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Original Paper

Puerarin Exerts a Delayed Inhibitory Effect on the Proliferation of Cardiomyocytes Derived from Murine ES Cells via Slowing Progression through G2/M Phase

Xueying Luo[a,b] Binlong Zhong[a,b] Xian Hong[a,b] Yurong Cui[a,b] Ying Gao[a,b] Mengmeng Yin[a,b] Ming Tang[a,b] Jürgen Hescheler[c] Jiaoya Xi[a,b]

[a]Department of Physiology and Chinese-German Stem Cell Center, School of Basic Medicine, Huazhong University of Science and Technology, The Key Laboratory for Drug Target Researches and Pharmacodynamic Evaluation of Hubei Province, Wuhan, [b]The institute of Brain Research, Huazhong University of Science and Technology, Wuhan, China; [c]Institute for Neurophysiology, University of Cologne, Cologne, Germany

Key Words
Puerarin • Murine embryonic stem cells • Cardiomyocytes • Proliferation • G2/M Phase

Abstract
Objective: Puerarin, which shows beneficial and protective effects on cardiovascular diseases, is the main isoflavone extracted from *Pueraria lobata (kudzu)* root. The aim of this study was to investigate the effects of puerarin on *in vitro* myocardial proliferation and its underlying mechanism. ***Methods:*** Myocardial differentiation of transgenic embryonic stem (ES) cells was performed by embryoid body-based differentiation method. The proliferation assay of cardiomyocytes (CMs) derived from ES cells (ES-CMs) was performed by EdU (5-Ethynyl-2′-deoxyuridine) staining. Flow cytometry was employed to determine the cell cycle distribution and apoptosis of purified ES-CMs. Quantitative real-time PCR was utilized to study the transcription of genes related to cell cycle progression. Signaling pathways relating to proliferation were studied by western blot analysis and application of specific inhibitors. ***Results:*** Puerarin exerted a delayed inhibitory effect on the proliferation of ES-CMs at the early-stage differentiation. Meanwhile, puerarin slowed progression through G2/M phase without inducing apoptosis of ES-CMs. Further assays showed that puerarin up-regulated the transcription of Cyclin A2, Cyclin B1 and Cdk1 in ES-CMs. The ERK1/2 specific inhibitor PD0325901 and the PI3K specific inhibitor Wortmannin successfully reversed puerarin-induced up-regulation of Cdk1 but not Cyclin A2 and B1. ***Conclusion:*** These findings suggest that puerarin inhibits CM proliferation via slowing progression through G2/M phase during early-stage differentiation.

X. Luo and B. Zhong have equal contribution to this manuscript.

Dr. Jiaoya Xi

Department of Physiology and Chinese-German Stem Cell Center, School of Basic Medicine, Huazhong University of Science and Technology, The Key Laboratory for Drug Target Researches and Pharmacodynamic Evaluation of Hubei Province; The institute of Brain Research, Huazhong University of Science and Technology, Wuhan, 430030, (China)
Tel. +86-27-83692622, Fax +86-27-83692608, E-Mail zhengyall@hotmail.com

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Introduction

Pueraria lobata (Willd) Ohwi (kudzu) has been utilized as a food source, medicine, and fodder in Southeast Asia for thousands of years [1]. Puerarin [7-hydroxy-3-(4-hydroxyphenyl)-1-benzopyran-4-one 8-(β-D-Glucopyranoside)] is the main isoflavone extracted from the root of it. The pharmacological properties of puerarin, as well as its beneficial and protective effects on cardiovascular diseases, have been widely investigated [2]. Puerarin exerts its myocardial protection effect by regulating cardiac Smad3 and Smad7 transcripts in spontaneously hypertensive rat [3], inducing vascular endothelial growth factor and endothelial nitric oxide synthase mediated angiogenesis in rats with myocardial infarction (MI) [4], inhibiting oxidative stress via regulation of superoxide dismutase activity in acute ischemic myocardial injury [5], or indirectly relieving myocardial ischemic damage by blocking the signaling transmission via P2X3 in SG and DRG [6].

Cardiomyocytes (CMs) derived from embryonic stem (ES) cells (ES-CMs) provide an alternative platform to study the mechanism of pathological remodeling, development and maturation of heart, and thus can serve as a relatively simple tool to investigate the detailed mechanism explaining pharmacological protective effects of puerarin on the heart. Previously, we reported that puerarin treatment suppresses the self-renewal of murine ES cells [7], and significantly enhances the population of functional murine ES-CMs [8]. Importantly, puerarin facilitates t-tubule development of these cells via up-regulating t-tubule development and formatting the related genes caveolin-3, amphiphysin-2 and junctophinlin-2 [9]. Accumulated data show that CMs are remodeled or "dedifferentiated" in pathological conditions, i.e. heart failure and other cardiovascular diseases, presenting enhanced proliferation ability and morphological and functional properties similar to embryonic CMs [10, 11]. Based on the myocardial protection effects of puerarin reported previously, we hypothesized that puerarin might also inhibit CM proliferation, thus further support its potential effect on reversing cardiac phenotypic remodeling in heart diseases.

It is widely accepted that the proliferation of CMs mainly occurs during prenatal development [12], and essentially stops early after birth. After birth, CMs further grow in volume and maturity in physiological function. Contemporary studies mostly focus on the mechanisms regulating cell cycle activity of CMs *in vitro* and *in vivo*, which are significant for the cardiovascular regenerative medicine community. Humoral factors have been shown to upregulate CM proliferation via critical signaling pathways, such as PI3K [13, 14], ERK [14], Npr3 and cAMP [15], and JAK/STAT [16] signaling pathways. In neonatal rat CMs, nuclear import of Cyclin D1 and cyclin-dependent kinase 4 (Cdk4) stimulates proliferation by activating the retinoblastoma regulatory pathway [17]. Inactivation of microRNA-34a enhances post-MI remodeling by dispelling its regulation on cell activity and apoptosis via target genes including Cyclin D1, Bcl2, and Sirt1 [18]. Thus CM proliferation, which plays a crucial role in *in vitro* cardiac differentiation [12], is related to several signaling pathways and regulated by cyclins.

Herein, we aimed to investigate the effects of puerarin on *in vitro* cardiac proliferation. This study may supplement the explanation of the myocardial protection effect of puerarin on reversing cardiac phenotypic remodeling in heart diseases.

Materials and Methods

Cultivation of ES cells and cardiac differentiation

The murine genetically modified D3 ES cell line αPIG (clone 44) was maintained as previously described [8, 19]. Briefly, undifferentiated ES cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 15% fetal bovine serum (FBS), 1% nonessential amino acids, 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mM β-mercaptoethanol, 100 μg/mL streptomycin and 1000 U/mL leukemia inhibitory factor (LIF, Chemicon). After removal of LIF, which inhibits the differentiation of ES cells [20], cardiac differentiation was performed by formation of embryonic bodies as shown in Fig. 1A. Puromycin (10 μg/mL, InvivoGen) was added at day 9 and day 12 of differentiation to collect purified ES-

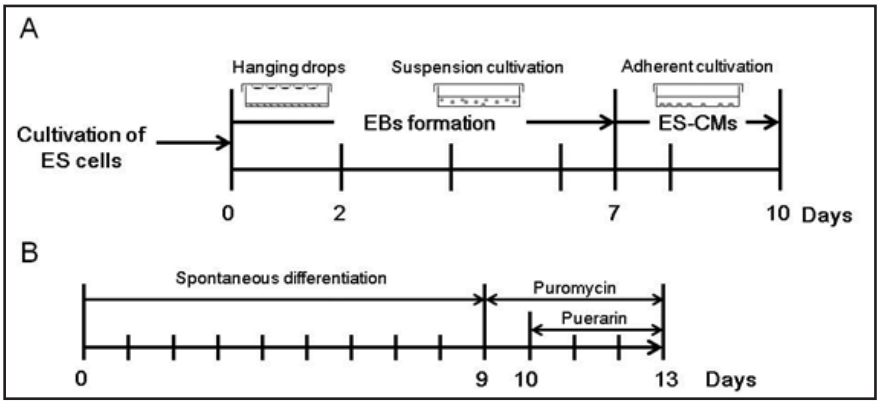

Fig. 1. Procedure of cardiac differentiation, puromycin selection and puerarin application. (A) Procedure of cardiac differentiation. (B) Scheme of puromycin selection and puerarin application.

CMs clusters [19]. Puerarin (50, 100 or 200 μM, the National Institute for Food and Drug Control, China) or DMSO (0.05%, Sigma) was added from day 10 to the endpoint of the experiment (Fig. 1B).

Proliferation assay

ES-CMs clusters were collected at day 10 of differentiation, and dissociated into single cells using 0.05% Trypsin/EDTA (Biosharp). 5×10^4 to 1×10^5 cells per well were seeded in a gelatin-coated 24-well-plate with or without 100 μM puerarin supplemented with 10 μM EdU (5-Ethynyl-2'-deoxyuridine) from the Click-iT® EdU Imaging Kits C10338 (Invitrogen). After 48 h or 72 h cultivation, the staining of ES-CMs with EdU was performed according to the instructions of the manufacturer. ES-CMs were identified by staining with mouse IgG anti-α-sarcomeric-actinin antibody (1:200, Sigma) overnight at 4°C. The secondary goat anti-mouse FITC-conjugated IgG (1:200, Proteintec) was used for 1 h at room temperature. Nuclei were stained with Hoechst33342 (5μg/mL, Invitrogen). Images were taken randomly using a fluorescence microscope (Nikon, TE2000-S). The total number of ES-CMs was determined by counting α-actinin/Hoechst33342 double positive cells. The percentage of proliferating ES-CMs was determined by dividing the number of α-actinin/EdU/ Hoechst33342 triple positive cells by the total number of ES-CMs. The counting was carried out independently by three operators. The proliferation assays were performed by 5 to 6 independent experiments, and total 3002 of control cells and 3166 of puerarin treated cells were counted.

Cell cycle and apoptosis analysis

ES-CM clusters with or without puerarin treatment were collected at day 13 of differentiation, and dissociated into single cells using 0.05% Trypsin/EDTA. Cells were washed with cold PBS, and fixed in ice-cold 70% ethanol for 2 hours or longer at 4°C. The cells were then stained with propidium iodide solution prepared according to the instructions of the manufacturer (Beyotime Biotehnology). After incubation at 37°C for 0.5 h in the dark, the samples were analyzed for DNA content by Flow Cytometry Caliber instrument (BD Biosciences, LSR Ⅱ). Cell distribution of ES-CMs among cell cycle phases and the percentage of apoptotic cells were evaluated as previously described [21]. The cell cycle distribution is shown as the percentage of cells containing 2n (G1 phase), 4n (G2 and M phases), and 4n>3>2n DNA amount (S phase) estimated by PI staining. The apoptotic cells are defined by the percentage of cells with DNA content lower than 2n (sub/G1phase) [22].

Quantitative real-time PCR

Total RNA was extracted from ES-CMs using Trizol (Life technology) according to the instructions of the manufacturer at day 13 of differentiation. The total RNA (5 μg) was reversely transcribed into cDNA using oligo (dT) primer and mmlv reverse transcriptase (Invitrogen). Real-time PCR was performed in the CFX Connect™ Real-Time PCR Detection System (Bio-Rad) using SYBR Premix Ex Taq (TOYOBO). CT values were automatically obtained. Relative expression of mRNA amount was calculated using the ΔΔCT method [23]. The primers used for real-time PCR are listed in Table 1.

Western blot analysis

ES-CMs at day 10 of differentiation were pretreated in Opti-MEM (Invitrogen) for 2 h, and proteins were extracted after DMSO (0.05%) or puerarin (100 μM) treatment for different time periods (0 min, 10

Table 1. Primers for real-time PCR analysis

Genes	Sense primers(5'→3')	Antisense primers(5'→3')	Product size(bp)
Cyclin D1	GCGTACCCTGACACCAATCTC	ACTTGAAGTAAGATACGGAGGGC	94
Cyclin A2	AAGAGAATGTCAACCCCGAAAAA	ACCCGTCGAGTCTTGAGCTT	118
Cyclin B2	AGCTCCCAAGGATCGTCCTC	TGTCCTCGTTATCTATGTCCTCG	116
Cyclin B1	CTTGCAGTGAGTGACGTAGAC	CCAGTTGTCGGAGATAAGCATAG	94
Cdk 1	AGAAGGTACTTACGGTGTGGT	GAGAGATTTCCCGAATTGCAGT	128
Cdk 2	CTCTCACGGGCATTCCTCTTC	CCCTCTGCATTGATAAGCAGG	133
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGC AGGGATGATGTTCT	132

min, 30 min, 1 h and 2 h). Protein concentration was determined using a BCA Protein Assay Kit (BOSTER). 25 μg protein samples were subjected to 12% SDS-PAGE and analyzed by blotting with antiphospho-ERK-1/2 MAb (1:2000, Cell Signaling Technology) or antiphospho-Ser^{473} Akt MAb (1:5000, Abcam) to detect the phosphorylation status of MERK/ERK and PI3K/Akt in ES-CMs.

Cell Signaling Inhibitors

Specific inhibitors were further used to investigate whether MERK/ERK and PI3K/Akt signaling pathways mediated the effect of puerarin on the transcription levels of cell cycle relevant genes. The PI3K inhibitor Wortmannin (1 μM, Sigma-Aldrich) or the EKR1/2 inhibitor PD0325901 (1 μM, Selleck) was added to differentiation medium, respectively. Total mRNA was extracted from cells at day 13 of differentiation, and the transcription levels of Cyclin A2, Cyclin B1 and Cdk1 were assessed by real-time PCR.

Statistical analysis

Unless otherwise stated, the results are expressed as mean ± SEM for at least three independent myocardium differentiations. Statistical significance was evaluated using paired or unpaired *t*-test where appropriate, $p < 0.05$ was considered as statistically significant.

Results

Puerarin exerts a delayed inhibitory effect on the proliferation of ES-CMs at day 10 of differentiation

CMs rapidly proliferate during fetal life but exit the cell cycle soon after birth in mammals, which limits the ability of the heart to restore function after any significant injury. To explore the effects of puerarin on the proliferation of CMs, ES-CMs at day 10 of differentiation were selected as study objects. The morphology of ES-CMs was comparable after cultivation with or without 100 μM puerarin for 48 h and 72 h, demonstrating that puerarin does not change the viability of ES-CMs (Fig. 2A). In the proliferation assay using EdU labeling, the percentage of proliferating ES-CMs was determined by dividing the number of α-actinin/ EdU/ Hoechst33342 triple positive cells by the total number of ES-CMs. We found that puerarin treatment for 48 h did not significantly affect the proliferation of ES-CMs (23.44 ± 1.97% in puerarin treated group *vs.* 23.50 ± 2.46% in control group, n = 6, $p = 0.3540$) (Fig. 2A and B). However, after 72 h puerarin treatment, the percentage of proliferating ES-CMs was attenuated compared with control group (25.61 ± 1.20% in puerarin treated group *vs.* 31.28 ± 1.17% in control group, n = 5, $p = 0.0096$) (Fig. 2A and B), indicating that puerarin exerts a delayed inhibitory effect on the proliferation of ES-CMs at the early-stage differentiation.

Puerarin slows the progression through G2/M phase without inducing apoptosis of ES-CMs

To obtain further insight into the mechanism of proliferation inhibitory effect of puerarin, we assessed cell cycle distribution of ES-CMs by flow cytometry. Flow cytometric analysis showed that the majority of the ES-CMs were in G0/G1 phase in the puerarin treatment group (82.9 ± 1.5%) and the control group (84.4 ± 1.3%). In addition, puerarin treatment for 72 h increased the percentage of the cells in G2/M phase compared with control group (11.1 ± 0.5% in the puerarin treatment group vs. 8.6 ± 0.4% in the control group, n = 5,

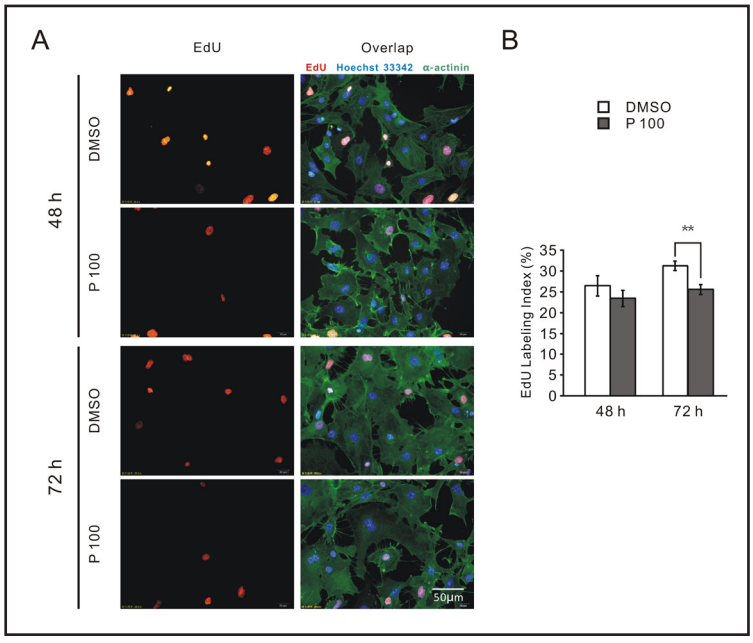

Fig. 2. Puerarin exerts a delayed inhibitory effect on the proliferation of ES-CMs at day 10 of differentiation. (A) Cells labeled with EdU, Hoechst33342 and α-actinin after treatment with DMSO or puerarin for 48 h and 72 h. (B) EdU labeling index with or without 100 μM puerarin treatment (P 100) for 48 h (n = 6) and 72 h (n = 5). All data are presented as mean ± SEM. **, $p < 0.01$ vs. control group.

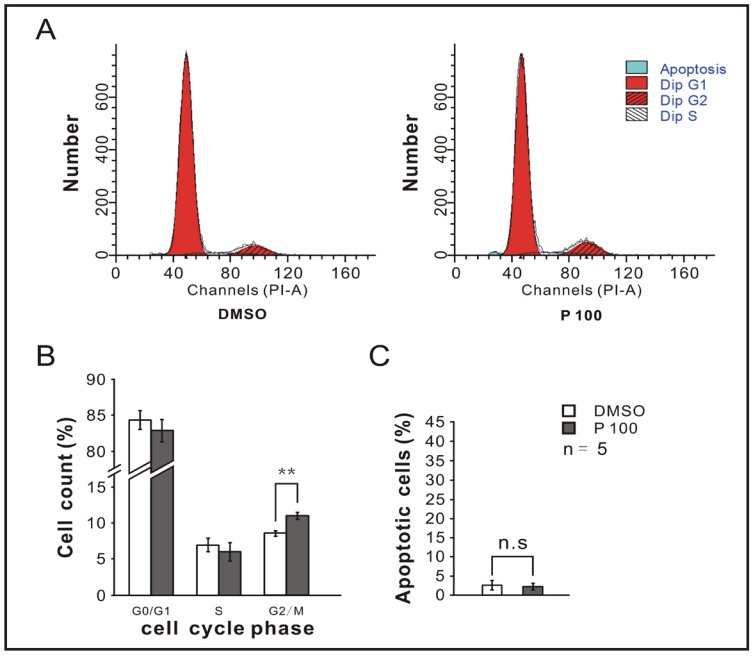

Fig. 3. Puerarin slows the progression through G2/M phase without inducing apoptosis of ES-CMs. (A) The representative data of flow cytometric analysis at day 13 of differentiation. (B) Flow cytometric analysis shows that the percentage of cells in G2/M phase is significantly higher in the puerarin treatment group than in the control (n = 5). (C) Apoptosis analysis shows no statistical difference between the puerarin and control groups (n = 5). All data are presented as mean ± SEM. **, $p < 0.01$ vs. control group.

p = 0.0045) at day 13 (Fig. 3A and B). Moreover, there is no significant difference between the percentage of the apoptotic ES-CMs in the puerarin treatment group (2.2 ± 0.9%) and that in the control group (2.6 ± 1.2%) (n = 5, $p = 0.8236$) (Fig. 3A and C). These data suggest that with or without puerarin treatment, the majority of CMs are arrested in G0/G1 phase naturally. More importantly, puerarin slows the progression through G2/M phase without inducing apoptosis of ES-CMs.

Puerarin up-regulates the transcription of Cyclin A2, Cyclin B1 and Cdk1 in ES-CMs

The cell cycle, which is controlled by lots of cytokines and growth factors, plays an important role in proliferation of CMs [12]. We next analyzed the effect of various concentrations of puerarin on the transcription levels of cell cycle relevant genes in ES-CMs by real-time PCR. As shown in Fig. 4, the transcription of Cyclin A2 was dose-dependently up-regulated by puerarin treatment. The mRNA expression levels of Cyclin B1 and Cdk1 in

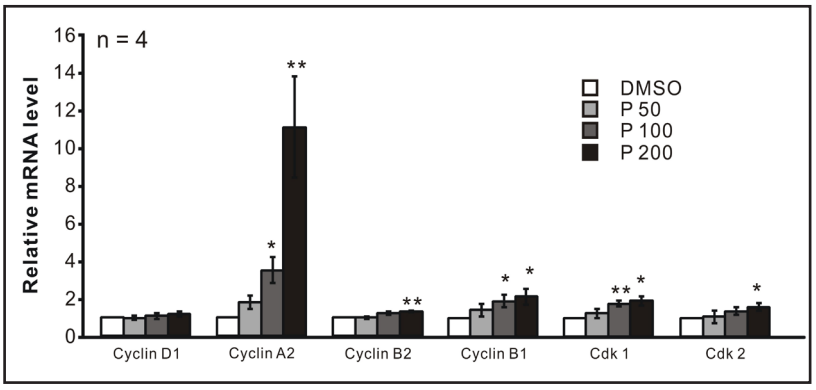

Fig. 4. Puerarin up-regulates the transcription of Cyclin A2, Cyclin B1 and Cdk1 in ES-CMs. Relative mRNA levels of Cyclin D1, Cyclin A2, Cyclin B2, Cyclin B1, Cdk1 and Cdk2 in ES-CMs after treatment with various concentrations of puerarin or DMSO for 72 hours. All data are presented as mean ± SEM. *, $p < 0.05$ vs. DMSO. **, $p < 0.01$ vs. DMSO.

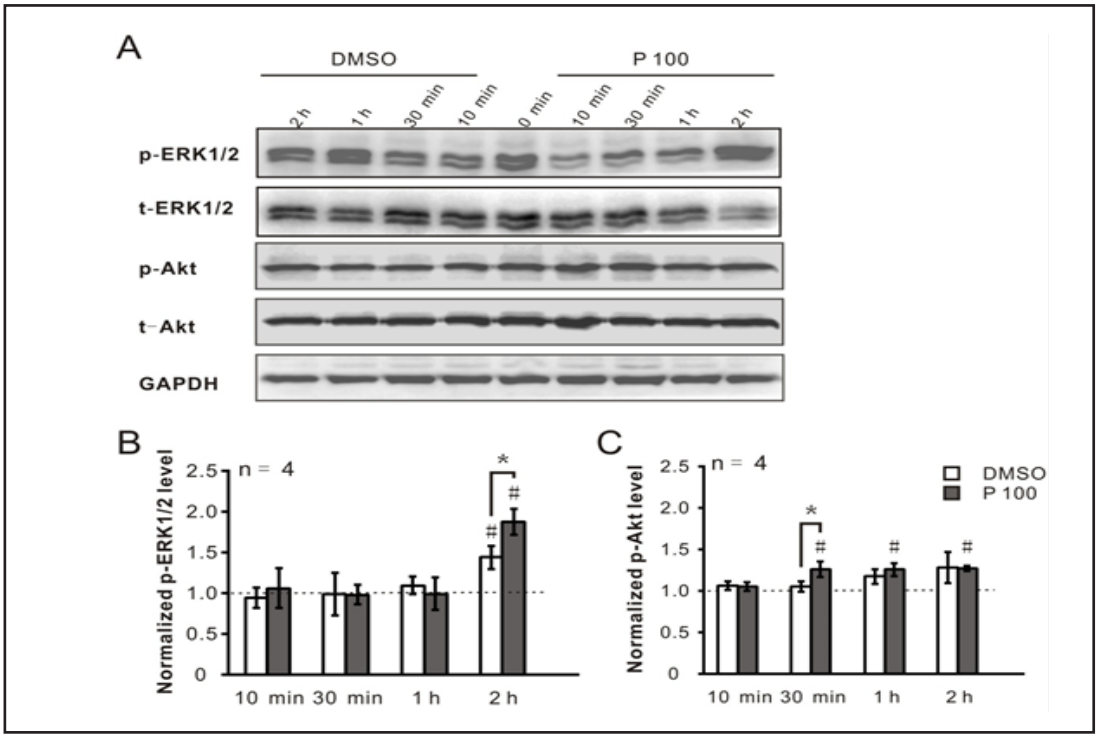

Fig. 5. Effects of puerarin on the phosphorylation of ERK and Akt of ES-CMs. (A) Representative western blots for phosphorylated and total ERK1/2, phosphorylated and total Akt, and GAPDH. (B) Normalized p-ERK1/2 levels at different time points by the p-ERK1/2 level at 0 min. The dished line demonstrated the p-ERK1/2 level at 0 min. (C) Normalized p-Akt levels at different time points by the p-Akt level at 0 min. The dished line demonstrated the p-Akt level at 0 min. All data are presented as mean ± SEM. *, p < 0.05 vs. control group. #, p < 0.05 vs. 0 min group.

the puerarin treatment group were significantly elevated by puerarin at 100 μM and 200 μM. The significant increases in transcription of Cyclin B2 and Cdk2 were also observed in 200 μM puerarin treatment group. Puerarin treatment did not change the transcription level of Cyclin D1.

ERK1/2 and PI3K/Akt signaling pathways are involved in puerarin-induced up-regulation of Cdk1

To explore mechanism how puerarin affects transcription of cell cycle relevant genes, western blot analyses of ERK1/2 and PI3K/Akt signaling pathways were performed. We found that the total-ERK1/2 levels and total-Akt levels were comparable at all observed time points in both control and puerarin treatment groups. The phospho-ERK1/2 (Thr202/

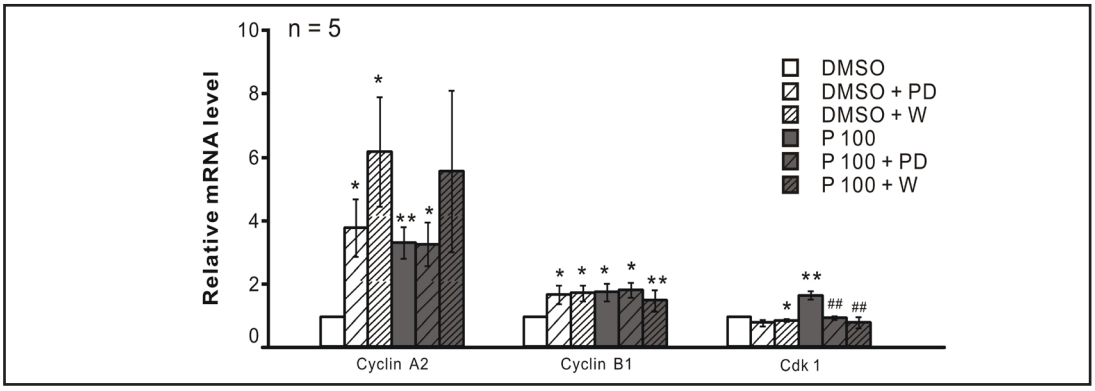

Fig. 6. ERK1/2 and PI3K/Akt signaling pathways were involved in puerarin-induced up-regulation of Cdk1. Relative mRNA levels of Cyclin A2, Cyclin B1 and Cdk1 in ES-CMs after treated with puerarin or DMSO companied with or without PD0325901 (PD) or Wortmannin (W) for 72 hours. All data are presented as mean ± SEM. *, $p < 0.05$ vs. DMSO. **, $p < 0.01$ vs. DMSO. ##, $p < 0.01$ vs. P 100 group.

Tyr204, p-ERK1/2) and phospho-Akt (Ser473, p-Akt) were detected even after 2 h pretreatment with medium without FBS. We normalized the p-ERK1/2 and p-Akt levels at different time points by the p-ERK1/2 or p-Akt level at 0 min, respectively, in both groups. Compared with the 0 min group, the p-ERK1/2 level was significantly raised after 2 h treated with or without puerarin ($p < 0.05$ vs. 0 min group), and even greater in puerarin treatment group (Fig. 5A and B, $p < 0.05$ vs. control group). The p-Akt levels at all observed time points in control group were almost comparable to p-Akt level at 0 min. However, the p-Akt level was increased after 30 min of puerarin treatment ($p < 0.05$ vs. 0 min group or control group), and maintained at high level up to 2 h, the endpoint of study (Fig. 5A and C, $p < 0.05$ vs. 0 min group). Consistently, as shown in Fig. 6, we found that solo application of ERK1/2 specific inhibitor PD0325901 (PD, 1 μM) or the PI3K specific inhibitor Wortmannin (W, 1 μM) increased the mRNA expressions of Cyclin A2 and Cyclin B1, respectively, but had no effect on Cdk1. For this reason, we failed to block the effect of puerarin on the transcription of Cyclin A2 and Cyclin B1 by applying PD or W. Nevertheless, both PD and W successfully reversed puerarin-induced up-regulation of Cdk1 (Fig. 6).

Discussion

The key findings of this study are: (1) puerarin exerts a delayed inhibitory effect on the proliferation of ES-CMs at the early-stage differentiation; (2) Puerarin slows the progression through G2/M phase without inducing apoptosis of ES-CMs; (3) puerarin up-regulates the transcription of Cyclin A2, Cyclin B1 and Cdk1 in ES-CMs; (4) the ERK1/2 specific inhibitor PD and the PI3K specific inhibitor W successfully reversed puerarin-induced up-regulation of Cdk1 but not Cyclin A2 and B1.

Before and after birth of mammals, the heart size increases through proliferation and hypertrophy in CMs, respectively. The cell cycle, which comprises four phases (G1, S, G2 and M), plays a key role in proliferation of CMs. Compared with the high proliferation level during early embryogenesis, the proliferation of mouse CMs starts to decrease significantly around embryonic day 10 to day 12 [12]. The cell cycle of almost all adult CMs is arrested in G1 phase [24], and only very few of the CMs can proliferate [25], which limits the cells' ability of self-repairing after damage. Considering *in vitro* myocardium differentiation is relatively delayed, we chose ES-CMs at day 10 of differentiation as study objects. We found that one third of early-stage ES-CMs keep proliferating, and puerarin treatment decreases the percentage of proliferating cells about 18.13% relative to control group. The majority of the early-stage ES-CMs were in G0/G1 phase. Meanwhile, puerarin treatment resulted in an elevated percentage of cells in G2/M phase without inducing cell apoptosis. Even

though the variations in absolute values of the EdU and flow cytometry assays seem small, the effects of puerarin should not be ignored since the majority of the CMs are arrested in G1 phase naturally and only one third of early-stage ES-CMs keep proliferating in our study. Nevertheless, future work still need to be made in parallel between embryonic CM proliferation and excessive adult myocyte hypertrophy, to further state the effect of puerarin on CM proliferation under pathological condition. Multiple reports suggest that puerarin might have opposite effects on the cell cycle and the proliferation of different cells. It is reported that puerarin down-regulates the Cyclin D1 and cdc25A transcription by promoting the recruitment of corepressors to estrogen receptor-α, and limiting the recruitment of coactivators in endometriotic stromal cells, and therefore arrests these cells in G1 phase to suppress estrogen-stimulated proliferation without inducing cell apoptosis [26]. Other research found that puerarin exhibits antitumor activity on MCF-7 cells via arresting cells in G2/M phase and inducing apoptosis [27]. Conversely, puerarin decreases human osteoblastic MG-63 cells in G1 phase while increasing the proportion of cells in G2 and S phases, thus significantly promotes MG-63 cells proliferation in a dose-dependent manner [28].

The cell cycle is regulated by cyclins, Cdks and Cdk inhibitors [12]. Cdks are activated by forming complexes with cyclins, following which they phosphorylate complex-specific substrates and allow cells to move past cell cycle check points and finish the cell cycle. Cell cycle phases can be arrested at check points due to abnormal cell cycle events [29]. Cyclin B is a mitotic cyclin and binds to Cdk1. The amount of cyclin B and the activity of the Cyclin B-Cdk1 complex rise through the cell cycle until mitosis. After anaphase, they fall suddenly due to degradation of cyclin B [30]. In the cell cycle phases, Cyclin A2 binds Cdk1 during the transition from G2 to M phase. It has been confirmed that the Cyclin A2-Cdk1 complex triggers Cyclin B1-Cdk1 activation [31], which causes break down of nuclear envelope to allow mitotic spindle to access the chromosomes [32]. At anaphase, highly active anaphase-promoting complexes support the degradation of Cyclin B1-Cdk1 complex and thereby inactivate the Cdk1 which initiates mitotic exit and reestablishment of the interphase state [30, 33]. Our study shows that the transcription levels of Cyclin A2, Cyclin B1 and Cdk1 are increased in puerarin treated ES-CMs. Taken together, we inferred that puerarin up-regulates the transcription of Cyclin B1 or activates the Cyclin B1-Cdk1 complex persistently, thus the progress through G2/M phase is delayed, and the proliferation of ES-CMs is inhibited.

Both ERK1/2 and PI3K/Akt signaling pathways are involved in cell cycle regulation. Activation of the PI3K/Akt pathway has been proved to inhibit embryonic CM proliferation [34], and to up-regulate Cdk1 in LNCaP cells treated with osteopontin-c [35]. Research on c-Met deficient hepatocytes showed that persistent ERK1/2 activation accounts for the up-regulation of Cdk1, which facilitates hepatocytes at the G2/M transition [36]. Our study found that the ERK1/2 and PI3K/Akt signaling pathways participate in puerarin-induced up-regulation of Cdk1. As regards increased transcriptions of CyclinA2 and CyclinB1 by puerarin treatment in the present study, further studies on the underlying mechanism are needed.

It should be noted that our study was *in vitro*, and did not examine effects of puerarin on pathological CMs *in vivo*. Although the present study and our previous study [9] suggest that the puerarin-induced inhibition of CM proliferation and promotion of CM maturity might attenuate the dedifferentiation process and heart remodeling, thereafter benefit the pumping function of the heart. Future work could be made in parallel between embryonic CM proliferation and excessive adult myocyte hypertrophy, to further study the effect of puerarin on CM proliferation under pathological condition and to better state the effect of puerarin on reversing excessive myocyte hypertrophy.

Conclusion

In summary, our study indicates that puerarin inhibits CM proliferation via slowing progression through G2/M phase during early-stage differentiation. This study does suggest

new pharmacological effects of puerarin on ES-CMs, which may supplement the explanation of the myocardial protection effect of puerarin on reversing cardiac phenotypic remodeling in heart diseases.

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Disclosure Statement

None.

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