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Original Article

The mechanism by which quercetin protects myocardial cells against apoptosis

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Abstract: Myocardial infarction (MI), commonly known as a heart attack, occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle. However, the detailed mechanism is still unclear, and a lack of effective drugs is a problem in the field. Quercetin is a plant polyphenol from the flavonoid group, and it is reported that it has ability to relieve MI. In this study, bioinformatics was used to discover the mechanism of quercetin protects myocardial cells against apoptosis. $CoCl_2$ was used to treat H9C2 cells to mimic a hypoxic/ischemic condition. Then quercetin was used to protect H9C2 cells pretreated with $CoCl_2$, and apoptosis and cell viability of H9C2 cells was determined for different treatments. Finally, bioinformatics was performed to reveal its potential mechanism. Quercetin was able to protect myocardial cells against apoptosis. Bioinformatics analysis results suggest that quercetin plays a protective role in the process of biological process, molecular function and cellular component in $CoCl_2$ induced cells apoptosis. These findings may provide a promising approach to treat the MI.

Keywords: Myocardial infarction, quercetin, apoptosis, bioinformatics

Introduction

Myocardial infarction (MI), commonly known as a heart attack, occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle [1]. The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Often it occurs in the center or left side of the chest and lasts for more than a few minutes. The discomfort may occasionally feel like heartburn [1]. An MI may cause heart failure, an irregular heartbeat, cardiogenic shock, or cardiac arrest. Worldwide, about 15.9 million myocardial infarctions occurred in 2015 [2]. More than 3 million people had an ST elevation MI and more than 4 million had an non-ST segment elevated myocardial infarction (NSTEMI) [3]. ST segment elevated myocardial infarction (STEMI) occur about twice as often in men as women [4]. About one million people have an MI each year in the United States. In the developed world the risk of death in those who have had an STEMI is about 10% [5]. Rates of MI for a given age have decreased globally between 1990 and 2010 [6]. In 2011, AMI was one of the top five most expensive conditions during inpatient hospitalizations in the US, with a cost of about $11.5 billion for 612,000 hospital stays [7]. Thus the MI has already seriously affected human health and burdened the government healthcare system. Effective medications are under urgent need.

Quercetin is a plant polyphenol from the flavonoid group, found in many fruits, vegetables, leaves, and grains. It can be used as an ingredient in supplements, beverages, or foods [8]. The reported biological functions of quercetin including anti-inflammatory, anti-coagulation, and oxygen radical-scavenging activity [9, 10]. Jin et al. discovered that the application of quercetin before ischemia or during reperfusion has been found to protect myocardium from ischemia-reperfusion injury in an acute myocardial ischemia-reperfusion injury rat model [11]. However, mechanisms underlying this protective effect remain unclear.

Bioinformatics is an interdisciplinary field that develops methods and software tools for under-

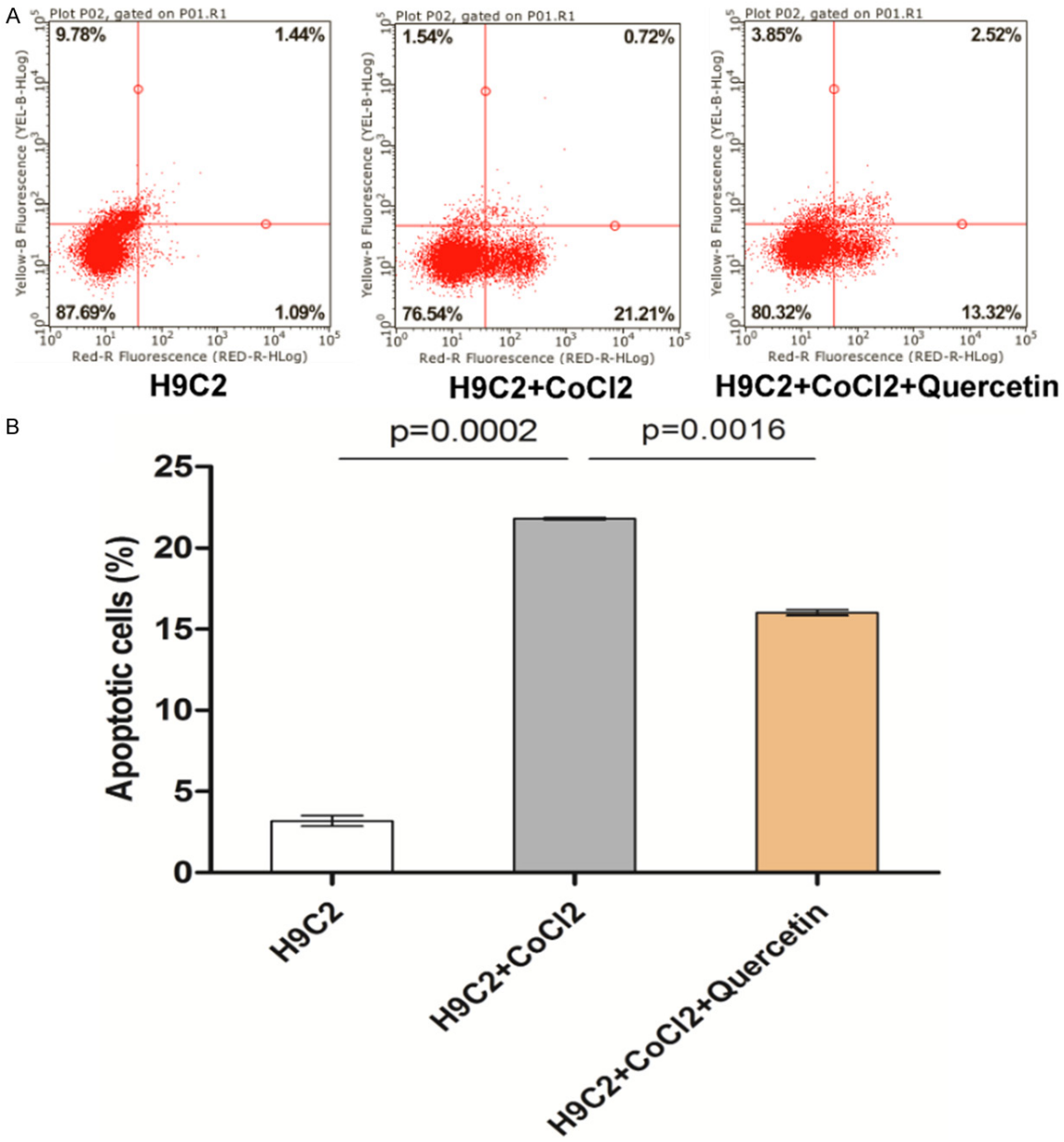


Figure 1. Quercetin protects myocardial cells against apoptosis. A. Apoptosis assay of H9C2 cells treated with $CoCl_2$, H9C2 cells treated with $CoCl_2$ plus quercetin, H9C2 cells without any treatment as control. B. Quantification of apoptotic H9C2 cells of control, $CoCl_2$ treated and $CoCl_2$ + quercetin treated groups.

standing biological data. As an interdisciplinary field of science, bioinformatics combines computer science, biology, mathematics, and engineering to analyze and interpret biological data [12]. Common uses of bioinformatics include the identification of candidate genes and single nucleotide polymorphisms (SNPs). Often, such identification is made with the aim of better understanding the genetic basis of disease, unique adaptations, desirable properties (esp. in agricultural species), or differences between populations [13].

According to the aforementioned, in this study, here bioinformatics was used to discover the mechanism of quercetin protects myocardial cells against apoptosis. $CoCl_2$ was used to treat H9C2 cells to mimic a hypoxic/ischemic condition. Then quercetin was used to protect H9C2 cells pretreated with $CoCl_2$, and apoptosis and

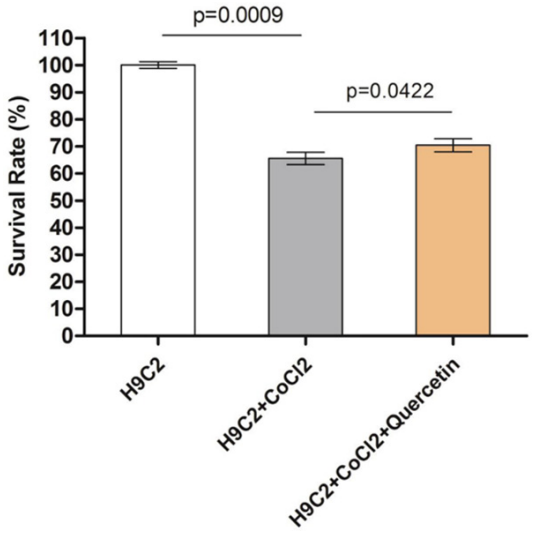


Figure 2. Survival rate of H9C2 cells in control, $CoCl_2$ treated and $CoCl_2$ + quercetin treated groups.

cell viability of H9C2 cells were detected with different treatments. Finally, the bioinformatics was performed to reveal its potential mechanism.

Methods and materials

H9C2 cells culture and treatment

H9C2 cells, obtained from American Type Culture Collection (Manassas, VA, USA), were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37°C. Before reaching confluence, the cells were split and plated at low density in culture medium containing 10% fetal bovine serum. Chemical hypoxia was achieved by adding $CoCl_2$ at 600 μM into the medium and cells were incubated in the presence of $CoCl_2$ for the indicated times.

Apoptosis assay

The apoptosis of the H9C2 cells treated with $CoCl_2$, $CoCl_2$ + quercetin were subsequently analyzed using an Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's instructions. The cells were seeded in 6-well plates at a density of 1×10^5 cells/well in DMEM medium for 24 hours. The cells were then digested with 0.25% trypsin (Invitrogen, Carlsbad, CA) and resuspended in 300 μl binding buffer (Beyotime Institute of Biotechnology) containing 5 μl Annexin V-FITC and 5 μl propidium iodide solution, and incubated at room temperature in the dark for 20 minutes. The stained cells were analyzed by flow cytometry (FACScan; BD Biosciences, Franklin Lakes, NJ, USA).

Cells viability assay

H9C2 cells were plated in 96-well plates at a density of 5,000 cells/well. When the cells were grown to ~70% confluence, the indicated treatments were administered. At the end of the treatment, the CCK-8 solution (10 μl) was added to each well followed by a further 3 hour incubation at 37°C. Absorbance (A) was measured at 450 nm with a microplate reader (Sunnyvale, CA, USA). Percentage of survival rate = (A treatment group - A Blank group)/(A Control group - A Blank group) × 100%. Experiments were performed six times.

Gene ontology and pathway enrichment analysis

R software (clusterprofiler) and DAVID (https://david.ncifcrf.gov/) were used to do GO functional enrichment analysis of differentially expressed genes.

Statistical analysis

The data are presented as the mean ± standard deviation, and the statistical analysis was performed using SPSS 13.0 software. Differences among three groups were assessed using one-way ANOVA followed by Fisher's Least Significance Difference test. The significance levels were set at $*P < 0.05$ and $**P < 0.01$.

Results

Quercetin protects myocardial cells against apoptosis

As shown in **Figure 1**, the apoptotic rate of H9C2 cells without any treatment is 1.09%. And it increases to 21.21% after using $CoCl_2$ to treat H9C2 cells, it is significant higher than control group ($P < 0.01$). Interestingly, the apoptotic rate of H9C2 cells reduces to 13.32% after using quercetin and $CoCl_2$ to co-treat H9C2 cells. Although the apoptotic rate of H9C2 cells treated with $CoCl_2$ + quercetin is still

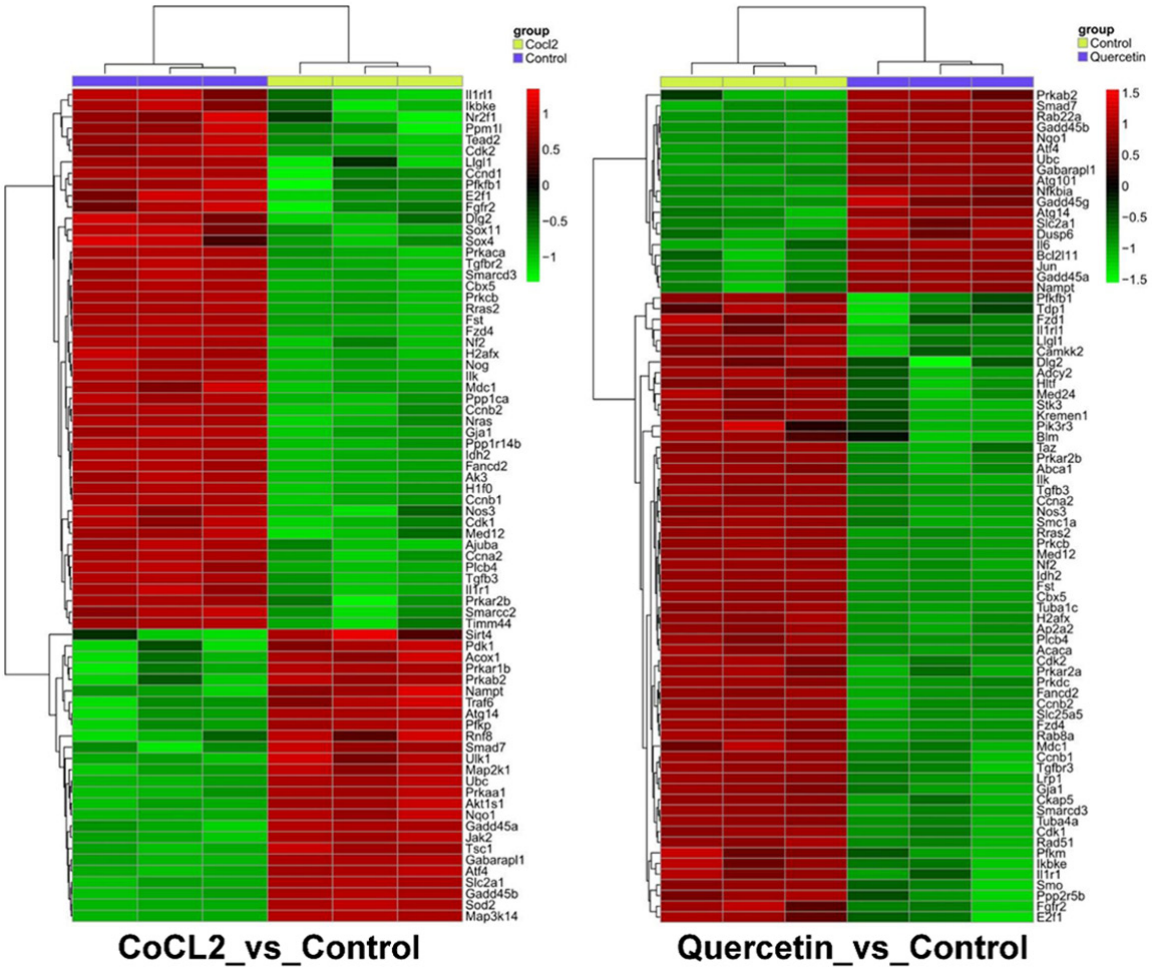


Figure 3. Heat-map shows the differentially expressed genes between Created group and control group, quercetin treated group and control groups.

higher than control group, it was obviously decreased compared with $CoCl_2$ along treated group ($P < 0.01$). Moreover, the proliferation assay results show the survival rate of H9C2 cells treated with $CoCl_2$ is about 68%, it is obvious lower than control group (100%, $P < 0.01$). However, the survival rate of H9C2 cells treated with $CoCl_2$ plus quercetin has increased compared with $CoCl_2$ along treated group ($P < 0.05$) (**Figure 2**).

Differentially expressed genes after quercetin treatment

As shown in **Figure 3**, the heat-map shows the differentially expressed genes between control and $CoCl_2$ treated or quercetin treated H9C2 cells. When compared with control group (without any treatment), there were 48 genes down-regulated and 26 genes up-regulated in $CoCl_2$ treated group. When compared with the $CoCl_2$ treated group, there were 19 genes up-regulated and 59 genes down-regulated in quercetin treated group. Moreover, the activated and inhibited signaling pathways were also analyzed. As shown in **Figure 4A**, the estrogen-mediated S-phase entry, cyclins and cell cycle regulation, eNOS signaling, HGF signaling, G beta gamma signaling were suppressed in $CoCl_2$ treated group compared to control group. In contrast, NRF2-mediated oxidative stress response, cell cycle: G1/S checkpoint regulation, PPARα/RXRα activation are promoted in $CoCl_2$ treated group compared to control group. In addition, the estrogen-mediated S-phase entry, cell cycle regulation by BTG family protein, cyclins and cell cycle regulation, HGF signaling, PPARα/RXRα activation are inhibited in quercetin treated group compared to the con-

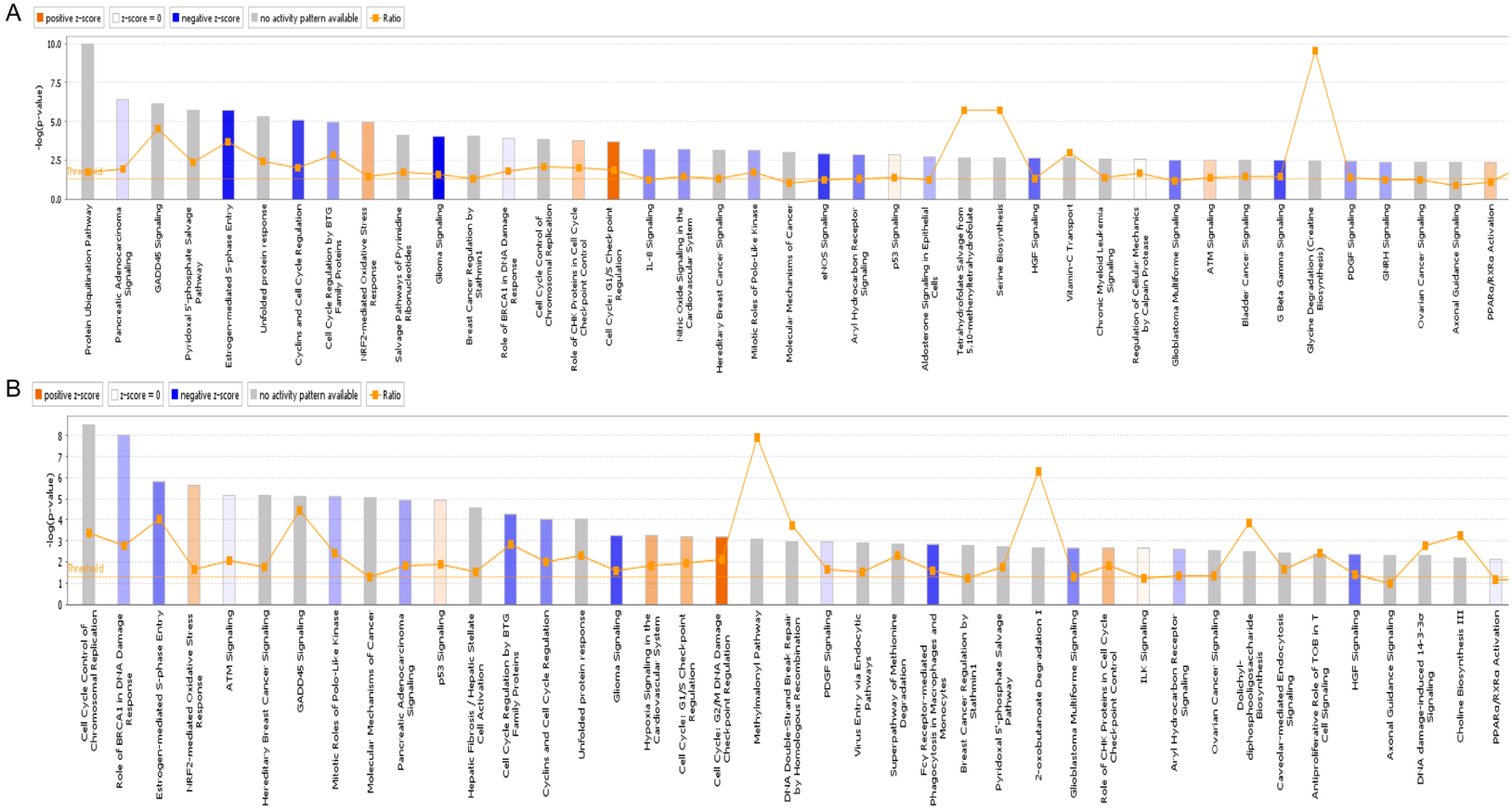
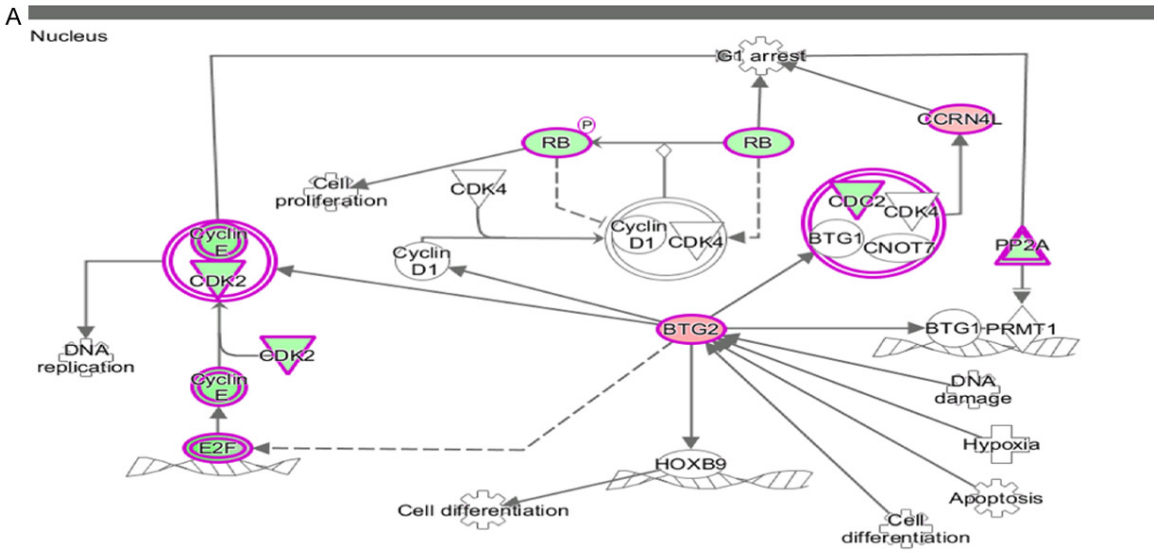


Figure 4. Activated and inhibited signaling pathway between $CoCl_2$ treated group and control group, quercetin treated group and control group.

A



B

Canonical_Pathway	Cocl2_vs_Control (z-score)	Quercetin_vs_Control (z-score)
Cell Cycle Regulation by BTG Family Proteins	-1.414	-2.121
ATM Signaling	0.905	-0.277
PPARα/RXRα Activation	1.147	-0.258
AMPK Signaling	0.775	-0.775
BMP signaling pathway	0.333	0
HIPPO signaling	1.342	0
Sirtuin Signaling Pathway	0.728	0
Wnt/β-catenin Signaling	1	0

Figure 5. IPA signaling pathway analysis.

trol group. The NRF2-mediated oxidative stress response, hypoxia signaling in the cardiovascular system, cell cycle: G1/S checkpoint regulation, cell cycle: G2/M DNA damage checkpoint regulation, role of CHK proteins in cell cycle checkpoint control were all accelerated in quercetin treated group (**Figure 4B**).

IPA signaling pathway analysis

In order to further explore its potential mechanism, the IPA signaling pathway analysis is performed. As shown in **Figure 5A**, the genes labeled with red color represent up-regulated genes, the genes labeled with green color represent down-regulated genes. The signaling of cell cycle regulation by BTG family proteins was significantly prohibited. In addition, stimulation of $CoCl_2$ was able to significantly activate the ATM Signaling, PPARα/RXRα Activation, AMPK Signaling, BMP signaling pathway, HIPPO signaling, Sirtuin Signaling Pathway, Wnt/β-catenin Signaling, resulting in promotion of apoptosis and inhibition of cell proliferation. In contrast, quercetin could suppress these signaling pathway to activate the protective roles (**Figure 5B**).

GO function analysis and KEGG pathway enrichment analysis

As shown in **Figure 6**, the GO function analysis results reveal that quercetin plays a protective role in the process of biological process, molecular function, and cellular component in $CoCl_2$ induced cells apoptosis.

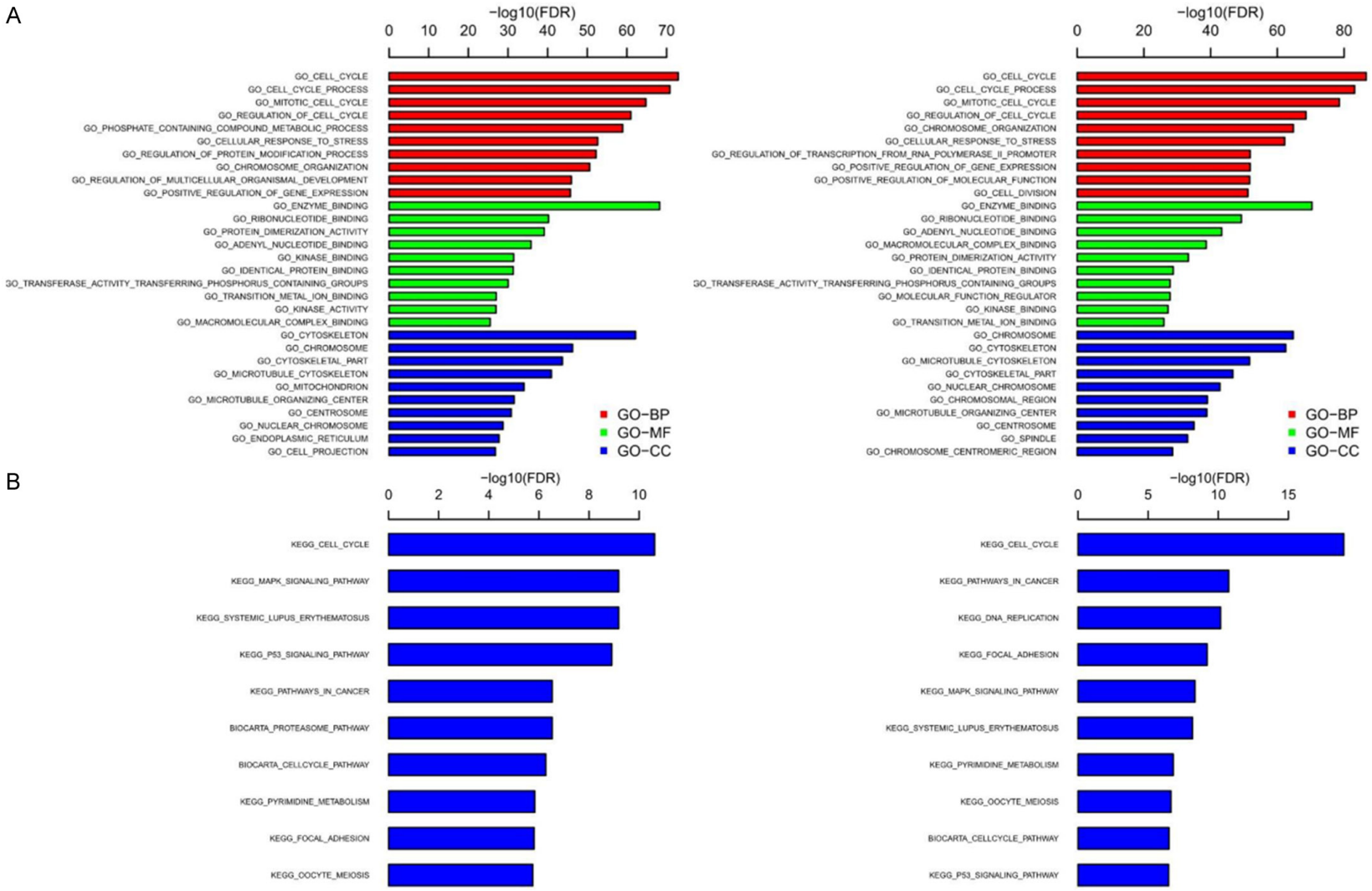


Figure 6. GO function analysis (A) and KEGG pathway enrichment analysis (B) between the $CoCl_2$ treated group and the control group, as well as the quercetin treated group and the control group.

Discussion

Myocardial infarction is a common presentation of coronary artery disease. The World Health Organization estimated in 2004, that 12.2% of worldwide deaths were from ischemic heart disease with it being the leading cause of death in high- or middle-income countries [14]. Currently, it is becoming a more common cause of death in the developing world. Especially in China [15]. Globally, disability adjusted life years lost to ischemic heart disease are predicted to account for 5.5% in 2030, making it the second-most-important cause of disability, as well as the leading cause of death by this date. However, there are no effective drugs to prevent MI or provide relief after myocardial infarction has occurred.

Quercetin has been studied in basic research and small clinical trials [16]. While quercetin supplements have been promoted for the treatment of cancer and various other diseases [17]. Mai et al. study reflected the protective effects of quercetin on isoprenaline-induced myocardial infarction in rats [18]. These results are in harmony with previous reports which stated that the biological actions of quercetin are, in part, connected to its anti-oxidant properties which are mainly due to its ability to scavenge ROS and to chelate transition metal ions [19, 20]. Moreover, quercetin protective effects could be also related to its ability to maintain heart calcium content and prevent isoprenaline-induced increase in heart calcium [21]. However, these reported potential mechanism can't fully reveal its mechanism.

Akula et al. showed quercetin had cardioprotective effects, however, they didn't explore its possible mechanism [22]. Zaafan et al. reported quercetin pretreatment attenuated oxidative stress and inflammatory reactions as well as declined tissue damage in isoprenaline-induced MI in rats [18]. Jin et al. also discovered quercetin was able to significantly attenuate MI injury through anti-inflammatory effects [11]. However, these studies just revealed the cardioprotective effects of quercetin with a few or a portion of mechanism information. While, bioinformatics can generally analyze differentially expressed genes and its possible involved signaling pathway. In this study, quercetin was able to protect myocardial cells against apoptosis and the bioinformatics analysis results suggest that quercetin plays a protective role in the process of biological process, molecular function and cellular component in $CoCl_2$ induced cells apoptosis. Altogether, this study has discovered the differentially expressed genes between quercetin treated group and $CoCl_2$ treated group, and its involved signaling pathway. These results obtained from a cell line and results from human cardiomyocytes will be important for future studies.

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Disclosure of conflict of interest

None.

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