferation, apoptosis, necrosis and autophagy (Klattenhoff et al. 2013; Wang et al. 2015). LncRNAs are functional RNA molecules transcribed by RNA polymerase a! with length over 200 nucleotides, which are located in cell nucleus and cytoplasm. LncRNAs can incorporate miRNAs, DNAs or RNA binding sites and target bases of promoters to regulate gene expressions and protein functions (Huang 2018). Research revealed that lncRNA EGOT had correlation with myocardial infarction, cardiac ischemia-reperfusion injury, cardiac hypertrophy and

**Address for correspondence*
Jie Zhao,
689 Guiping Road, Xuhui District,
Shanghai 200081, China
Phone: 86-18019031877
E-mail: 19531958@qq.com

heart failure, which also played an important role in cell differentiation and cell transformation (Zhang et al. 2018). LncRNA HOTAIR was measured in hypoxia in several studies, which was expressed lower in oxidative stress-induced H9c2 cells and alleviated injuries of cells through absorbing miR-125 (Li et al. 2018). Besides that, lncRNA HOTAIR could sponge miR-519d-3p to prevent myocardial infarction of rats (Zhang et al. 2019). In order to enrich functions of lncRNA HOTAIR in hypoxia injury of myocardial cells, this study would select lncRNA HOTAIR as the object to figure out more mechanisms and functions of this long noncoding RNA for further treatments.

Objectives

This study aimed at detecting interactions among lncRNA HOTAIR, miR-217 and GPD2 in hypoxic-induced AC16 cells and functions of them in modulating cell viability, apoptosis and protein expressions related to apoptosis and inflammation.

Experimental

Main Reagents

DMEM medium (Gibco, USA), 10 percent fetal bovine serum (Gibco), Beyozol reagent (Beyotime, Shanghai, China), BeyoRT™II First Strand cDNA Synthesis Kit (Beyotime), Lipofectamine 2000 reagent (Invitrogen, USA), RIPA Lysis Buffer (Beyotime), Annexin V-FITC Apoptosis Detection Kit (Invitrogen), QuickBlock™ Blocking Buffer (Beyotime), cleaved-caspase-3 (1:1000; ab32042), Bax(1:1000; ab182733), Bcl-xL(1:1000; ab32370), IL-1β(1:1000; ab9722), IL-6 (1:1000; ab6672), p-PI3K (1:1000; ab182651), p-AKT (1:1000; ab38449), GAPDH(1:2000; ab181602), Goat Anti-Rabbit IgG H&L (HRP) (1:800; ab6721).

METHODOLOGY

Cell Culture

AC16, a myocardial cell line of human was utilized from American Type Culture Collection (ATCC, USA). Incubation of cells was in strict accordance with instructions of ATCC to offer high glucose DMEM medium with 10 percent fetal bovine serum (Gibco, USA) and temperature was 37^0C. Cells were divided into three groups: normal group, short-time hypoxia group and long-time hypoxia group. Cells in normal group were cultured with 5 percent CO_2, 20 percent O_2 and 75 percent N_2. Cells in short-time hypoxia group were filled with 5 percent CO_2 and 95 percent N_2 for 12h. Cells in long-time hypoxia group were filled with 5 percent CO_2 and 95 percent N_2 for 24h.

RT-qPCR

Based on the manufacturer's instruction of Beyozol reagent (Beyotime, Shanghai, China), total RNA was extracted from cells. Afterwards, complementary deoxyribonucleic acid (cDNA) was acquired through reverse transcription of RNA using BeyoRT™II First Strand cDNA Synthesis Kit (Beyotime, Shanghai, China). PCR was applied for amplification later. Related expressions of genes were calculated with $2^{-\Delta\Delta Ct}$ method. Sequences of related primers were shown as below: lncRNA HOTAIR, forward: 5'-CAGTGGGGAACTCTGACTCG-3', reverse: 5'-GTGCCTGGTGCTCTCTTACC-3'; GAPDH forward: 5'-CATGCCGCCTGGAAACCTGCCA-3', reverse: 5'-TGGGCTGGGTGGTCCAGGGGTTTC-3'(Lu et al. 2018); miR-217 forward: 5'-TTGAGGTTGCTTCAGTGA-3', reverse: 5'-GGAGTAGATGATGGTTAGC-3' and U-6 forward:5'-CTCGCTTCGGCAGCACA-3', reverse:5'-AACGCTTCACGAATTTGCGT-3(Wang H. et al. 2018). GPD2: forward: 5'-GGCAGTGAAAGGGACGATTC-3', reverse: 5'-CTGAAATGCAGTCTGCTGCT-3'. Reaction conditions of PCR were displayed as follows: predenaturation: 95^0C, 5min; denaturation, 95^0C, 30s; =annealing, 55^0C, 30s; extension, 72^0C, 30s, 40 cycles. Then, products were stored at 4^0C.

Cell Transfection

Small interfering RNAs of lncRNA HOTAIR and GPD2 and inhibitor of miR-217 were purchased form GenePharma (Suzhou, China). Meanwhile, pcDNA3.1 for overexpressing lncRNA HOTAIR and GPD2 and mimics of miR-217 were utilized from GenePharma. After cell con-

fluences reached 80 percent, Lipofectamine 2000 reagent (Invitrogen, USA) was used for cell transfection in accordance with instructions. After transfection for 48h, RT-qPCR was applied to measure the efficiency.

Dual luciferase Report Assay

3'UTR of GPD2 were inserted into pmirGLO plasmid (Promega, USA) to create GPD2-wt. Mutations of GPD2 were treated as negative control. In the help with Lipofectamine 2000 (Invitrogen), NC/miR-217 mimics were co-transfected into AC16 cells with GPD2-wt/mut. Afterwards, luciferase activities were examined by GloMax® 20/20 Luminometer (Promega, USA).

CCK-8

Cells in log phase were selected and seeded into a 96-well plate with 1×10^3 cells per well. After that, normal group cells were stored in 37^0C, 5 percent CO_2 while hypoxia groups cells were cultured at 37!^{0}C, 5 percent CO_2 and 95 percent N_2. Then, 10ìl CCK-8 was added into each plate to culture with cells for another 1h. Optical density (OD) values of cells were checked at 450nm wavelength with microplate reader (Thermo Scientific, USA).

Cell Apoptosis

Cells were digested with 0.25 percent trypsin first and rinsed through PBS twice to separate single cells. Annexin V-FITC Apoptosis Detection Kit (Invitrogen, USA) was applied to measure cell apoptosis. Cells were mixed with 7-ADD and Annexin V-FITC under dark environment for 20min. Flow cytometry of ACEA NovoCyte (USA) were used for evaluating apoptosis of cells.

Western Blot

Cells were rinsed with PBS twice and RIPA Lysis Buffer (Beyotime, Shanghai, China) was employed to extract total protein. Thereafter, SDS-PAGE was applied to separate proteins and PVDF membranes (Beyotime) were used to transfer proteins. After blocking with QuickBlock™ Blocking Buffer (Beyotime), membranes were cultivated with primary antibodies at 4^0C overnight to detect expressions of cleaved-caspase-3 (1:1000; ab32042), Bax(1:1000; ab182733), Bcl-xL(1:1000; ab32370), IL-1â(1:1000; ab9722), IL-6 (1:1000; ab6672), p-PI3K (1:1000; ab182651), p-AKT (1:1000; ab38449) and GAPDH(1:2000; ab181602). Subsequently, membranes were cultured with Goat Anti-Rabbit IgG H&L (HRP) (1:800; ab6721) at room temperature for 1h. Finally, grey values of proteins were checked through Image Lab (Bio-Rad).

Statistical Analysis

Data were shown as mean±SD and analyzed with SPSS 19.0 (IBM, USA). Student's t-test and one-way ANOVA were used for detecting differences of groups. $P<0.05$ was significant in statistics.

RESULTS

Hypoxia Induced Injuries of AC16 Cells

At first, AC16 cells were placed in a hypoxic environment. CCK-8 test indicated that hypoxia decreased of AC16 cell viability compared with cells in normal condition (Fig. 1A). Meanwhile, hypoxia caused a higher apoptosis rate of AC16 cells (Fig. 1B). Beyond that, relative expressions of factors related to apoptosis as well as inflammation were evaluated. Expressions of cleaved caspase-3 and Bax were upregulated while Bcl-xL was downregulated. At the same time, IL-1â and IL-6 protein expressions were promoted after cells were treated with hypoxia (Fig. 1C). Therefore, hypoxia could restrain progressions of myocardial cells time dependently.

Overexpressed lncRNA HOTAIR Released Hypoxia Injuries While Suppressed lncRNA HOTAIR Enhanced Hypoxia Injuries

Furthermore, RT-qPCR results revealed that hypoxia could reduce lncRNA HOTAIR expression in a time dependent manner (Fig. 2A). The long-time hypoxia group was chosen for measuring functions of lncRNA HOTAIR. Overexpressed lncRNA HOTAIR increased its RNA expression while suppressed lncRNA HOTAIR caused low expression (Fig. 2B). Additionally, lncRNA HOTAIR overexpression facilitated

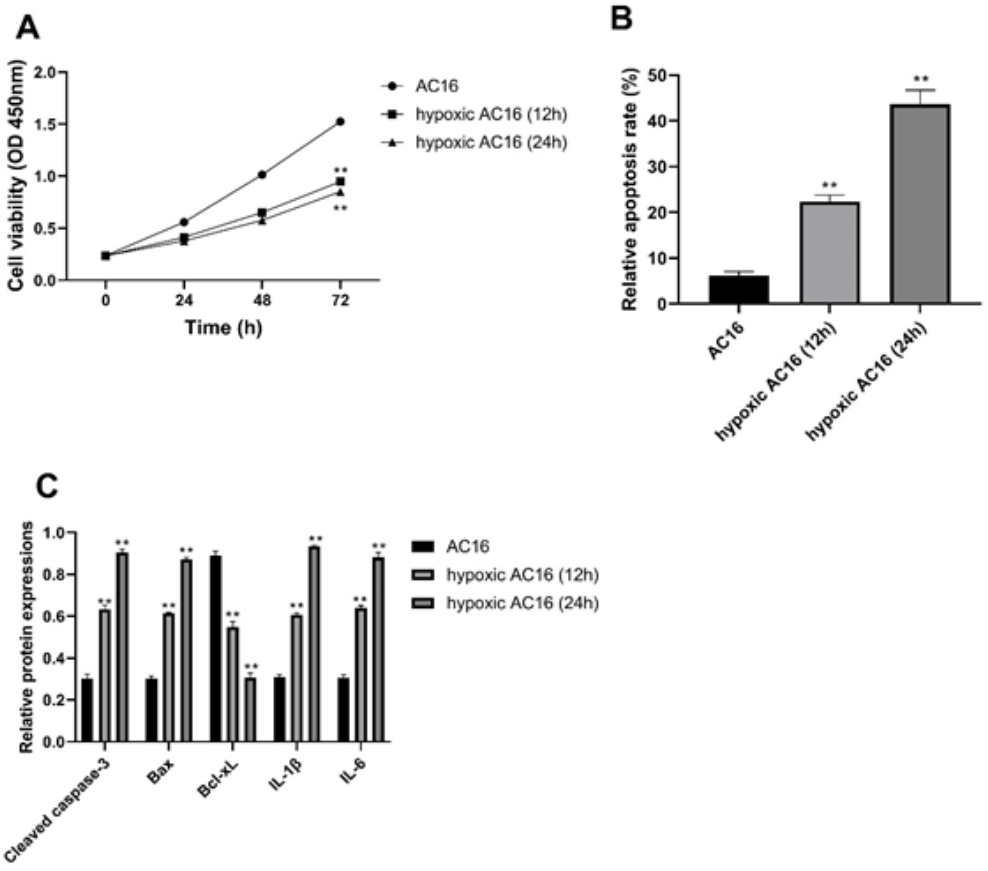

Fig. 1. Hypoxia induced injuries of AC16 cells
A: CCK-8 was applied to check cell viabilities in normal AC16 and hypoxic AC16 (12h) and hypoxic AC16 (24h), P<0.05
B: Apoptosis rate was analyzed with flow cytometry, P<0.05
C: Expressions of cleaved caspase-3, Bax, Bcl-xL, IL-1β and IL-6 were detected through western blot, P<0.05

AC16 cell viability, but its suppression reduced AC16 cell viability (Fig. 2C). Meanwhile, overexpressed lncRNA HOTAIR decreased the cell apoptosis while its suppression accelerated the cell apoptosis (Fig. 2D). Furthermore, cleaved caspase-3 and Bax expressions were reduced and IL-1β and IL-6 expressions were also downregulated by the overexpression of lncRNA HOTAIR. Bcl-xL expression was promoted with lncRNA HOTAIR upregulation (Fig. 2E). In contrast, lncRNA HOTAIR suppression caused opposite results of protein expressions (Fig. 2E).

LncRNA HOTAIR Negatively Regulated miR-217 Expression and Upregulated lncRNA HOTAIR Reduced Hypoxia Damage through Downregulating miR-217

In AC16 cells with hypoxia treatment, miR-217 expression was evaluated to be downregulated with overexpressed lncRNA HOTAIR while knockdown of lncRNA HOTAIR prompted miR-217 expressions (Fig. 3A). Furthermore, miR-217 mimics reversed impacts of HOTAIR overexpres-

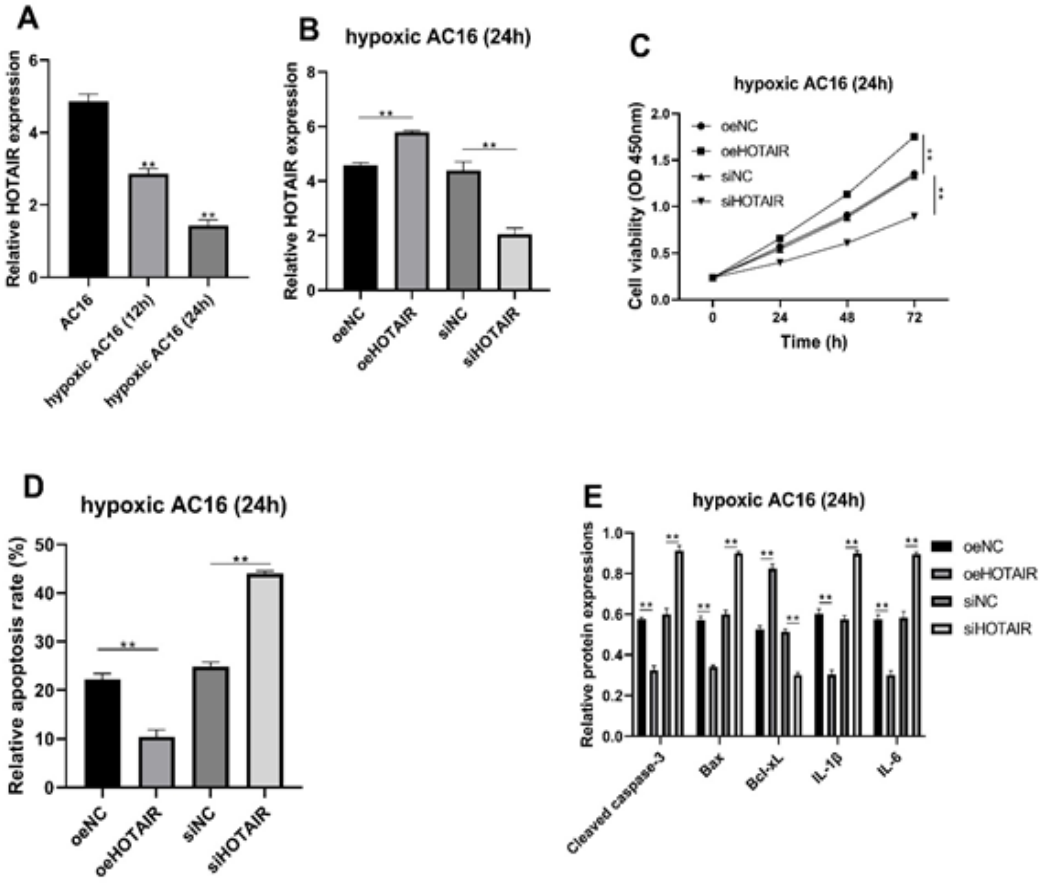

Fig. 2. Overexpressed lncRNA HOTAIR released hypoxia injuries while suppressed lncRNA HOTAIR enhanced hypoxia injuries
A: RT-qPCR was for checking expressions of HOTAIR in AC16 cells in normal and hypoxia conditions, P<0.05
B: Levels of overexpressed HOTAIR and silenced HOTAIR were examined with RT-qPCR, P<0.05
C: Cell viabilities with overexpressed HOTAIR and inhibited HOTAIR were evaluated by CCK-8, P<0.05.
D: Flow cytometry was used to analyze apoptosis rate in hypoxic AC16, P<0.05. E: Levels of cleaved caspase-3, Bax, Bcl-xL, IL-1β and IL-6 were detected through western blot, P<0.05

sion, resulting in increased miR-217 (Fig. 3B). Moreover, miR-217 mimics reduced cell viabilities and increased apoptosis (Figs. 3C, D). Proteins related to apoptosis and inflammation were examined. Results showed enhanced cleaved caspase-3 and Bax expression but suppressed Bcl-xL expressions after miR-217 overexpression (Fig. 3E). Additionally, IL-1β and IL-6 expressions were also increased after miR-217 upregulation (Fig. 3E).

GPD2 targeted by miR-217

Overexpressed miR-217 could decrease level of GPD2 while suppression of miR-217 caused high expression of GPD2 in AC16 cells (Fig. 4A). ENCORI (https://starbase.sysu.edu.cn/) predicted potential target of miR-217, which was GPD2. Results of luciferase reporter test revealed that miR-217 could downregulate fluorescence in AC16 cells through binding 3'UTR of GPD2 (Fig.

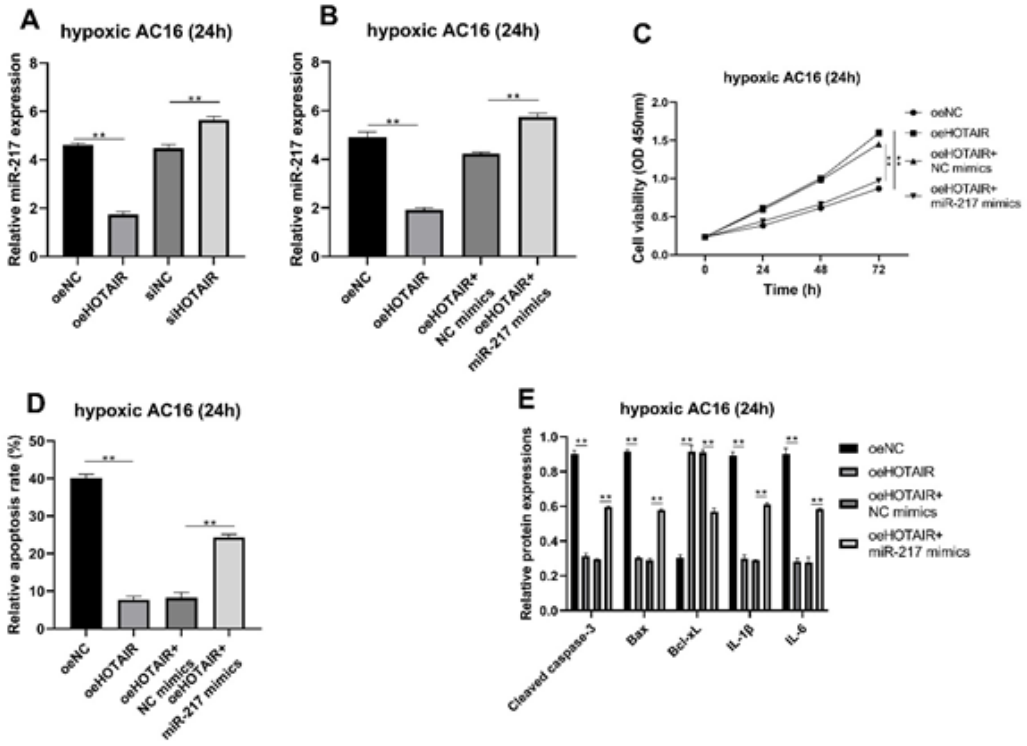

Fig. 3. LncRNA HOTAIR negative regulated level of miR-217 and upregulated lncRNA HOTAIR reduced hypoxia damage through downregulating miR-217
A: Expressions of miR-217 was measured using RT-qPCR, P<0.05
B: Expressions of miR-217 were examined with mimics of miR-217 added, P<0.05
C: Cell viabilities after mimics of miR-217 applied were evaluated with CCK-8, P<0.08. D: Apoptosis rate was validated by flow cytometry, P<0.05
E: Levels of cleaved caspase-3, Bax, Bcl-xL, IL-1β and IL-6 were detected through western blot, P<0.05

4B). Afterwards, interplays between miR-217 and GPD2 were measured as well. Moreover, overexpressed GPD2 enhanced expression of GPD2 while inhibited GPD2 reduced GPD2 expression in hypoxic AC16 cells (Fig. 4C). To confirm functions of miR-217 in regulating GPD2, cell viabilities were checked. Results indicated that overexpressed GPD2 reversed low cell viability caused by miR-217 overexpression (Fig. 4D). Meanwhile, high apoptosis rate caused by miR-217 upregulation was restored with upregulated GPD2 (Fig. 4E). Moreover, factors in apoptosis and inflammation with GPD2 overexpression were checked as well, which indicated that promoted cleaved caspase-3 and Bax in apoptosis caused by miR-217 upregulation were reversed by overexpressed GPD2, so were IL-1β and IL-6. However, Bcl-xL downregulated by miR-217 mimics was restored to be promoted by GPD2 upregulation (Fig. 4F). In order to figure out potential mechanism of GPD2, PI3K/AKT signaling pathway were measure as well, which showed that phosphorylated PI3K and AKT were increased with GPD2 upregulation (Fig. 4G).

DISCUSSION

Coronary heart disease is the most common and severe type of cardiovascular diseases, whose clinical manifestations are angina pectoris, myocardial infarction and heart failure from moderate to severe (Shiroma et al. 2017). The

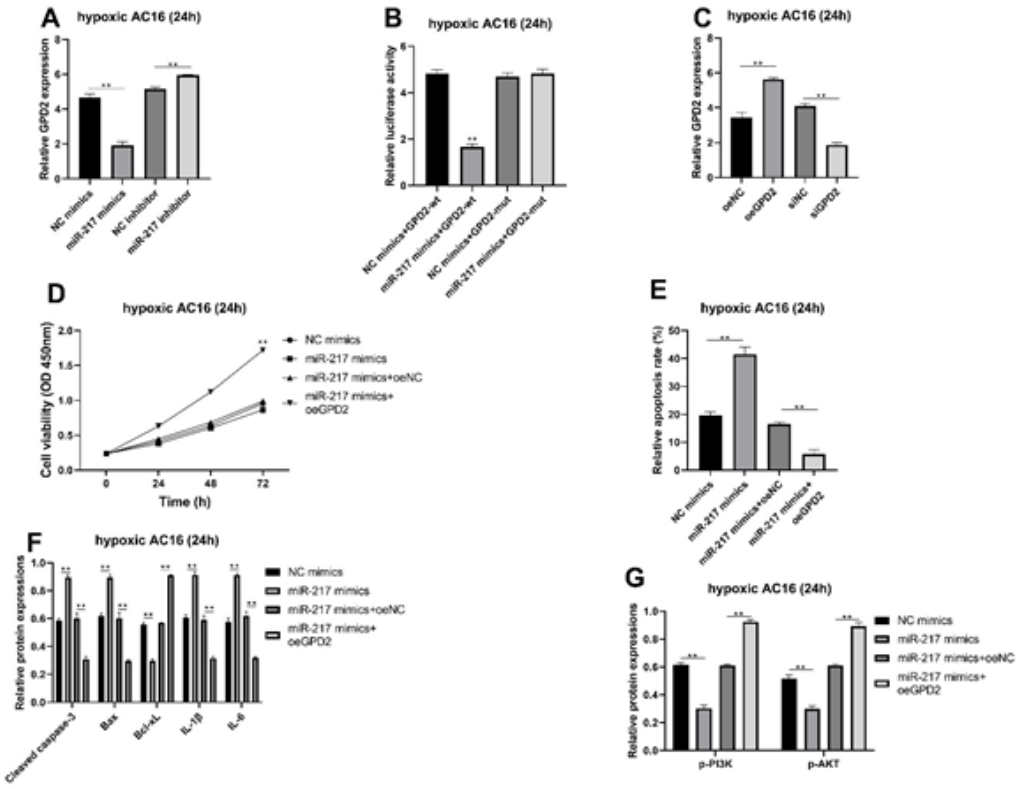

Fig. 4. GPD2 is the target gene of miR-217 and negatively regulate functions of miR-217
A: Levels of GPD2 with overexpressed and suppressed miR-217 were checked by RT-qPCR, P<0.05
B: Luciferase activity was applied to check binding situation between miR-217 and GPD2, P<0.05
C: Expressions of GPD2 with overexpressed and inhibited GPD2 were analyzed by RT-qPCR, P<0.05
D: Cell viabilities with mimics of miR-217 were analyzed after overexpression of GPD2 through CCK-8, P<0.05
E: Apoptosis rate with mimics of mi-217 and overexpressed miR-217 were detected by flow cytometry, P<0.05
F: Levels of cleaved caspase-3, Bax, Bcl-xL, IL-1β and IL-6 were detected through western blot, P<0.05
G: Expressions of phosphorylated PI3K and AKT were examined with mimics of miR-217 and overexpressed GPD2

development of atherosclerosis of coronary artery could induce severe narrowing or blockage of the lumen in a coronary artery, causing hypoxia and ischemia of myocardium and even heart failure (Gao et al. 2012a). Heart failure means that heart does not provide the necessary blood supply to the tissues in time, leading to a decrease in blood flow to the heart (Lee and Auld 2015). Moreover, long-term hypoxia was verified to induce damages and necrosis of myocardial cells, resulting in heart failure (Cruz et al. 2012). Meanwhile, other researchers have determined that hypoxia could lead to severe damages of cardiomyocyte functions and changes of cell ultrastructure such as cell membrane integrity loss, myocardial enzyme release and declined cell growth and survival rates and so on (Rosenson et al. 2016). Until now, oxidative stress, inflammation and cell apoptosis are key points in mechanisms of hypoxia injuries of myocardial cells (Bing et al. 1971; Chen et al. 2020). This study also measured mechanism of hypoxia in detecting cell viabilities, apoptosis as well as inflammation, which revealed that hypoxia could

decrease viabilities of cells in time dependence. Meanwhile, apoptosis rate was increased and expressions of caspase-3 and Bax were increased but Bcl-xL expression was reduced. Furthermore, levels of IL-1β and IL-6, factors in inflammation, were also increased. Results showed that hypoxia could make contribution to apoptosis as well as inflammation to myocardial cells through suppressing cell viabilities.

Many kinds of lncRNAs were detected to have close connections with myocardial ischemia as well as cell apoptosis in myocardial cells. LncRNA CARL upregulated expressions of PHB2 by sponging miR-539, resulting in suppressed cardiomyocytes apoptosis (Wang et al. 2014a). Besides that, lncRNA MDRL could promote expressions of miR-484 through absorbing miR-361 to release apoptosis induced by hypoxia (Wang et al. 2014b). LncRNA TUG1 could enhance hypoxia-induced injury of myocardial cells through sponging miR-145-5p and increasing expressions of Binp3 (Wu et al. 2018). LncRNA GAS5 enhanced hypoxia damage of HL-1 cells by reducing cell viability and promoting apoptosis via sponging miR-222-3p (He et al. 2020). Previous research has measured expressions of HOTAIR related to hypoxia-induced myocardial cells, revealing that HOTAIR expressed lower in patients with acute myocardial infarction(Gao et al. 2017b). In order to make further detection, HOTAIR was selected in this study, whose expression was decreased with hypoxia. After transfection, overexpressed lncRNA HOTAIR increased its expression and cell viabilities. Meanwhile, apoptosis was reduced and caspase-3 as well as Bax levels were suppressed while Bcl-xL expression was upregulated. As for inflammation, IL-1β and IL-6 were reduced as well. Suppressed lncRNA HOTAIR was checked as well, which displayed a reverse result. Through detection, HOTAIR acted as a suppressor in hypoxia injury of myocardial cells, which could protect cells from death through downregulating apoptosis as well as inflammation.

MicroRNAs are short, endogenic and single stranded RNAs with about 22 nucleotides in length which are considered as negative regulators of gene expressions through binding 3'UTR of mRNAs (Fabian et al. 2010; Humphreys et al. 2005; Kiriakidou et al. 2007). Recent studies have revealed that some of miRNAs were upregulated in cardiovascular system with regulating cardiovascular development and diseases (Pedersen et al. 2007). MiR-217 was found to enhance hypoxia injury of H9c2 cells under correlation with lncRNA MALAT1 (Yao et al. 2019). Moreover, miR-217 was detected to be sponged by lncRNA HOTAIR. Therefore, this study would choose miR-217 to measure its correlation with lncRNA HOTAIR. Expressions of miR-217 were first verified that lncRNA HOTAIR could negatively regulate levels of miR-217. With evaluation of overexpressed miR-217, negative interactions between miR-217 and lncRNA HOTAIR were determined through cell viabilities, apoptosis and factors in inflammation. Mimics of miR-217 could reverse high cell viabilities caused by upregulation of lncRNA HOTAIR. Meanwhile, it could also enhance apoptosis rate as well as caspase-3 and Bax expressions. In inflammation, low levels of IL-1β and IL-6 were also increased. Thus, miR-217 was a factor that could enhance process of hypoxia in myocardial cells.

GPD2 (glycerol-3-phosphate dehydrogenase2) is a nuclear coding protein located in the outer surface of the mitochondrial membrane (Klingenberg 1970), which has been detected in protecting survival of Caenorhabditis elegans in hypoxia environment. At the same time, GPD2 was evaluated to have binding sites with miR-217. Thus, functions of GPD2 were checked as well, which indicated that GPD2 could reverse functions of miR-217 in hypoxia-treated cells. Overexpressed GPD2 enhanced cell viabilities by repressing expressions of miR-217. High apoptosis and inflammation by mimics of miR-217 were suppressed with upregulation of GPD2. Besides that, overexpressed GPD2 was discovered to released hypoxia injuries through PI3K/AKT signaling pathway, which has been verified to relieve hypoxia damages in myocardial cells (Song et al. 2018; Tang et al. 2017). Results showed that GPD2 could be a factor to release hypoxia injury in myocardial cells by suppressing apoptosis as well as inflammation.

CONCLUSION

LncRNA HOTAIR upregulated GPD2 to accelerated hypoxia-induced AC16 cell viability and restrained apoptosis through sponging miR-217, suggesting lncRNA HOTAIR/miR-217/GPD2

axis might be a promising way to treat hypoxia damage. However, in vivo research should be made to confirm functions of these three in the whole system.

RECOMMANDATIONS

LncRNA HOTAIR and GPD2 were downregulated after hypoxia treatment while miR-217 was promoted. MiR-217 was verified to be sponged by lncRNA HOTAIR and GPD2 was targeted by miR-217. LncRNA HOTAIR upregulated GPD2 through sponging miR-217, accelerating cell viability and hindering cell apoptosis and inflammation.

ABBREVIATION LIST

LncRNA HOTAIR: long noncoding RNA HOX Transcript Antisense RNA;
GPD2: Glycerol-3-phosphate dehydrogenase 2;
IRI: Ischemia reperfusion injury;
ATP: Adenosine triphosphate;
ATCC: American Type Culture Collection;
DMEM: Dulbecco's modified eagle medium;
PI3K: phosphatidylinositol 3-kinase.

FUNDING

This study was supported by the Natural Science Foundation of 2021 "Science and Technology Innovation Action Plan" of Shanghai (21ZR1459400), The key disciplines of Yangpu District 2019 2022(YP19ZA07).

ACKNOWLEDGMENTS

I wish to thank Mr. Zhou Zhen for his helpful suggestions and inspiration on relevant experimental materials and experimental design, as well as his review of the accuracy of the final text.

REFERENCES

Bing OH, Keefe JF, Wolk MJ, Finkelstein LJ, Levine HJ 1971. Tension prolongation during recovery from myocardial hypoxia. *J Clin Invest*, 50(3): 660-666.

Chen D, Wang X, Huang J, Cui S, Zhang L 2020. Cdkn1b mediates apoptosis of neuronal cells and inflammation induced by oxyhemoglobin via mir-502-5p after subarachnoid hemorrhage. *J Mol Neurosci*, 70(7): 1073-1080.

Chiong M, Wang ZV, Pedrozo Z, Cao DJ, Troncoso R, Ibacache M, Criollo A, Nemchenko A, Hill JA, Lavandero S 2011. Cardiomyocyte death: Mechanisms and translational implications. *Cell Death Dis*, 2(12): 1-11.

Cruz JA, Bauer EM, Rodriguez AI, Gangopadhyay A, Zeineh NS, Wang Y, Shiva S, Champion HC, Bauer PM 2012. Chronic hypoxia induces right heart failure in caveolin-1-/- mice. *Am J Physiol Heart Circ Physiol*, 302(12): H2518-2527.

Fabian MR, Sonenberg N, Filipowicz W 2010. Regulation of mrna translation and stability by micrornas. *Annu Rev Biochem*, 79: 351-379.

Frank A, Bonney M, Bonney S, Weitzel L, Koeppen M, Eckle T 2012. Myocardial ischemia reperfusion injury: From basic science to clinical bedside. *Semin Cardiothorac Vasc Anesth*, 16(3): 123-132.

Gao L, Chen Q, Zhou X, Fan L 2012. The role of hypoxia-inducible factor 1 in atherosclerosis. *J Clin Pathol*, 65(10): 872-876.

Gao L, Liu Y, Guo S, Yao R, Wu L, Xiao L, Wang Z, Liu Y, Zhang Y 2017. Circulating long noncoding rna hotair is an essential mediator of acute myocardial infarction. *Cell Physiol Biochem*, 44(4): 1497-1508.

Hausenloy DJ, Yellon DM 2013. Myocardial ischemia-reperfusion injury: A neglected therapeutic target. *J Clin Invest*, 123(1): 92-100.

He J, Dai H, Zhao Q, Guo J, Chen C 2020. Lncrna gas5 suppression protected hl-1 cells against hypoxia injury by sponging mir-222-3p. *Exp Mol Pathol*, 115: 1-19.

Huang Y 2018. The novel regulatory role of lncrna-mirna-mrna axis in cardiovascular diseases. *J Cell Mol Med*, 22(12): 5768-5775.

Humphreys DT, Westman BJ, Martin DI, Preiss T 2005. Micrornas control translation initiation by inhibiting eukaryotic initiation factor 4e/cap and poly(a) tail function. *Proc Natl Acad Sci U S A*, 102(47): 16961-16966.

Ibanez B, Heusch G, Ovize M, Van De Werf F 2015. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol*, 65(14): 1454-1471.

Kiriakidou M, Tan GS, Lamprinaki S, De Planell-Saguer M, Nelson PT, Mourelatos Z 2007. An mrna m7g cap binding-like motif within human ago2 represses translation. *Cell*, 129(6): 1141-1151.

Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S, Abo R, Tabebordbar M, Lee RT, Burge CB, Boyer LA 2013. Braveheart, a long noncoding rna required for cardiovascular lineage commitment. *Cell*, 152(3): 570-583.

Klingenberg M 1970. Localization of the glycerol-phosphate dehydrogenase in the outer phase of the mitochondrial inner membrane. *Eur J Biochem*, 13(2): 247-252.

Kurian GA, Rajagopal R, Vedantham S, Rajesh M 2016. The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: Revisited. *Oxid Med Cell Longev*, 2016: 1-14.

Lee CS, Auld J 2015. Heart failure: A primer. *Crit Care Nurs Clin North Am*, 27(4): 413-425.

Li L, Zhang M, Chen W, Wang R, Ye Z, Wang Y, Li X, Cai C 2018. Lncrna-hotair inhibition aggravates oxidative stress-induced h9c2 cells injury through suppression of mmp2 by mir-125. *Acta Biochim Biophys Sin (Shanghai)*, 50(10): 996-1006.

Li Y, Zhu X, Liu X, Du A, Yu B 2017. Mir-200a mediates protection of thymosin beta 4 in cardiac microvascular endothelial cells as a novel mechanism under hypoxia-reoxygenation injury. *J Cell Biochem*, 120(11): 19098-19106.

Lu W, Zhu L, Ruan ZB, Wang MX, Ren Y, Li W 2018. Hotair promotes inflammatory response after acute myocardium infarction by upregulating rage. *Eur Rev Med Pharmacol Sci*, 22(21): 7423-7430.

Martindale JJ, Metzger JM 2014. Uncoupling of increased cellular oxidative stress and myocardial ischemia reperfusion injury by directed sarcolemma stabilization. *J Mol Cell Cardiol*, 67: 26-37.

Mironova GD, Rozova EV, Belosludtseva NV, Man'kovskaya IN 2019. Dynamic restructuring of the myocardial mitochondria in response to uridine modulation of the activity of mitochondrial atp-dependent potassium channel under conditions of acute hypoxic hypoxia. *Bull Exp Biol Med*, 166(6): 806-810.

Pedersen IM, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, David M 2007. Interferon modulation of cellular micrornas as an antiviral mechanism. *Nature*, 449(7164): 919-922.

Portman MA, Standaert TA, Ning XH 1996. Developmental changes in atp utilization during graded hypoxia and reoxygenation in the heart in vivo. *Am J Physiol*, 270(1 Pt 2): H216-223.

Rosenson RS, Brewer HB, Jr., Ansell BJ, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, Webb NR 2016. Dysfunctional hdl and atherosclerotic cardiovascular disease. *Nat Rev Cardiol*, 13(1): 48-60.

Rozova EV, Mankovskaya IN, Mironova GD 2015. Structural and dynamic changes in mitochondria of rat myocardium under acute hypoxic hypoxia: Role of mitochondrial atp-dependent potassium channel. *Biochemistry (Mosc)*, 80(8): 994-1000.

Shiroma EJ, Cook NR, Manson JE, Moorthy MV, Buring JE, Rimm EB, Lee IM 2017. Strength training and the risk of type 2 diabetes and cardiovascular disease. *Med Sci Sports Exerc*, 49(1): 40-46.

Song HP, Chu ZG, Zhang DX, Dang YM, Zhang Q 2018. Pi3k-akt pathway protects cardiomyocytes against hypoxia-induced apoptosis by mitokatp-mediated mitochondrial translocation of pakt. *Cell Physiol Biochem*, 49(2): 717-727.

Tang L, Mo Y, Li Y, Zhong Y, He S, Zhang Y, Tang Y, Fu S, Wang X, Chen A 2017. Urolithin a alleviates myocardial ischemia/reperfusion injury via pi3k/akt pathway. *Biochem Biophys Res Commun*, 486(3): 774-780.

Wang H, Ke J, Guo Q, Barnabo Nampoukime KP, Yang P, Ma K 2018. Long non-coding rna crnde promotes the proliferation, migration and invasion of hepatocellular carcinoma cells through mir-217/mapk1 axis. *J Cell Mol Med*, 22(12): 5862-5876.

Wang K, Long B, Zhou LY, Liu F, Zhou QY, Liu CY, Fan YY, Li PF 2014a. Carl lncrna inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing mir-539-dependent phb2 downregulation. *Nat Commun*, 5: 1-13.

Wang K, Sun T, Li N, Wang Y, Wang JX, Zhou LY, Long B, Liu CY, Liu F, Li PF 2014b. Mdrl lncrna regulates the processing of mir-484 primary transcript by targeting mir-361. *PLoS Genet*, 10(7): 1-12.

Wang K, An T, Zhou LY, Liu CY, Zhang XJ, Feng C, Li PF 2015. E2f1-regulated mir-30b suppresses cyclophilin d and protects heart from ischemia/reperfusion injury and necrotic cell death. *Cell Death Differ*, 22(5): 743-754.

Whelan RS, Kaplinskiy V, Kitsis RN 2010. Cell death in the pathogenesis of heart disease: Mechanisms and significance. *Annu Rev Physiol*, 72: 19-44.

Wu Z, Zhao S, Li C, Liu C 2018. Lncrna tug1 serves an important role in hypoxia-induced myocardial cell injury by regulating the mir1455pbinp3 axis. *Mol Med Rep*, 17(2): 2422-2430.

Yao Y, Fan X, Yu B, Li T, Zhang Y 2019. Knockdown of long noncoding rna malat1 aggravates hypoxia-induced cardiomyocyte injury by targeting mir-217. *Adv Clin Exp Med*, 28(6): 719-728.

Zhang C, Pan S, Aisha A, Abudoukelimu M, Tang L, Ling Y 2018. Recombinant human brain natriuretic peptide regulates pi3k/akt/mtor pathway through lncrna egot to attenuate hypoxia-induced injury in h9c2 cardiomyocytes. *Biochem Biophys Res Commun*, 503(3): 1186-1193.

Zhang D, Wang B, Ma M, Yu K, Zhang Q, Zhang X 2019. Lncrna hotair protects myocardial infarction rat by sponging mir-519d-3p. *J Cardiovasc Transl Res*, 12(3): 171-183.

Paper received for publication in May, 2022
Paper accepted for publication in April, 2022