Blockage of Insulin-Like Growth Factor (IGF)-I Survival Signaling by IGFBP3 Enhanced by ROS-dependent HIF1α Activation Mediates High Glucose-Induced Cardiac Cell Apoptosis

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Abstract

Backgrounds:

High glucose can cause intracellular ROS generation, inactivate insulin-like growth factor-I (IGF-I) cell survival signaling, leading to cell apoptosis. IGF-binding protein-3 (IGFBP-3) is the most concentrated carrier protein for IGF-I in blood. Recently, IGFBP-3 was reported to mediate high glucose-induced cell apoptosis. HIF-1 alpha, a transcription factor, is an upstream protein of IGFBP3. In this study, we investigated the role of IGFBP-3 in high glucose-induced apoptosis in cardiac cells.

Methods and Results:

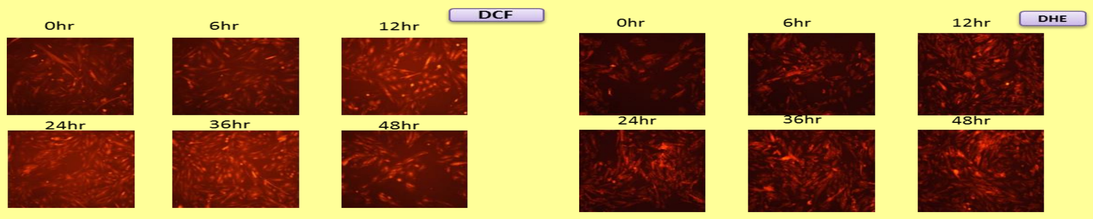
H9c2 cells were treated with 5.5 mM and 33mM (high glucose, HG) glucose for 36 hr. We found HG resulted in a time-dependent increase in ROS generation, intracellular and extracellular (secreted) IGFBP-3, as well as reduced IGF-I signaling activity. The results of co-immunoprecipitation (Co-IP) assay showed that compared with 5.5 mM glucose, HG enhanced the extracellular association of IGF-I with IGFBP-3, which was also observed in serum sample of STZ-administrated rats. Interestingly, Treatment of IGFBP-3 antibody in medium reversed the decreased IGF-I signaling activity and the apoptosis development in HG-exposed cells. IGFBP-3 siRNA treatment showed the similar results. Additionally, HG time-dependently promoted HIF-1 α nuclear translocation examined by immunofluorecence and western blot. However, the RNA level was not affected. HIF-I α siRNA treatment decreased intra- and extracellular IGFBP-3, apoptosis level and enhanced the reduced IGF-I signaling activity induced by HG. In contrast, the treatment of overexpressed HIF-Iα reversed altered protein levels induced by HG. Using the apocynin, a cytosolic ROS inhibitor, and rotenone, a mitochondria ROS inhibitor, the results showed that increased levels of HIF-Iα, secreted IGFBP-3 and apoptosis as well as the decreased IGF-I survival signaling by HG were significantly reversed by the ROS scavengers, and mitochondria is the major ROS source in cells exposed to HG.

Conclusion:

Our findings suggest that increased IGFBP-3 expression and secretion by oxidative stress mediate high glucose-induced apoptosis in H9c2. The increased oxidative stress from high glucose stabilized HIF1 alpha protein expression to regulate IGFBP-3 expression and extracellular secretion, which further induce cell apoptosis.

Results

FIG.1 (A)



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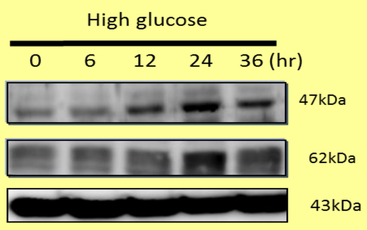

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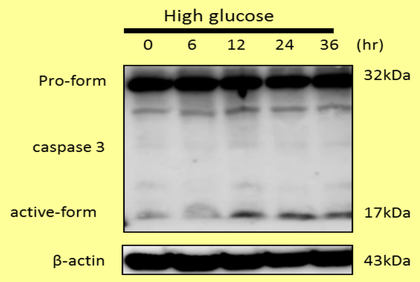
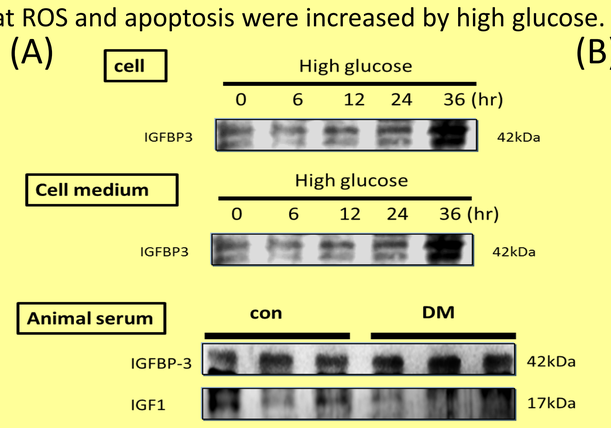


Fig.1. Effects of high glucose-induced H9c2 cell damage. Cells were cultured in high glucose medium (33nm) for different time periods as indicated. (A) Fluorescence images detected by DCF and DHE, (B) NADPH oxidase subunits, p47 and NOX2, and (C) cleaved caspase 3 levels detected by western blot show that ROS and apoptosis were increased by high glucose.

FIG.2 (A)



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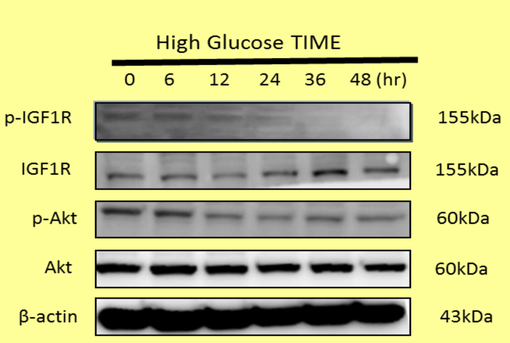
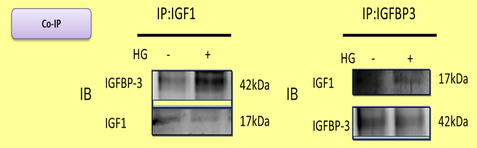


Fig.2. High glucose increases IGFBP3 expression and decreases cell survival in H9c2 cardiomyoblasts exposed to high glucose. (A)High glucose increases IGFBP3 releasing in both cell medium and diabetic animal serum. (B)High glucose decreased the activity of IGF1 survival pathway.

FIG.3 (A)



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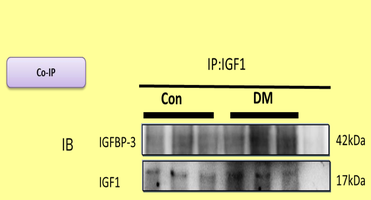

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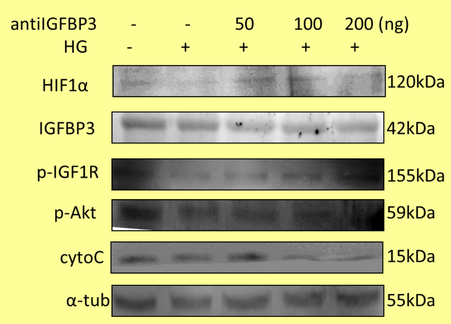
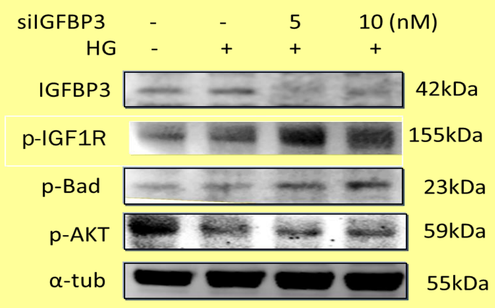


Fig.3. High glucose enhances the extracellular binding of IGFBP3 and IGF1. The association of IGF-I with IGFBP3 detected by immunoprecipitation was increased by high glucose both in (A) cell medium and (B) diabetic animal serum(C)added the antiIGFBP3 in the medium the IGF1 pathway are blocked.

FIG.4

(A)



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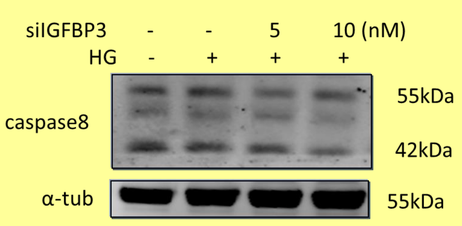
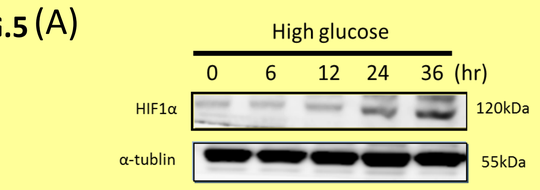


Fig.4. IGFBP3 enhances cell damage by decreasing cell survival and increasing cell apoptosis. High glucose-exposed H9c2 cells were treated with IGFBP3 siRNA, and the (A) survival and (B) apoptotic protein levels were detected by western blot.

FIG.5 (A)



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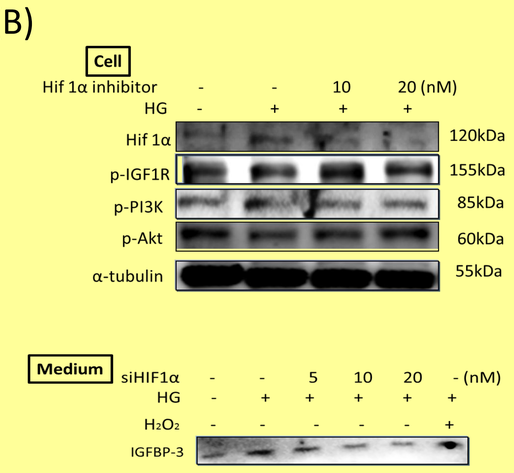

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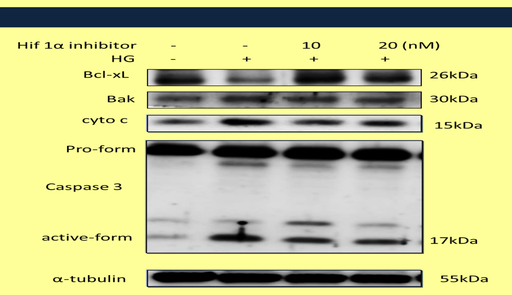

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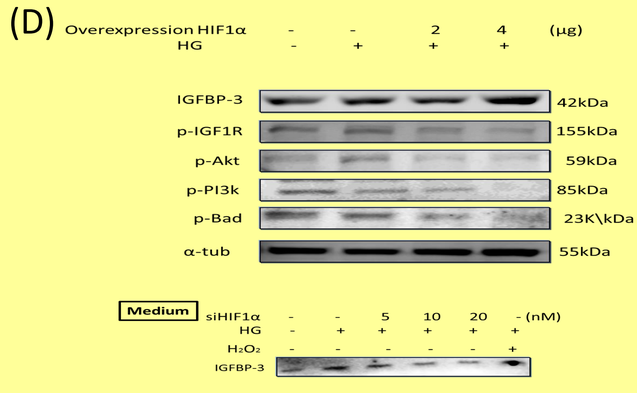
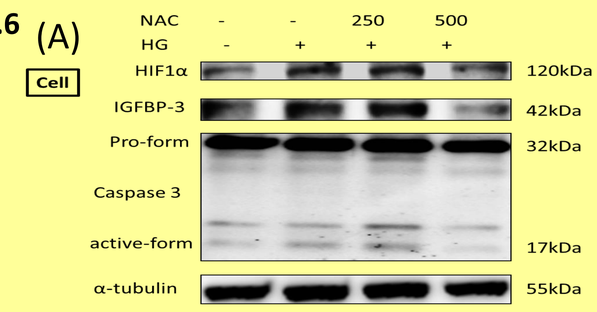


Fig.5. HIF-1α-dependent IGFBP3 releasing is involved in high glucose-induced cell apoptosis. High glucose-exposed H9c2 cells were treated with (A & B) HIF1α inhibitor, siRNA and (B) overexpression plasmid. The survival and apoptotic protein levels were detected by western blot.

FIG.6 (A)



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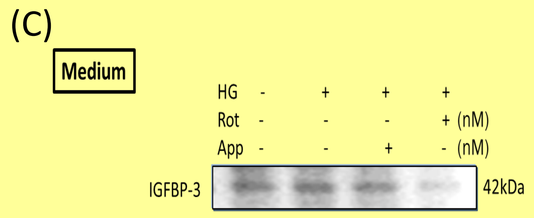

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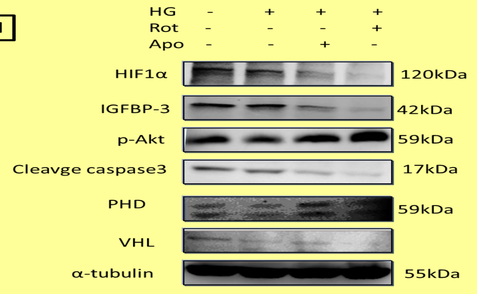

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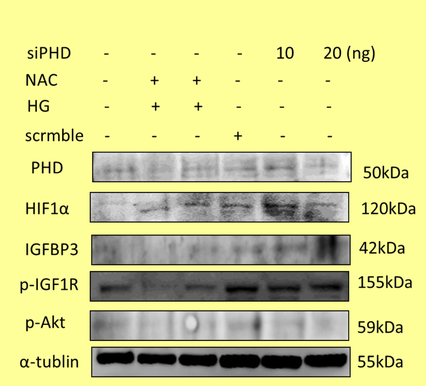
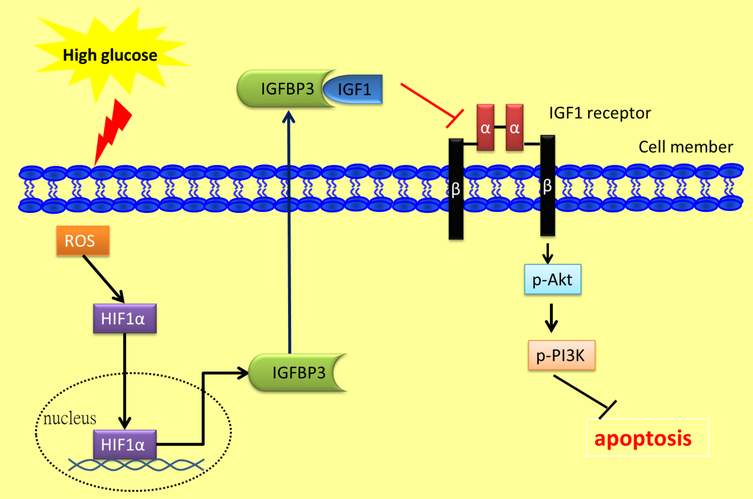


Fig.6. ROS majorly from mitochondria stabilized HIF-1α involves in the mediation of IGFBP3 in high glucose-induced cardiac cell apoptosis. High glucose-exposed H9c2 cells were treated with (A) NAC, a ROS scavenger, or (B) rotenone (Rot), a mitochondria complex I inhibitor, and apocynin (Apo), a NADPH oxidase inhibitor. Survival and apoptotic protein levels were detected by western blot. (D)when we use the siPHD in normal glucose we can find that the HIF1α and IGFBP3 are increased and IGF1 pathway are decreased .

Summary



Insulin-Like Growth Factor-Binding Protein-3 Mediates High Glucose-Induced Apoptosis by Increasing Oxidative Stress to stabilized HIF1 alpha to inhibited IGF1 survival pathway in H9c2 cell

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