Long non-coding RNA DANCR alleviates hypoxia-caused H9c2 cells damage through up regulation of HIF-1α

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ABSTRACT
Hypoxia is an important cause of myocardial cell loss, further inducing various heart illnesses, including acute myocardial infraction (AMI). Long non-coding RNA (LncRNA) discrimination antagonising non-protein coding RNA (DANCR) was firstly identified as epidermal cell differentiation suppressor. Here, we aimed to explore the effects and mechanism of DNACR in hypoxia-induced H9c2 cells. Hypoxic cells were made through 94% N₂, 5% CO₂, and 1% O₂ environment for 24 h. Cell viability and apoptosis were detected via methyl thiazolyl tetrazolium (MTT) method and flow cytometry analysis, respectively. The expression of DANCR and HIF-1α was examined via qRT-PCR. The expression of proteins related to cell apoptosis and PI3K/AKT/mTOR and ERK1/2 signal pathways was examined through western blot analysis. We found that hypoxia induced obvious cell activity inhibition and apoptosis increasing in H9c2 cells. DANCR was negatively regulated under hypoxia condition. Overexpression of DANCR rescued activity and attenuated apoptosis. Moreover, the overexpression of DANCR elevated the activation of PI3K/AKT/mTOR and ERK1/2 pathways. Further study indicated that DANCR could up-regulate the expression of HIF-1α. Si-HIF-1α transfection could remove the beneficial effects of DANCR overexpression in hypoxia-caused H9c2 cells damage. In conclusion, DANCR alleviated hypoxia-caused H9c2 cells damage through positive regulation of HIF-1α.

GRAPHICAL ABSTRACT

Introduction
With the increasing pressure of life and mental stress, cardiovascular disease has become one of the important diseases that threaten human health, and the main cause of death is myocardial infarction (MI) [1]. In general, MI is mostly caused by persistent ischaemia and hypoxia in the coronary arteries, which leads to long-term insufficiency of blood supply to the heart [2]. As one of the highest mortality diseases in the...
world, acute myocardial infarction (AMI) causes myocardial cell necrosis, heart failure, malignant arrhythmia and even sudden death, resulting in over 4 million deaths in Europe and northern Asia [3,4]. At present, main diagnostic methods for AMI are the combination of physical examination and electrocardiogram, and the detection of certain gold standard cardiac biomarkers. However, the specificity and sensitivity of these methods are far from meeting the current needs [5]. Therefore, it is of great importance to find specific markers for early diagnosis of AMI and effective solutions to alleviate myocardial damage caused by hypoxia.

In the early days of molecular biology, ribonucleic acid was usually divided into two categories: coding RNA and non-coding RNA [6]. Long non-coding RNA (LncRNA) is a transcription product which is longer than 200 nt but without proteins encoding capabilities [7]. At present, IncRNA has been found to be irreplaceable in other diseases besides its role in the pathophysiology of cancer, such as ischaemic heart disease (containing myocardial infarction and angina pectoris) [8,9]. For example, Li X et al. detected that IncRNA myocardial infarction related transcript 1 (Mirt1) was highly expressed in cardiac fibroblasts, and Mirt1 knockdown elevated cardiac roles, reduced cardiomyocytes apoptosis and infiltration of inflammatory cells [10]. Besides that, Gong LC et al. demonstrated that hypoxia induced overexpression of IncRNA H19 and H19 knockdown aggravated hypoxia-caused damage presented as raising cell activity, migration and incursion but reducing cell apoptosis via miR-139-mediated positive regulation of Sox8 [11]. Therefore, in-depth exploration of IncRNAs functions in the occurrence and development of MI as well as the related molecular mechanism are of great significance to the development of molecular targeted drugs, the improvement of therapeutic methods and the increasing of prognosis and survival rate of MI.

LncRNA discrimination antagonising non-protein coding RNA (DANCR) encodes human chromosome 4q12 and it was firstly identified as epidermal cell differentiation suppressor [12], but further study revealed that it acted as an oncogene in various kinds of cancers, such as colon cancer [13], prostate cancer [14] and hepatocellular carcinoma [15]. However, the effect of DANCR in cardiovascular diseases has never been explored. In our current research, we aimed to explore the roles and action mechanism of DANCR to provide a novel target for diagnosis and therapy of cardiovascular diseases.

Materials and methods

Cell

The H9c2 cell line was cultivated in DMEM contained with 10% (v/v) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a 37 °C environment with 5% CO₂. To simulate the myocardial ischaemia, H9c2 cells were handled with hypoxia.

Figure 1. Hypoxia caused damage in H9c2 cells. (A) Cell activity was valued through MTT method. (B) Apoptosis was tested through flow cytometry. (C–D) Levels of apoptosis related factors were tested through western blot analysis. CTRL, control. *p < .05, **p < .01 and ***p < .001 contrasted with control group.
H9c2 cells were incubated in hypoxic incubator containing 94% N₂, 5% CO₂ and 1% O₂ to simulate hypoxia for 24 h.

**Cell viability experiment**

Cell activity was tested through methyl thiazolyl tetrazolium (MTT) method. Cells (1 x 10⁵ cells/well) were seeded in 96-well cell culture plates. When cells were treated as indicated, 20 μl of the MTT solution (5 mg/ml; Sigma-Aldrich) was put into every well and then the mixture were incubated for 2 h. After removing the medium, 100 μl DMSO was put into every well and then the absorbance was read at 570 nm using a Microplate reader (Bio-rad, Hercules, CA, USA).

**Apoptosis experiment**

Cell apoptosis analysis was conducted through PI and FITC-conjugated Annexin V staining. After cells were cleaned in phosphate buffered saline and re-suspended by using binding buffer, the cells were stained in PI and FITC-Annexin V containing 50 μg/ml RNase A (Sigma-Aldrich) following the producer’s directions. Flow cytometry analysis was done through a FACScan (Beckman Coulter, Fullerton, CA, USA) and the data was analysed using FlowJo software (Tree Star, SanCarlos, CA, USA).

**Western blot**

Protein in H9c2 cells was collected through RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) containing protease inhibitors and quantified through BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). After separation through a Bio-Rad Bis-Tris Gel system following the producer’s directions, proteins in the gels were transferred into polyvinylidene difluoride (PVDF) membranes. After blocking with non-fat milk, primary antibodies specific against Bcl-2 (ab32124, Abcam, Cambridge, MA, USA), Bax (ab32503), pro-caspase-3 (ab183179), cleaved-caspase-3 (ab49822), β-actin (ab8226), t-PI3K (ab140307), p-PI3K (ab182651), t-AKT (ab179463), p-AKT (ab38499), t-mTOR (ab2732), p-mTOR (ab84400), t-ERK1/2 (ab54230), p-ERK1/2 (ab32538) and HIF-1α (ab51608) were cultured with the membrane at room temperature for 10 min and at 4 °C overnight. Then, the membranes were incubated with secondary antibody signed by horseradish peroxidase at room temperature for 1 h. At last, semaphores were caught and strength of the bands was quantified through Image Lab™ Software (Bio-Rad, Shanghai, China).

**qRT-PCR**

Overall RNA was collected through Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) referring to the producer’s directions. NanoDropND-2000 spectrophotometer (Thermo Scientific, Rockford, IL, USA) was employed to quantify RNA. Reverse transcriptase (Promega, Madison, WI, USA) was employed to do reverse transcription. The One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) was employed for Real-Time PCR analysis to detect expression of DANCR and HIF-1α. Relative expression was normalised with GAPDH through 2⁻ΔΔCt cycle threshold way. Primers used were: DANCR, forward 5'-CCTATCCCCCTTCTCTAAGAA-3' and reverse 5'-ACTCTTGCAAAAAC-GTGCTG-3'; HIF-1α, forward 5'-CAGCAACGACAAGAACTG-3' and reverse 5'-AAATGCAGTCTGGA-3'; and GAPDH, forward 5'-ATGGAAAGCTGTCATCAAC-3' and reverse 5'-GGATGCAGGGATGT TTCT-3'.

**Cell transfection**

Full length of human IncRNA DANCR was linked into the pcDNA3.1 plasmid (pcDNA3.1-DANCR, GenePharma, Shanghai, China) and the empty pcDNA3.1 plasmid was used as a blank. The small interfering (si) RNA for HIF-1α (si-HIF-1α) and the relative negative control (NC) were designed and compounded specifically by GenePharma Co. (Shanghai, China) and the sequence was as follows: si-HIF-1α, 5'-GCUGUUCACUAAGUGAAUC-3'. Cells were cultured in 6-well plates until reached 80% confluency in a fresh culture medium without FBS before transfection. The pcDNA3.1-DANCR (1 μg/ml) and si-HIF-1α (100 mM) were transfected into cells through Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA) following the producer's direction. 48 h post-transfection was considered as the harvest time in the subsequent assays because the highest transfection efficiency was appeared at 48 h.

**Statistic analysis**

Our consequences of multiple assays are revealed as mean ± SD. Statistical analysis were done through SPSS 19.0 (IBM, Armonk, NY, USA). P values were counted through Student’s t-test or one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test. A p value <.05 was statistically notable result.
Results

Hypoxia caused damage in H9c2 cells

After hypoxia treatment, we found that cell activity was largely suppressed in hypoxia group contrasted with CTRL ($p < .01$, Figure 1(A)). Besides, apoptosis was notably increased by hypoxia ($p < .001$, Figure 1(B)). Similarly, levels of cleaved-caspase-3 and apoptotic protein Bax was apparently elevated ($p < .001$ and $p < .01$), while anti-apoptotic protein Bcl-2 was notably suppressed after hypoxia stimulation contrasted with CTRL ($p < .05$, Figure 1(C,D)). Thus, hypoxia induced obvious cell damage in H9c2 cells presented as the inhibition of cell activity and the increasing of apoptosis.

Hypoxia suppressed the expression of DANCR

To investigate the function of DANCR in hypoxia-caused H9c2 cell damage, qRT-PCR was employed to examine the
expression of DANCR in cells treated by hypoxia. As shown in Figure 2, the expression of DNACR was notably decreased by hypoxia contrasted with CTRL (p < .01), suggesting that hypoxia could negatively regulate DANCR.

**Overexpression of DANCR alleviated hypoxia-caused H9c2 cell damage**

Based on the result in Figures 1 and 2, we supposed that the overexpression of DANCR might protect H9c2 cells against hypoxia-caused damage. To verify our hypothesis, the DNACR overexpression cell model was successfully established through transfecting pcDNA3.1-DANCR into H9c2 cells (Figure 3(A)). After transfection with pcDNA3.1-DANCR, the expression level of DANCR was notably increased (p < .001, Figure 3(A)). Then we observed that overexpression of DANCR apparently raised the decreasing viability caused by hypoxia (p < .05, Figure 3(B)). In addition, pcDNA3.1-DANCR transfection notably reduced apoptosis (p < .05, Figure 3(C)). And similarly, the increasing levels of Bax and cleaved-caspase-3 were notably reduced by pcDNA3.1-DANCR transfection (both p < .01), while that of Bcl-2 did the opposite (p < .05, Figure 3(D,E)). Thus, the overexpression of DANCR could attenuate H9c2 cells damage caused by hypoxia.

**Overexpression of DANCR activated the PI3K/AKT/mTOR and ERK1/2 signal pathways**

Associated signal pathways were also explored through western blot analysis. Figure 4(A,B) indicated that levels of p/t-PI3K, p/t-AKT and p/t-mTOR was notably suppressed by hypoxia stimulation (p < .001, p < .05 and p < .01), while were apparently elevated by pcDNA3.1-DANCR transfection (p < .05, p < .001 and p < .01), indicating that up-regulation of DANCR elevated the activation of PI3K/AKT/mTOR signal pathway under hypoxic conditions. Similarly, level of p/t-
ERK1/2 was also suppressed by hypoxia stimulation \((p < .01)\), while was obviously raised after transfection with pcDNA3.1-DANCR \((p < .01\), Figure 4(C,D)). These finding suggested that overexpression of DANCR activated these two pathways.

**Overexpression of DANCR elevated the level of HIF-1α in hypoxia-caused H9c2 cells**

HIF-1α, a core regulator of hypoxia response [16], was detected in our study to further examine the functional mechanism of DANCR. qRT-PCR and western blot assays showed that expression level of HIF-1α was apparently raised under hypoxia condition \((p < .05)\), and it was further increased by the transfection of pcDNA3.1-DANCR \((p < .05\), Figure 5(A,B)). This finding suggested that DANCR could up-regulate the expression of HIF-1α.

**Overexpression of DANCR alleviated hypoxia-caused H9c2 cell damage through up-regulating HIF-1α**

Based on the positive regulation relationship of DANCR on HIF-1α, si-HIF-1α was transfected into H9c2 cells to further research the function of DANCR. qRT-PCR and western blot assays indicated that the expression of HIF-1α was notably decreased by transfection of si-HIF-1α \((p < .001\), Figure 6(A,B)). The raising viability by the overexpression of DANCR was largely weakened through the combination of si-HIF-1α \((p < .05\), Figure 6(C)). Besides, the inhibiting effect of overexpressed DANCR on cell apoptosis was also abolished by the knockdown of HIF-1α \((p < .05\), Figure 6(D)). Similarly, the transfection of si-HIF-1α notably raised the levels of Bax and cleaved-caspase-3 \((both \ p < .01)\), while notably reduced the level of Bcl-2 \((p < .05\), Figure 6(E,F)). Our above consequences showed that overexpressed DANCR alleviated hypoxia caused H9c2 cell damage by elevating HIF-1α.

**Discussion**

Previous studies have pointed out that hypoxia is a key inducing factor for myocardial cell loss, which further induces kinds of heart illnesses and possibly eventually causes heart exhaustion [17,18]. Trying to block the apoptosis and cell death caused by hypoxia possibly helps to weaken or even avoid the occurrence and developing of heart exhaustion. So it is important to find key molecules that protect cardiomyocytes from hypoxia-caused apoptosis. Here, we were committed to research the probable molecular mechanism of lncRNA DANCR in hypoxia-caused cell damage in cardiomyocytes. Hypoxia mould in vitro in our study was established as described previously [19]. Our present study suggested that significant cell viability decrease and obvious cardiomyocyte apoptosis were induced in the H9c2 cells which were treated in hypoxia for 24h.

With the in-depth research on lncRNAs, some lncRNAs have been revealed as important biomarkers and hopeful targets for AMI [20]. For example, Yan Y et al. detected that plasma circulating lncRNA urothelial carcinoma-associated 1 (UCA1) level of early AMI patients was declined, while that was raised after AMI at 3rd day [12]. Besides, Hu H et al. also reported that the lncRNA metastasis-associated lung adeno-carcinoma transcript 1 (MALAT1) were positively regulated in mouse AMI [21]. DANCR function has been researched in some damaging illnesses. For instance, DANCR was found to act as an oncogene in oesophageal squamous cell carcinoma (ESCC) cells by promoting cell proliferation, migration, invasion and resistance to apoptosis [22]. Here, DANCR expression was suppressed in hypoxia-treated H9c2 cells and overexpression of DANCR effectively attenuated hypoxia-caused cell damage presented as rescuing cell activity and reducing apoptosis. Then, associated regulation mechanism was further investigated.

HIF-1α is a kind of nuclear transcription factor and is important in maintaining oxygen homeostasis in the body. HIF-1α can form a complex network regulation system with other signalling pathways and exert a key function in cell proliferation, angiogenesis and remodelling [23]. In cardiac diseases, increased expression of HIF-1α is the compensation for the body’s adaptation to myocardial ischaemia and hypoxia. HIF-1α can increase blood oxygen capacity and collateral branches by regulating the expression of related genes in the nucleus, activating multiple genes, regulating cell survival and differentiation, vascular remodelling and
production, regulating redox balance and sugar metabolism, increasing oxygen supply and hypoxic local blood flow, thus improving myocardial oxygen status [24]. In our study, we also found that HIF-1α expression was elevated through the stimulation of hypoxia. Besides that, Wen X et al. reported that LncRNA DANCR raised mRNA stability of HIF-1α through interacting with NF90/NF45, causing metastasis and illness developing of nasopharyngeal carcinoma (NPC) [25]. Similarly, in our study, we also found that overexpression of DANCR elevated the level of HIF-1α in hypoxia-induced H9c2 cells.

Figure 6. Overexpressed DANCR alleviated hypoxia-caused H9c2 cell damage through elevating HIF-1α. Specific si-HIF-1α and pcDNA3.1-DANCR were co-transfected into H9c2 cells. The expression of HIF-1α was examined through qRT-PCR (A) and western blot (B). (C) Cell activity was valued through MTT method. (D) Apoptosis was tested through flow cytometry. (E–F) Levels of apoptosis relative factors were examined through western blot analysis. CTRL, control. *p < .05, **p < .01 and ***p < .001 contrasted with indicated group.
cells. Further study revealed that si-HIF-1α abolished the protective influence of DANCR in hypoxia-caused H9c2 cell damage, indicating that overexpression of DANCR alleviates hypoxia-caused damage in H9c2 cells through elevating HIF-1α.

We then explored the possible signal pathways through which DANCR exerted protection role in hypoxia-caused injury in H9c2 cells. In previous studies, Lin KH et al. also demonstrated that Tetramethylpyrazine (TMP) exerted protective effect in hypoxia-mediated cell apoptosis and cell death through actuating IGF1 acceptor path of survival, which was mainly dependent on PI3K/Akt signal pathway [26]. Xiao et al. detected that hydrogen sulphide (H2S) attenuated hypoxia-reoxygenation injury in cardiomyocytes via mTOR activation [27]. And Dong XB et al. reported that H2S exerted cardioprotective effects against chemical hypoxia-caused damage in H9c2 cells via the suppression of ROS-actuated ERK1/2 signal pathways [28]. For the first time, we found the regulation mechanism between DANCR and these pathways that up-regulation of DANCR might attenuate hypoxia-caused H9c2 cell damage through actuating the PI3K/AKT/mTOR and ERK1/2 signal pathways. Our findings will be verified through more assays. Furthermore, the regulation between HIF-1α and these signal pathways has been reported in the previous researches. For example, hypoxia-caused the up-regulation of HIF-1α could be inhibited by the inactivation of PI3K/AKT/mTOR pathway in human glioblastoma cells [29]. Besides, erianin-caused the inhibition of ERK1/2 pathway resulted in its blockage on HIF-1α [30]. These findings revealed the positive regulation mechanism of these pathways on HIF-1α. Further work will be done to explore the functional mechanism among DANCR, HIF-1α and PI3K/AKT/mTOR and ERK1/2 pathways to provide theoretical basis for the application of DANCR on treating AMI.

Conclusion

In our present study, we found that hypoxia caused obvious cell damage in H9c2 cells and the down-regulation of the expression of DANCR. Then we found that overexpression of DANCR effectively attenuated hypoxia-caused cell damage through positively regulating HIF-1α. At the same time, PI3K/AKT/mTOR and ERK1/2 signal pathways were activated by overexpression of DANCR in the stimulation of hypoxia. Our study for the first time revealed the protective effect and the possible signal pathway of DANCR in hypoxia-caused cell damage, providing a novel diagnostic and therapeutic target for cardiovascular diseases.

Authors’ contributions

Conceived and designed the experiments: JCC; Performed the experiments and analysed the data: LBQ, QZ and ASZ; Contributed reagents/materials/analysis tools: FXF, LLD and SSZ; Wrote and revised the manuscript: JCC and LLD. All authors read and approved the final version of the manuscript and ensure this is the case.

Disclosure statement

The authors declare that there are no conflicts of interest.

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Data availability

The dataset(s) supporting the conclusions of this article is(are) included within the article.

References

[1] Okwuosa IS, Lewsey SC, Adesiyun T, et al. Worldwide disparities in cardiovascular disease: challenges and solutions. Int J Cardiol. 2016;202:433–440.
[2] Nazir S, Tachamo N, Lohani S, et al. Acute myocardial infarction and antiphospholipid antibody syndrome: a systematic review. Coron Artery Dis. 2017;28(4):332–335.
[3] Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart disease and stroke statistics–2017 update: a report from the American Heart Association. Circulation. 2017;135(10):e146–e603.
[4] Nichols M, Townsend N, Scarborough P, et al. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J. 2014;35(42):2950–2959.
[5] Park JP, Park MK, Yun JW. Proteomic biomarkers for diagnosis in acute myocardial infarction. Biomarkers. 2011;16(1):1–11.
[6] Palazzo AF, Lee ES. Non-coding RNA: what is functional and what is junk? Front Genet. 2015;6:2.
[7] Perkel JM. Visiting “noncoddarnia. BioTechniques. 2013;54(6):303–304.
[8] Huarte M. The emerging role of IncRNAs in cancer. Nat Med. 2015;21(11):1253–1261.
[9] Greco S, Zaccagnini G, Perfetti A, et al. Long noncoding RNA dysregulation in ischemic heart failure. J Transl Med. 2016;14(1):183.
[10] Li X, Zhou J, Huang K. Inhibition of the IncRNA Mirt1 attenuates acute myocardial infarction by suppressing NF-kappaB activation. Cell Physiol Biochem. 2017;42(3):1153–1164.
[11] Gong LC, Xu HM, Guo GL, et al. Long non-coding RNA H19 protects H9c2 cells against hypoxia-induced injury by targeting MicroRNA-139. Cell Physiol Biochem. 2017;44(3):857–869.
[12] Yan Y, Zhang B, Liu N, et al. Circulating long noncoding RNA UCA1 as a novel biomarker of acute myocardial infarction. BioMed Res Int. 2016;2016:1–7.
[13] Liu Y, Zhang M, Liang L, et al. Over-expression of IncRNA DANCR is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol. 2015; 8(9):11480–11484.
[14] Jia J, Li F, Tang XS, et al. Long noncoding RNA DANCR promotes invasion of prostate cancer through epigenetically silencing expression of TIMP2/3. Oncotarget. 2016;7(25):37868–37881.
[15] Wang J, Pu J, Zhang Y, et al. DANCR contributed to hepatocellular carcinoma malignancy via sponging miR-216a-5p and modulating KLF12. J Cell Physiol. 2019;234(6):9408–9416.
[16] Rankin EB, Glaccia AJ. Hypoxic control of metastasis. Science. 2016;352(6282):175–180.
[17] Nakayama H, Chen X, Baines CP, et al. Ca2++ - and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. J Clin Invest. 2007;117(9):2431–2444.
[18] Kakinuma Y, Ando M, Kuwabara M, et al. Acetylcholine from vagal stimulation protects cardiomyocytes against ischemia and hypoxia involving additive non-hypoxic induction of HIF-1alpha. FEBS Letters. 2005;579(10):2111–2118.
[19] Wang X, Takahashi N, Uramoto H, et al. Chloride channel inhibition prevents ROS-dependent apoptosis induced by ischemia-reperfusion in mouse cardiomyocytes. Cell Physiol Biochem. 2005; 16(4-6):147–154.
[20] Zhang Y, Sun L, Xuan L, et al. Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. Sci Rep. 2016;6(1):22384.

[21] Hu H, Wu J, Li D, et al. Knockdown of IncRNA MALAT1 attenuates acute myocardial infarction through miR-320-Pten axis. Biomed Pharmacother. 2018;106:738–746.

[22] Shi H, Shi J, Zhang Y, et al. Long non-coding RNA DANCR promotes cell proliferation, migration, invasion and resistance to apoptosis in esophageal cancer. J Thorac Dis. 2018;10(5):2573–2582.

[23] Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). Mol Pharmacol. 2006;70(5):1469–1480.

[24] Semenza GL. Hypoxia-inducible factor 1 and cardiovascular disease. Annu Rev Physiol. 2014;76(1):39–56.

[25] Wen X, Liu X, Mao YP, et al. Long non-coding RNA DANCR stabilizes HIF-1alpha and promotes metastasis by interacting with NF90/NF45 complex in nasopharyngeal carcinoma. Theranostics. 2018;8(20):5676–5689.

[26] Lin KH, Kuo WW, Jiang AZ, et al. Tetramethylpyrazine ameliorated hypoxia-induced myocardial cell apoptosis via HIF-1alpha/JNK/ p38 and IGFBP3/BNIP3 inhibition to upregulate PI3K/Akt survival signaling. Cell Physiol Biochem. 2015;36(1):334–344.

[27] Xiao J, Zhu X, Kang B, et al. Hydrogen sulfide attenuates myocardial hypoxia-reoxygenation injury by inhibiting autophagy via mTOR activation. Cell Physiol Biochem. 2015;37(6):2444–2453.

[28] Dong XB, Yang CT, Zheng DD, et al. Inhibition of ROS-activated ERK1/2 pathway contributes to the protection of H2S against chemical hypoxia-induced injury in H9c2 cells. Mol Cell Biochem. 2012;362(1–2):149–157.

[29] Huang W, Ding X, Ye H, et al. Hypoxia enhances the migration and invasion of human glioblastoma U87 cells through PI3K/Akt/ mTOR/HIF-1alpha pathway. Neuroreport. 2018;29(18):1578–1585.

[30] Yu Z, Zhang T, Gong C, et al. Erianin inhibits high glucose-induced retinal angiogenesis via blocking ERK1/2-regulated HIF-1alpha-VEGF/VEGFR2 signaling pathway. Sci Rep. 2016;6(1):34306.