# 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

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.

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.

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**

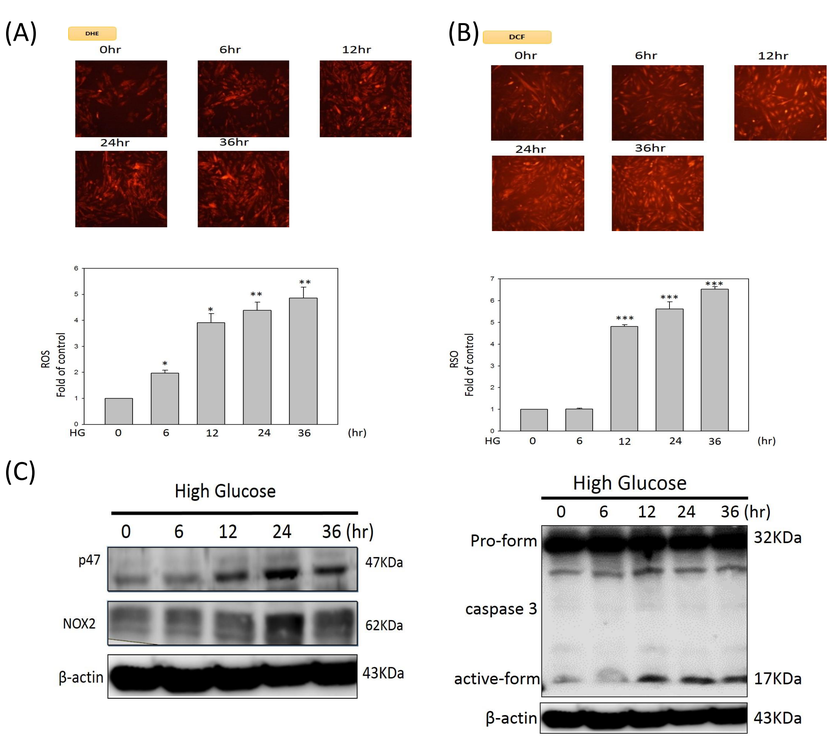



Fig.1. **Effects of HG on ROS generation and apoptosis in cell.** Cells were cultured in HG medium (33mM) for different time periods as indicated. (A) Fluorescence images detected by microscope DCF and (B) DHE as probes, (C) NADPH oxidase subunits, p47 and NOX2, and cleaved caspase 3 levels detected by western blot show that ROS and apoptosis were increased by HG.

**FIG.2**

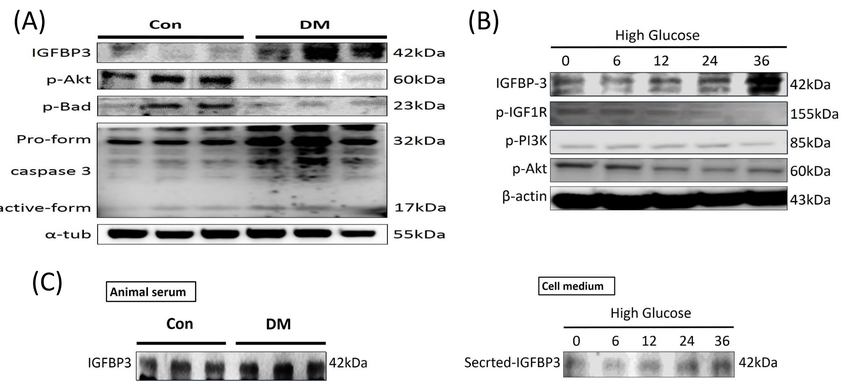



Fig.2. **HG increases IGFBP3 expression and secretion, as well as decreases cell survival in H9c2 cardiomyoblasts exposed to high glucose.** (A) HG increased IGFBP3 expression and decreased the activity of IGF1 survival pathway. (B)Cardaic IGFBP3 expression was increased and survival pathway was suppressed in diabetic rats. (C) HG increases IGFBP3 releasing in both cell medium and diabetic animal serum.

**FIG.3**

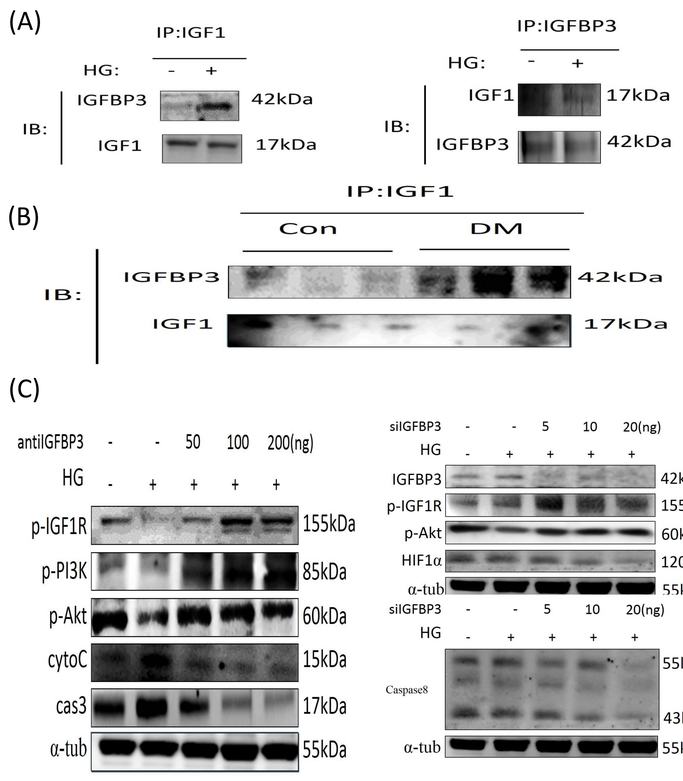



Fig.3. **HG enhances the extracellular binding of IGFBP3 and IGF1 to black IGF1 survuval signaling and cause cell apoptosis.** (A) The association of IGF-I with IGFBP3 detected by immunoprecipitation assay both in cell medium and diabetic animal serum was increased by HG. (B) HG-exposed H9c2 cells were treated with IGFBP3 siRNA at concentrations as indicated. (C) We treated IGFBP3 antibody in the high glucose medium and the survival and apoptotic protein levels were detected measure by western blot.

**FIG.4**

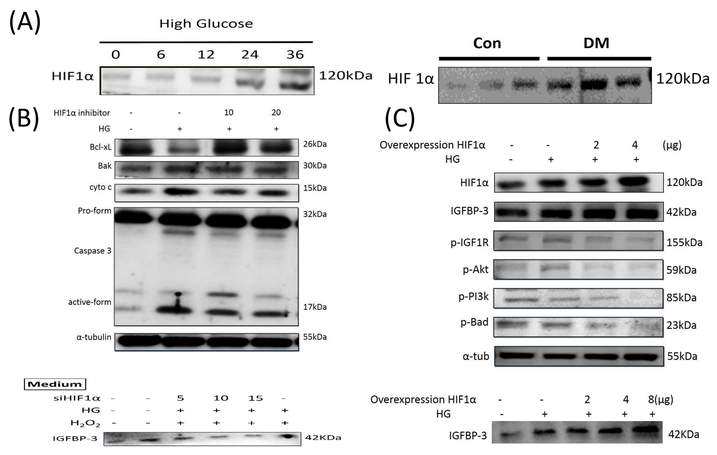



Fig.4. **HIF-1α-dependent IGFBP3 releasing to down-regulated IGF1 survival signaling is involved in high glucose-induced cell apoptosis. (A) Cells were cultured in high glucose medium (33mM) for different time periods as indicated. HIF1α protein examined in both cells and cardiac tissue was analyzed by western blot. HG-exposed H9c2 cells were treated with (B) HIF1α inhibitor, siRNA and (C) overexpression plasmid. The survival and apoptotic protein levels and IGFBP3 level in medium were detected by western blot.**

**FIG.5**

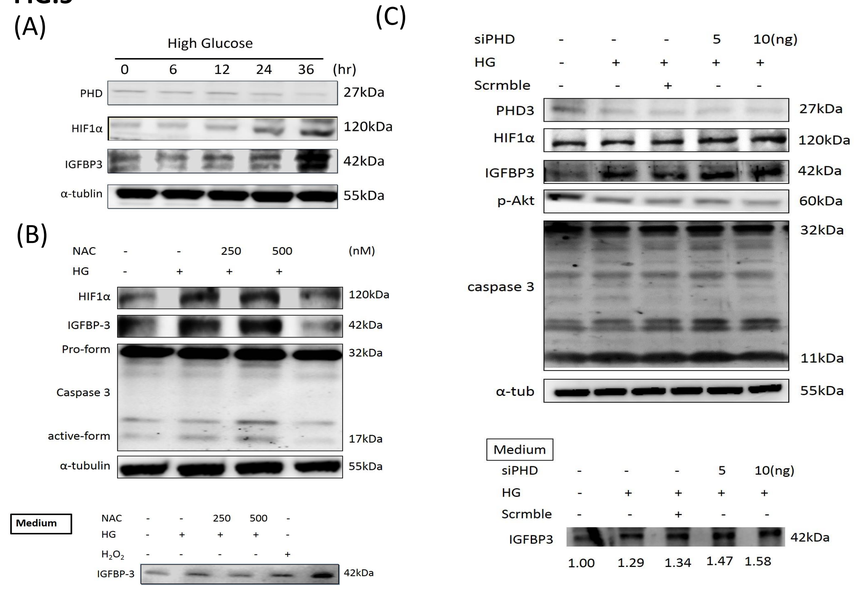
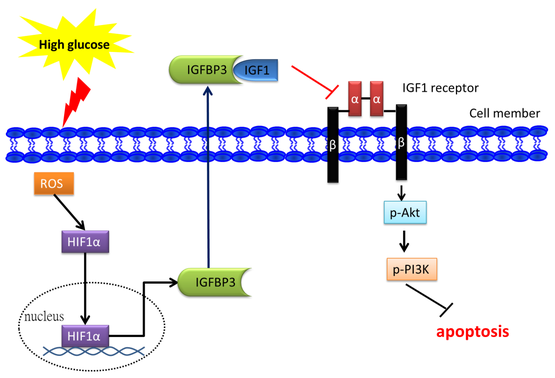



Fig.5. **ROS majorly from mitochondria stabilizes HIF-1α through down-regulation of PHD involves in the mediation of IGFBP3 in high glucose-induced cardiac cell apoptosis.** (A)Cells were cultured in HG medium (33mM) for different time periods as indicated. **HG-exposed H9c2 cells were treated with (B) NAC, a ROS scavenger, or (C) rotenone (Rot), a mitochondria complex I inhibitor, or apocynin (Apo), a NADPH oxidase inhibitor. (D) High glucose-exposed H9c2 cells were treated with PHD siRNA**

## 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 .**