EXPERIMENTAL STUDY

STAT3 Phosphorylation Mediating DMSO’s Function on Fetal Cardiomyocyte Proliferation with Developmental Changes

Haitao Hu,1,2* BSMed, Jin Xue,1,2,3* MS, Renshun Dong,1,2* BSMed, Yanan Zhao,1,2 MS, Chunyan Song,1,2 BSMed, Hongjian Zhao,1,2 BSMed, Jürgen Hescheler,4 MD, Yan Zhang,1 MD and Huamin Liang,1,2 MD

Summary

Endogenous cardiac regeneration has been focused for decades as a potential therapy for heart diseases with cell loss, and dimethyl sulfoxide (DMSO) has been proposed as a treatment for many diseases. In this study, we aimed to investigate the function of DMSO on fetal cardiomyocyte proliferation. By tracing BrdU+/α-actinin+ cells or Ki67+/α-actinin+ cells with immunohistochemical staining, we found that DMSO remarkably promoted fetal cardiomyocytes proliferation, and at the late developmental stage (LDS), such effects were more efficient than that at early developmental stage (EDS). Western blot data revealed a significant increase in STAT3 phosphorylation under DMSO treatments at LDS, while not at EDS. Consistently, STAT3 phosphorylation blocker STA21 could greatly reverse DMSO’s function at LDS whereas hardly at EDS. Moreover, hearts at the EDS had less total STAT3 protein, but relatively much higher level of phosphorylated STAT3. This suggests that DMSO promote fetal cardiomyocytes proliferation, and STAT3 phosphorylation play a pivotal role in DMSO’s function. With maturation, DMSO exerted a better ability to favor cardiomyocyte proliferation depending on STAT3 phosphorylation. Therefore, DMSO could serve as an effective, economic, and safe therapy for heart diseases with cell loss.

Key words: Dimethyl sulfoxide, Cardiomyocyte regeneration, JAK/STAT3 pathway, Ischemic heart diseases, Cell therapy

Since the first evidence of endogenous cardiac regeneration,1-3 activating endogenous cardiac regeneration has been focused for decades as a potential therapy for heart diseases with cell loss.4-6 Aside from the cardiac stem/progenitor cell and preexisting cardiomyocytes,4-7 some cell sources for endogenous cardiac regeneration exist in other parts of the body: bone marrow mesenchymal stem cell, endothelial progenitor cell, and mesenchymal stem cell.4-7 The most convincing and vital cell sources are cardiomyocytes themselves.8,9 STAT3 phosphorylation has been reported to play essential roles in the re-enter cell cycle in many models including cardiomyocyte proliferation.10-13 Hence, it could serve as a valuable target for clinical trials to activate endogenous cardiac regeneration.

Dimethyl sulfoxide (DMSO) is a well-known solvent for water-insoluble substances and also as a cryoprotectant for cells.14 It has multiple effects on cellular functions, cell growth, cell proliferation, and cell differentiation.15-17 Importantly, DMSO has been used as a topical analgesic,18 an anti-inflammatory,19 an antioxidant,20 or a collaborator with other medications to increase the rate of absorption.21 Notably, DMSO can induce the differentiation of P19 CL6 embryonic carcinoma cells to cardiomyocytes.22 Cardiac differentiation from pluripotent cells is generally accompanied with both cardiomyocyte proliferation and cardiomyocyte maturation.23 Therefore, we proposed that DMSO could promote cardiomyocyte proliferation, and STAT3 phosphorylation may underlie this function. Taking

From the 1Department of Physiology, Hubei Key Laboratory of Drug Target Research and Pharmacodynamic Evaluation, School of Basic Medicine, Huazhong University of Science and Technology, Wuhan, China, 2Institute of Brain Research, Huazhong University of Science and Technology, Wuhan, China, 3Department of Pathology, School of Basic Medicine, Huazhong University of Science and Technology, Wuhan, China, 4Institute of Physiology, University of Cologne, Cologne, Germany and 5Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

*These authors contributed equally to this study.

The study was founded by the National Nature Science Foundation of China (31271040) and the Fundamental Research Funds for the Central Universities (HUST: 2012TS036).

Address for correspondence: Huamin Liang, MD, Department of Physiology, Hubei Key Laboratory of Drug Target Research and Pharmacodynamic Evaluation, School of Basic Medicine, Huazhong University of Science and Technology, Hangkong Road 13, Wuhan 430030, China. E-mail: hliang@mail.hust.edu.cn or Yan Zhang, MD, Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, China. E-mail: 2011xh0852@hust.edu.cn

Received for publication April 1, 2018. Revised and accepted July 26, 2018.

Released in advance online on J-STAGE February 8, 2019.

doi: 10.1536/ijh.18-206

All rights reserved by the International Heart Journal Association.
fetal cardiomyocytes as a model, we found that DMSO enhanced the cardiomyocyte proliferation at both the early developmental stage (EDS) and late developmental stage (LDS). However, STAT3 phosphorylation significantly contributed to the effect only at LDS.

Methods

Cell culture of fetal cardiomyocytes: Six to eight-week old female mice (provided by the Center of Animal Experimentation of Tongji Medical College, Huazhong University of Science and Technology, China) were superovulated and the hearts were harvested at the EDS (E10.5-E12.5) and LDS (E16.5-E18.5). Ventricles were enzymatically dissociated to get single cells as previously described.24-30 The isolated cells were plated on sterile gelatin-coated glass cover slips, cultured in Dulbecco’s Modified Eagle’s Medium (Gibco) containing 20% fetal bovine serum (Gibco), and kept in the incubator for 24 hours for further treatment according to the following experiments. DMSO (from 1:100,000 to 1:1000, Sigma, USA), STA21 (selective STAT3 DNA binding inhibitor) (Santa Cruz Biotechnology, USA) or BrdU (0.03 mg/mL) (Boster, China) was applied where necessary.

Immunohistochemical staining: Single cardiomyocytes enzymatically dissociated from fetal hearts were subjected to immunostaining. Cells were treated with BrdU (0.03 mg/mL) for 24 hours to trace the cycling cardiomyocytes. After 10 minutes of fixation with 4% paraformaldehyde, 15 minutes of permeabilization with 0.5% Triton X-100, and 1 hour of blocking with 3% BSA at room temperature, the cells were incubated with the primary antibodies rabbit anti-mouse α-actinin (1:100, Proteintech Group, USA), or mouse monoclonal anti-mouse BrdU (1:100, Proteintech Group, USA) overnight at 4°C, followed by a further incubation of the secondary antibody goat anti-rabbit IgG-FITC (1:50, Proteintech Group, USA) or goat anti-mouse IgG-TRITC (1:50, Proteintech Group, USA) for 60 minutes at room temperature. Primary antibody mouse monoclonal anti-mouse α-actinin (1:100, Bosterbio, USA) or rabbit anti-mouse Ki67 (1:50, Abcam, USA), secondary antibody goat anti-mouse IgG (H+L)-Light488 conjugated (1:100, Boster, China) and Alex Fluor 594 Goat Anti-Rabbit IgG (H+L) (1:50, Proteintech Group, USA) were used to trace ki67+ cardiomyocytes. α-diamidino-2-phenylindole (Beyotime, China) was used to stain the nuclei for 10 minutes. The cells were then mounted onto glass slides and the pictures were captured using Immuno Floure (Olympus, Japan).

Western blot: Protein extracts of cardiomyocytes or whole hearts were prepared in RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 1 mM phenylmethylsulfonyl fluoride) according to the standard methods. Protein concentration was determined using the BCA protein assay kit (Pierce, Rockland, USA). 30 μg of total protein per lane was resolved by 10% SDS-PAGE and transferred to PVDF membrane. Primary antibody mouse monoclonal anti-GAPDH antibody (1:5000, Biossci, China) and mouse monoclonal anti-p-STAT3 antibody (1:200, Santa Cruz Biotechnology, USA) were diluted and detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG, and the enhanced chemiluminescent reagent (Pierce Biotechnology, USA). Immunoreactive bands were detected using Kodak BioMax ML film. The results were characteristic of at least three independent experiments.

Results

DMSO promoted fetal cardiomyocyte proliferation: With BrdU as a label, different concentrations of DMSO were applied to LDS cardiomyocytes to evaluate the concentration dependence on fetal cardiomyocyte proliferation. We found that DMSO promoted fetal cardiomyocyte proliferation in a partial dose-dependent manner. The obvious toxic effect was observed in 10% DMSO where almost all the cells were killed, and no proliferation could be traced (Figure 1A). Cell proliferation ability decreased with the mature progress of the fetal cardiomyocyte (Figure 1C, E, control panel) as reported.2 0.1% DMSO was taken as the optimal concentration in the following experiments to investigate the underlying mechanisms. It significantly promoted cardiomyocyte proliferation at both EDS (Figure 1B, C, P < 0.05, n = 4) and LDS (Figure 1D, E, P < 0.05, n = 4). Similar effects were repeated by tracing Ki67+ mononuclear cardiomyocytes (Figure 2). The favorable effects of DMSO on cell proliferation were more evident and significant at LDS than at EDS.

Distinct effects of DMSO on STAT3 phosphorylation in cardiomyocytes at early and LDS: We observed that the influence of DMSO on STAT3 phosphorylation experienced changes during development from EDS to LDS. DMSO significantly increased STAT3 phosphorylation at LDS (Figure 3B, P > 0.05, n = 3) while ineffectively at EDS (Figure 3A, P < 0.05, n = 3). Previous publication showed the specific inhibitory effects of STA21 on STAT3 phosphorylation, and did not find significant difference between 20 μM and 30 μM STA21.27 We found that for 0.1% DMSO-treated LDS cardiomyocytes, STA21 decreased the percentage of BrdU+ mononuclear cardiomyocytes in a dose-dependent manner (Figure 4A, P < 0.05, n = 4), and 30 μM STA21 remarkably suppressed LDS STAT3 phosphorylation (Figure 4B, P < 0.05, n = 3). Consistent with the changes in STAT3 phosphorylation, 30 μM STA21 slightly reversed the positive effects of DMSO on total BrdU+ nuclei without significance at EDS (Figure 4C, P > 0.05, n = 5), whereas it further increased the percentage of BrdU+ mononuclear cardiomyocytes (Figure 4C, P < 0.05, n = 5). At LDS, STA21 remarkably attenuated the function of DMSO (Figure 4D, P < 0.05 or P < 0.01, n = 4).

Basic status of STAT3 phosphorylation changed with maturation from early developmental stage to LDS: Developmental changes of DMSO on cardiomyocytes proliferation and STAT3 phosphorylation along with maturation from EDS to LDS was probably based on the basic status of STAT3. Therefore, whole hearts from EDS and LDS were harvested and subjected to western blot to evaluate the basic activity of STAT3. Figure 5 revealed that EDS hearts had less amount of total STAT3 protein, with relatively much more phosphorylated STAT3 (p-STAT3) (Figure 5, P < 0.05, n = 3).
Discussion

In this study, we found the positive effect of DMSO on the fetal cardiomyocyte proliferation with the involvement of STAT3 phosphorylation in this function. The functions of DMSO on fetal cardiomyocyte proliferation and STAT3 phosphorylation changed with maturation from EDS to LDS.

Cardiomyocyte proliferation was the foundation of cardiac regeneration and the result of multimodal regulation, which was an attractive therapeutic method to cure heart injuries and heart failure. However, the ability of cardiomyocyte proliferation changed through a series of developmental stages. Based on immunohistochemical staining results, we confirmed that the ability of cardiomyocyte proliferation at EDS was much better than LDS, which was demonstrated in previous studies.

Through the results shown in Figures 1 and 2, we believed that DMSO had certain favorable effect on fetal cardiomyocyte proliferation. Preexisting cardiomyocytes was the main cell source for both immature and adult hearts or in the infarcted heart. Additionally, it was clear in our study that the effect of DMSO was more distinct at LDS than at EDS, which could be explained by

---

**Figure 1.** DMSO increased BrdU fetal cardiomyocytes. A: Dose dependence of DMSO effects on BrdU+% mononuclear cardiomyocytes at LDS. B, C: 0.1% DMSO moderately promoted fetal cardiomyocyte proliferation at EDS. B: Immunohisto-chemical staining of control and cells subjected to 0.1% DMSO. C: Statistical analysis on B, D, E: 0.1% DMSO greatly promoted fetal cardiomyocyte proliferation at LDS. D: Immunohistochemical staining of control and cells subjected to 0.1% DMSO. E: Statistical analysis on D. EDS indicates early developmental stage; and LDS, late developmental stage. *P < 0.05, **P < 0.01 versus control.
DMSO favors cardiomyocyte proliferation via STAT3

Figure 2. DMSO increased Ki67+ fetal mononuclear cardiomyocytes. A, B: 0.1% DMSO moderately promoted fetal cardiomyocyte proliferation at EDS. A: Immunohistochemical staining of control and cells subjected to 0.1% DMSO. B: Statistical analysis on A. C, D: 0.1% DMSO greatly promoted fetal cardiomyocyte proliferation at LDS. C: Immunohistochemical staining of control and cells subjected to 0.1% DMSO. D: Statistical analysis on C. EDS indicates early developmental stage; and LDS, late developmental stage. *P < 0.05 versus control.

Figure 3. Effects of DMSO on STAT3 phosphorylation. A: At EDS, DMSO minimally activated STAT3 phosphorylation. Left panel: Representative western blot data of control and cells subjected to 0.1% DMSO. Right: Statistical analysis of left panel by evaluating the ratio of p-STAT3/GAPDH and p-STAT3/STAT3. B: At LDS, DMSO remarkably increased STAT3 phosphorylation. Left: Representative western blot data of control and cells subjected to 0.1% DMSO. Right: Statistical analysis of left panel by evaluating the ratio of p-STAT3/GAPDH and p-STAT3/STAT3. EDS indicates early developmental stage; and LDS, late developmental stage. *P < 0.05, **P < 0.01 versus control.
the fact that DMSO exerted stronger effects on STAT3 phosphorylation. By using STA21 to test the possible role of STAT3 phosphorylation in DMSO’s function, we found that STA21 failed to reverse DMSO’s effect on EDS cardiomyocytes while successful on LDS cells. This was well explained by the influence of DMSO on STAT3 phosphorylation: DMSO significantly elevated STAT3 phosphorylation only at LDS. EDS hearts had less amount of total STAT3 protein while STAT3 was relatively much more phosphorylated. Thereby, there was much less extent for DMSO to further increase STAT3 phosphorylation. Similar events could also be found in tumor cells where the
activity of STAT3 varied with tumor development.31,32) STAT3 phosphorylation were well-known in tumor cell proliferation, which could make up the tumor microenvironment, mediate tumorigenic effects in many cell types, and regulate gene expression through epigenetic mechanisms. Therefore, STAT3 phosphorylation was among the most promising targets to cure cancer.31,33) Moreover, STAT3 phosphorylation mattered significantly in the re-enter cell cycle of cardiomyocytes in many models.10,34,35) A presentative study10) found that after apical resection or IL-6 intervention, IL-6 promoted cardiomyocyte proliferation mainly through the activation of the JAK/STAT3 signaling pathway. We showed that likewise, DMSO also impressively activated STAT3 phosphorylation at LDS. As a result, we could assume that DMSO could induce STAT3 phosphorylation by activating STAT3 related signaling pathways in LDS cardiomyocytes.

STAT3 phosphorylation is important; however, it is not the only possible mechanism underlying the regulation of cardiac regeneration. Other signaling pathways, such as Ras/ERK,36) PI3K/AKT,37) Hippo-YAP,38) NRG/ErbB4,39) and some microRNAs40) are reported to be responsible for the cardiac proliferation, and possibly interact with each other in this process.70) As a result, the cardiomyocyte cell cycle activity is higher in EDS cardiomyocytes compared with LDS cells,2) regardless of the similar STAT3 phosphorylation as found in our data. Our study suggested that other signaling pathways rather than STAT3 phosphorylation mediate DMSO’s function in EDS fetal cardiomyocyte proliferation. This is evidenced by the finding that DMSO moderately elevated EDS cardiomyocyte proliferation without significant change in STAT3 phosphorylation. It is out of expectation that additional application of STA21 with DMSO moderately furthered the positive effects on mononuclear cardiomyocytes proliferation at EDS, which could be due to the complicated signaling network in the regulation of cardiomyocytes cell cycle activity. More efforts should be devoted to unveil more details of this network, especially with the possible interactions in cardiac regeneration.

DMSO has been reported to have favorable effects on cardiac differentiation and cardiomyocyte protection in multiple models.1-46) Several signaling pathways are involved in such functions such as p38 MAPK.42) DMSO could interfere with other signaling pathways in non-cardiovascular systems.45) Our data revealed that DMSO could elevate STAT3 phosphorylation, therefore enhance LDS fetal cardiomyocyte proliferation. As a lipid soluble chemical, DMSO could directly interact with STAT3 and possibly, indirectly act on STAT3 through its upstream proteins or interaction with other special signaling pathways. Although the previous publications and the present data have unveiled that DMSO could interact with these signaling pathways, more details need to be addressed on how these interactions could be processed.

Conclusions

DMSO has been proposed as a treatment for some diseases41-46,47) and its new biological activities have been found in cell research.41-46,47,48) Our findings revealed that DMSO promoted fetal cardiomyocytes proliferation partially through STAT3 phosphorylation. DMSO had stronger impacts on cells at LDS than at EDS, suggesting that DMSO could also activate preexisting cardiomyocytes proliferation in the adult heart where the cardiomyocytes are more mature. Hence, DMSO could serve as an effective, economic, and safe therapy for heart diseases with cell loss.

Disclosures

Conflicts of interest: None.

References

1. Soonpaa MH, Field LJ. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. Am J Physiol 1997; 272: H220-6.
2. Senyo SE, Lee RT, Kühn B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. Stem Cell Res 2014; 13: 532-41.

3. Yuan X, Braun T. Multimodal regulation of cardiac myocyte proliferation. Circ Res 2017; 121: 293-309.

4. Dixit P, Katare R. Challenges in identifying the best source of stem cells for cardiac regeneration therapy. Stem Cell Res Ther 2015; 6: 26.

5. Suuronen EL, Price J, Veinot JP, et al. Comparative effects of mesenchymal progenitor cells, endothelial progenitor cells, or their combination on myocardial infarct regeneration and cardiac function. J Thorac Cardiovasc Surg 2007; 134: 1249-58.

6. Wang Z, Schnull S, Zheng H, Shan J, Zou R, Xue S. Ascend- ing aortic constriction promotes cardiomyocyte proliferation in neonatal rats. Int Heart J 2017; 58: 264-70.

7. Mitsutake Y, Pyun WB, Rouy D, et al. Improvement of local cell delivery using helix transcendocardial delivery catheter in a porcine heart. Int Heart J 2017; 58: 435-40.

8. Senyo SE, Steinhauser ML, Pizzimenti CL, et al. Mammalian heart renewal by pre-existing cardiomyocytes. Nature 2013; 493: 433-6.

9. Naqvi N, Li M, Calvert JW, et al. A proliferative burst during preadolescence establishes the final cardiomyocyte number. Cell 2014; 157: 795-807.

10. Han C, Yu N, Hong L, et al. Acute inflammation stimulates a regenerative response in the neonatal mouse heart. Cell Res 2015; 25: 1137-51.

11. Ferrarelli LK. Focus issue: networking cancer treatment strategies. Sci Signal 2013; 6: eg5.

12. Fang Y, Gupta V, Karra R, Holdway JE, Kikuchi K, Poss KD. Adult murine cardiomyocytes exhibit regenerative activity with cell cycle reentry through stat3 signalling in breast cancer cells. Proc Natl Acad Sci U S A 2015; 2012; 154: 47-50.

13. Przybytek E, Krenning G, Harmsen MC. Adipose cell delivery using helix transendocardial delivery catheter in a porcine heart. Int Heart J 2017; 58: 13416-21.

14. Stopp S, Gründl M, Fackler M, et al. Deletion of gas2I3 in mice leads to specific defects in cardiomyocyte cytokinesis during development. Proc Natl Acad Sci U S A 2017; 14: 8029-34.

15. Han C, Yu N, Hong L, et al. Acute inflammation stimulates a regenerative response in the neonatal mouse heart. Cell Res 2015; 25: 1137-51.

16. Ferrarelli LK. Focus issue: networking cancer treatment strategies. Sci Signal 2013; 6: eg5.

17. Fang Y, Gupta V, Karra R, Holdway JE, Kikuchi K, Poss KD. Adult murine cardiomyocytes exhibit regenerative activity with cell cycle reentry through stat3 signalling in breast cancer cells. Proc Natl Acad Sci U S A 2015; 2012; 154: 47-50.

18. Jacob SW, Herschler R. Pharmacology of dimso. Cryobiology 1986; 23: 14-27.

19. Ahn H, Kim J, Jeung EB, Lee GS. Dimethyl sulfoxide inhibits nlrp3 inflammasome activation. Immunobiology 2014; 219: 315-22.

20. Damoncova JPL, Giuberti CDS, Kitagawa RR. Preformulation study and influence of dimso and propylene glycol on the anti- oxidant action of isocoumarin paenapalentine isolated from paenapalanthus bromelioides. Rev Bras Farmacogn 2015; 25: 395-400.

21. Stoughton RB, Fritsch W. Influence of dimethylsulfoxide (dmos) on human percutaneous absorption. Arch Dermatol 1964; 90: 512-7.

22. Habara-Ohkubo A. Differentiation of beating cardiac muscle cells from a derivative of p19 embryonal carcinoma cells. Cell Struct Funct 1996; 21: 101-10.

23. Špäter D, Hansson EM, Zangi L, Chien KR. How to make a cardiomyocyte. Development 2014; 141: 4418-31.

24. Nie L, Tang M, Zeng Y, et al. Properties and functions of K (ATP) during mouse perinatal development. Biochem Biophys Res Commun 2012; 418: 74-80.

25. Liu A, Tang M, Xi J, et al. Functional characterization of inward rectifier potassium ion channel in murine fetal ventricular cardiomyocytes. Cell Physiol Bioche 2010; 26: 413-20.

26. Lie N, Gao SJ, Zhao YN, et al. Thymosin β4 impeded murine stem cell proliferation with an intact cardiovascular differentiation. J Huazhong Univ Sci Technolog Med Sci 2016; 36: 328-34.

27. Song H, Wang R, Wang S, Lin J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. Proc Natl Acad Sci U S A 2005; 102: 4700-5.

28. Jarocha D, Zuba-Surma E, Majka M. Dimethyl sulfoxide (dmso) attenuates nitric oxide generation via heme oxygenase-1. J Cell Physiol 2011; 226: 433-43.

29. Eulalio A, Mano M, Berselli K, Walsh S, et al. Cardiomyocyte proliferation contributes to heart growth in young humans. Proc Natl Acad Sci U S A 2013; 110: 1446-51.

30. Kornakova E, Fackler M, et al. Inhibition of differentiation and function of osteoclasts by dimethyl sulfoxide (dmso). Cell Physiol Bioche 2010; 26: 413-20.

31. Duarte F, Alcántara D, Barreira Silva M, et al. Comparative effects of mesenchymal progenitor cells, endothelial progenitor cells, or their combination on myocardial infarct regeneration and cardiac function. J Thorac Cardiovasc Surg 2007; 134: 1249-58.

32. Stopp S, Gründl M, Fackler M, et al. Deletion of gas2I3 in mice leads to specific defects in cardiomyocyte cytokinesis during development. Proc Natl Acad Sci U S A 2017; 14: 8029-34.

33. Tian Y, Liu Y, Wang T, et al. A microrna-hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. Sci Transl Med 2015; 7: 279ra238.

34. Liang H, Chiou HK, Chung Y, Lim GL, Park YN, Park SW. Predominant activation of jak/stat3 pathway by interleukin-6 is implicated in hepatocarcinogenesis. Neoplasia 2015; 17: 586-97.

35. Miyawaki A, Obana M, Mitsuhara Y, et al. Adult murine cardiomyocytes exhibit regenerative activity with cell cycle reentry through stat3 signalling in the healing process of myocarditis. Sci Rep 2017; 7: 1407.

36. Xu W, Wu W, Chen J, et al. Exogenous hydrogen sulfide protects H9c2 cardiac cells against high glucose-induced injury by inhibiting the activities of the g38 MAPK and ERK1/2 pathways. Int J Mol Med 2013; 32: 917-25.

37. Lin Z, Zhou P, Von Gise A, et al. P3Kcbk links Hippo-YAP and PI3K-AKT signaling pathways to promote cardiomyocyte proliferation and survival. Circ Res 2015; 116: 35-45.

38. von Gise A, Lin Z, Schlegelmilch K, et al. YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proc Natl Acad Sci U S A 2012; 109: 2394-9.

39. Rupert CE, Coulombe KL. The roles of neuregulin-1 in cardiac development, homeostasis, and disease. Biomark Insights 2015; 10: 1-9.

40. Eulalio A, Mano M, Dal Ferro M, et al. Functional screening identifies miRNAs inducing cardiac regeneration. Nature 2012; 492: 376-81.
45. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochem Pharmacol 2003; 65: 1035-41.
46. Capriotti K, Capriotti JA. Dimethyl sulfoxide: History, chemistry, and clinical utility in dermatology. J Clin Aest Dermatol 2012; 5: 24-6.
47. Czysz K, Minger S, Thomas N. DMSO efficiently down regulates pluripotency genes in human embryonic stem cells during definitive endoderm derivation and increases the proficiency of hepatic differentiation. PLoS One 2015; 10: e0117689.
48. Choi SC, Choi JH, Cui LH, et al. Mixl1 and Flk1 are key players of Wnt/TGF-β signaling during DMSO-induced mesodermal specification in P19 cells. J Cell Physiol 2015; 230: 1807-21.