Diallyl trisulfide (DATS) Suppresses HG-induced H9c2 Cardiomyoblast Apoptosis by Targeting ROS- mediated HIF-1α-IGFBP-3 Activation

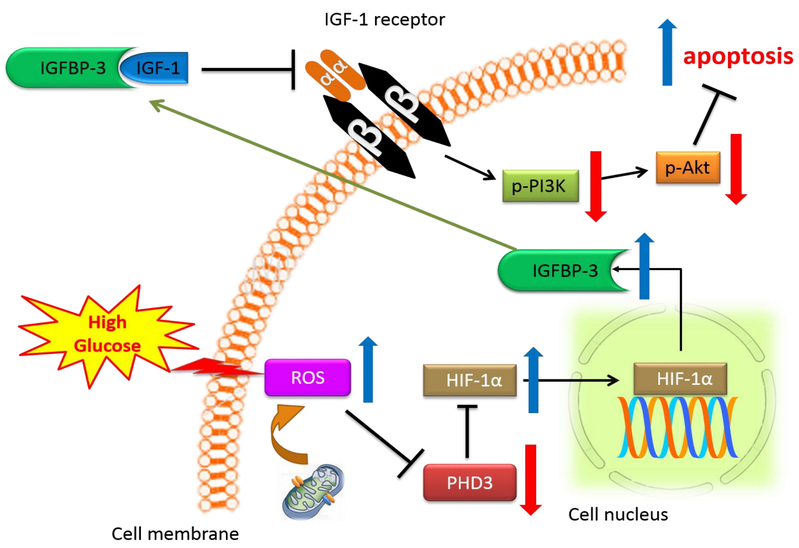
Yu-Min Wei.[1], Chih-Yang Huang. [2,3] , Wei-Wen Kuo.[1]

[1]*Department of Biological Science and Technology, China Medical University, Taichung*
[2]*Graduate Institute of Basic Medical Science, China Medical University, Taichung*
[3]*Department of Biological technology, Asia University, Taichung*

Abstract

Backgrounds:
Diabetes is one of the most common diseases to lead death in Taiwan and more than 80% patients are dead due to cardiovascular diseases. In our previous study, it is demonstrated that cardiac activation of HIF-1α-IGFBP-3 signaling mediated by ROS-regulated PHD is involved in HG-induced apodosis. Diallyl trisulfide (DATS) is the component in garlic oil with the strongest inhibitory effect on DCM. In this study, we will further investigate whether HIF-1α-IGFBP-3 signaling governs the anti-apoptotic effect of DATS on HG-exposed H9c2 cardiomyoblast cells.
Methods and Results:
H9c2 cells were treated with 5.5 mM and 33mM glucose for 36 hr. It was observed that significant increased levels of the cell apoptosis, ROS production, HIF-1α, IGFBP-3 and down-regulated phosphorylated Akt phosphorylation induced by HG were reversed by the treatment of DATS in a dose-dependent manner. The results of co-immunoprecipitation (Co-IP) assay showed that DATS suppressed the extracellular association of IGF-1 with IGFBP-3 of H9c2 cardiomyoblast exposed to HG. The treatment of H_2O_2 and PHD siRNA increased HIF-1α and IGFBP-3 protein levels which was decreased by DATS. Medium sample showed the similar results. The overexpression HIF-1α and IGFBP-3 reversed the level of cell apoptosis which was suppressed by the treatment of DATS in HG-exposed cells.
Conclusion:
Taken together, these findings show that the mediation of ROS-regulated PHD on HIF-1α-IGFBP-3 signaling activation governs the anti-apoptotic effect of DATS on HG-exposed H9c2 cardiomyoblast cells.

Previous Studies



Schematic illustrating extracellular downregulation of cardiac IGF-1 survival signaling through binding and sequestering activity of increased secreted-IGFBP-3 induced by HG. Additionally, ROS-regulated PHD mediated the intracellular activation of HIF-1a - IGFBP-3 signaling to increase IGFBP-3 secretion, involving gin HG-induced cardiomyocyte apoptosis.

Results

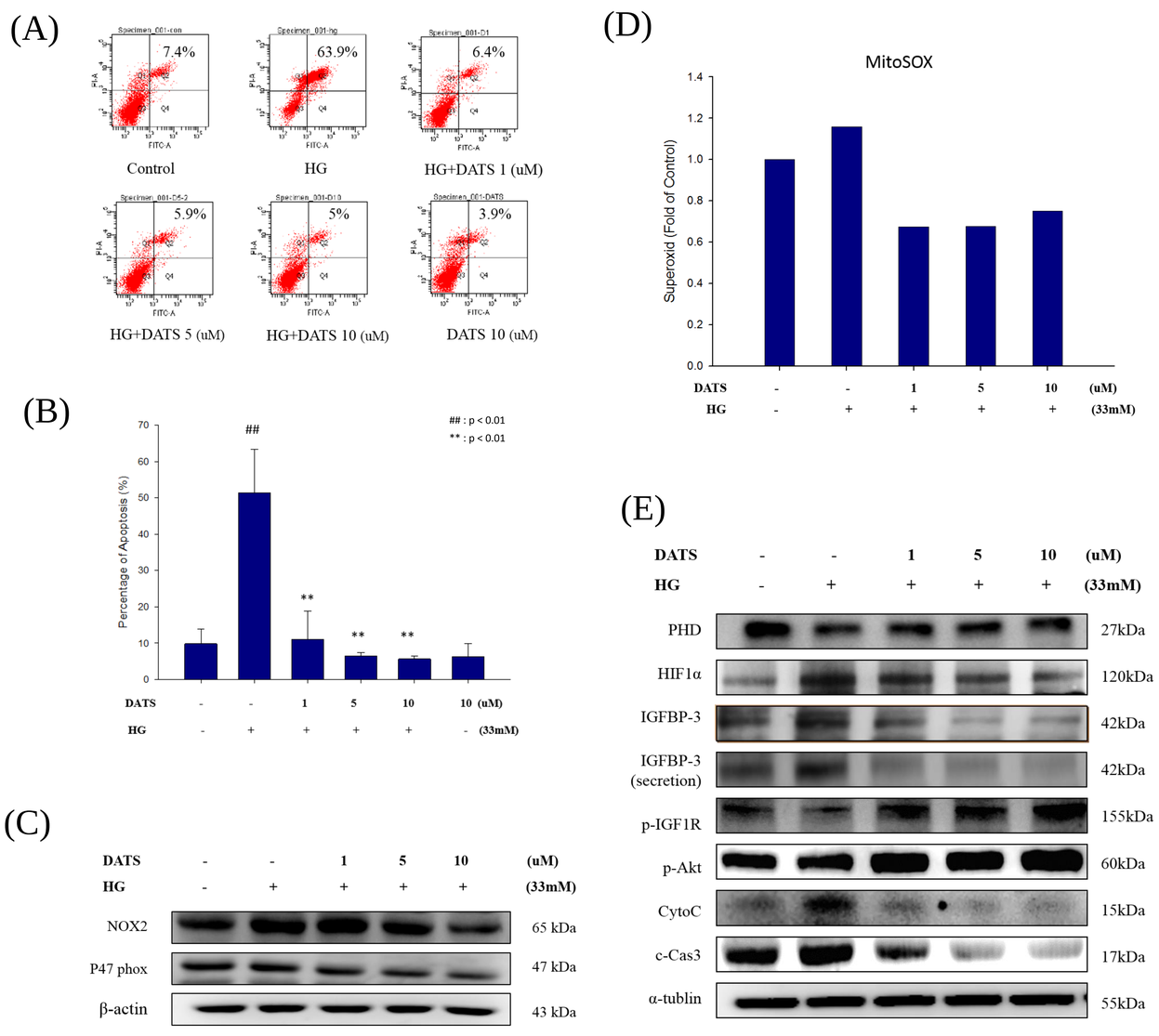


Fig.1.The mediation of ROS regulated PHD on HIF-1α- IGFBP-3 signaling activation may govern the anti-apoptotic effect of DATS on HG-exposed H9c2 cardiomyoblast cells. Cell were culture in high glucose medium (33mM) for 36 hours. DATS were treated at doses 1, 5 and 10 (μM), (A)(B) Apoptosis detection and quantitative results by Flow Cytometry under different doses of DATS, (C) p47 phox and NOX2 detected by western blotting, (D) MitoSOX analysis, (E) HIF-1α- IGFBP-3 signaling and apoptotic proteins analysed by western blotting.

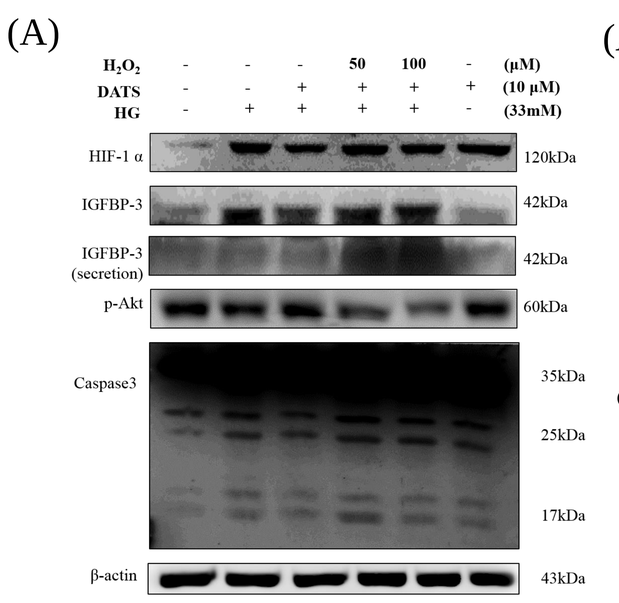


Fig.2 Reduction of ROS generation to enhance HIF-1α-IGFBP-3 signaling activation is involved in anti-apoptotic effect of DATS on cardiac cell exposed to HG. Protein levels were examined by western blotting.

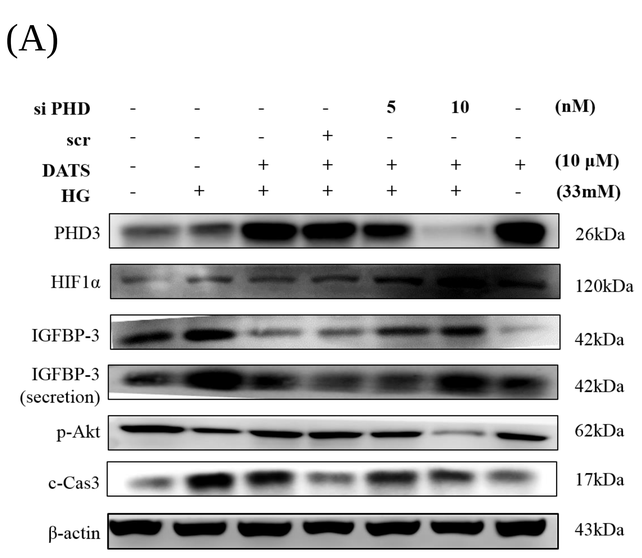


Fig.3 DATS reduces the HG-induced apoptosis through upregulating PHD expression then further activating its downstream. Protein levels were detected by Western Blotting.

Previous Studies

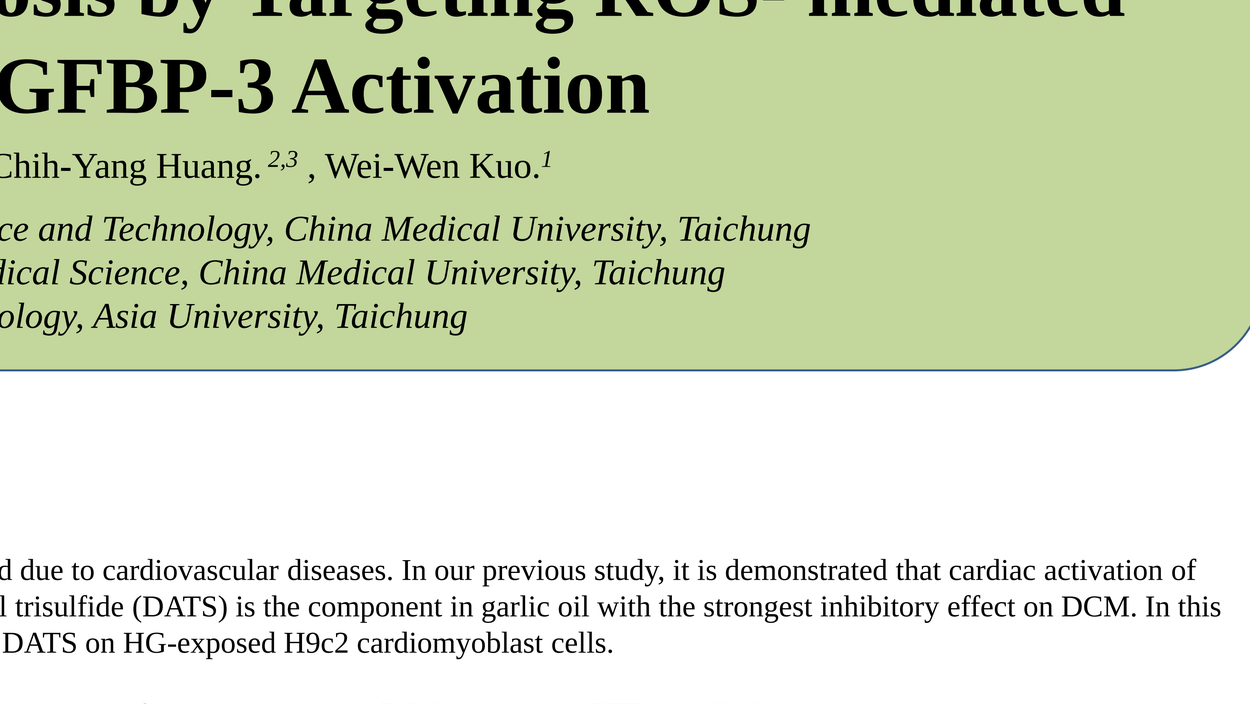


Fig.4 The anti-apoptotic effects of DATS on High glucose-treated H9c2 cells is through inhibiting HIF-1α expression. (A) animal models (B) Successful transfection of HIF-1α plasmid examined by western blotting, (C) Protein levels of high glucose-exposed H9c2 cells were treated with DATS and HIF-1α overexpression plasmid (6,10 μg) were detected by Western Blotting.

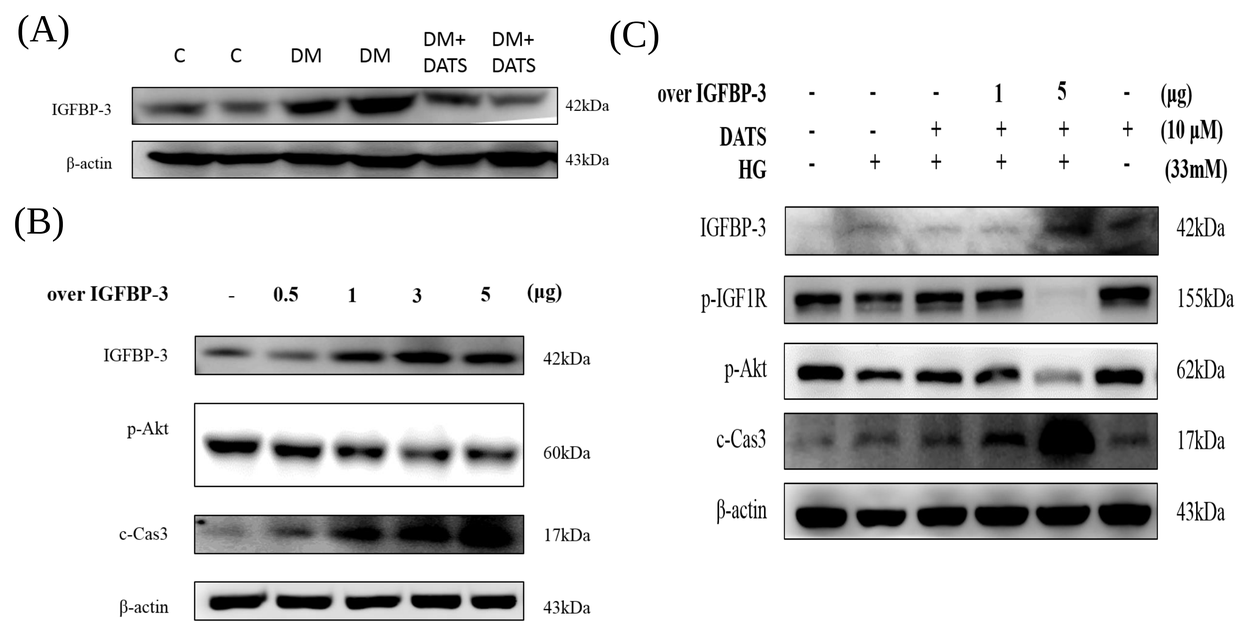


Fig.5 The anti-apoptotic effects of DATS on High glucose-treated H9c2 cells is through inhibiting HIF-1α expression. (A) animal models (B) Successful transfection of IGFBP-3 plasmid examined by western blotting, (C) Protein levels of high glucose-exposed H9c2 cells were treated with DATS and IGFBP-3 overexpression plasmid (1,5 μg) were detected by Western Blotting.

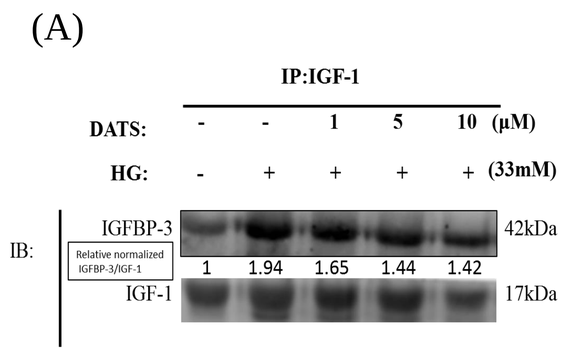
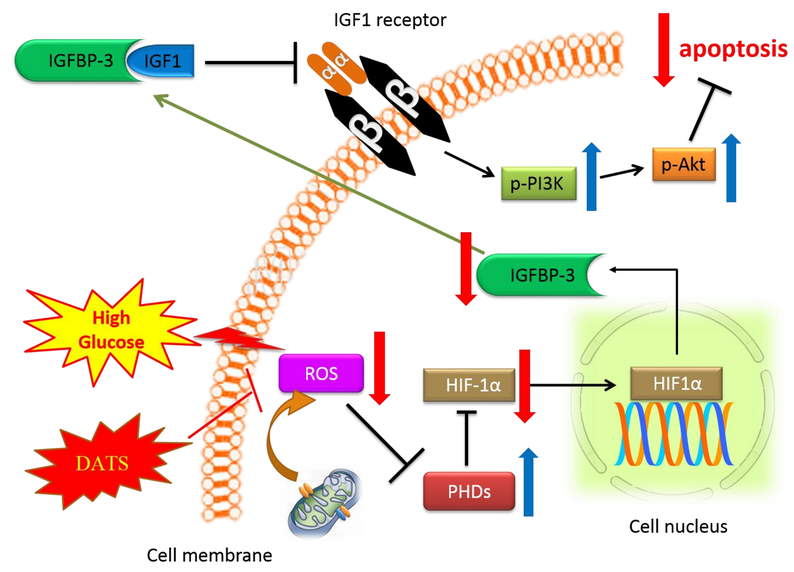


Fig.6 DATS reduced the extracellular binding of IGFBP3 and IGF1 caused by high glucose. The association of IGF-I with IGFBP3 detected by immunoprecipitation was decreased by DATS in cell medium.

Summary



Inhibitions of ROS generation, PHD protein reduction and HIF-1α- IGFBP-3 signaling activation are involved in anti-apoptotic effects of DATS on cardiomyoblast cells exposed to HG. Furthermore, decreased secreted IGFBP-3 by DATS enhances more extracellular unbound IGF-1 to promote cardiac cell survival under HG condition.