# NFIL3 Protect the Streptozotocin-Induced Diabetes in Rat Hearts and High Glucose-Induced Apoptosis in H9c2 Cardiomyoblast Cells is Associated IGF2R Signaling Pathway Inhibition

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## Abstract

The cation-independent mannose 6-phosphate receptor/insulin-like growth factor II receptor (CI-MPR/IGF2R) is a multifunctional protein able to interact with intracellular and extracellular ligands, some of which are important growth regulatory factors. Much evidences has shown that in spite of degradation of IGF2 in the lysosome, IGF2R may leads intracellular signaling transduction in cell behavior regulation. In this study, we investigated whether IGF2R signaling pathway play a critical role in streptozotocin (STZ)-induced diabetic rat hearts. In STZ-induced diabetic rat hearts, western blot revealed that protein level of IGF2, IGF2R and Gαq increased and an increased in the phosphorylation of PKCα and CaMKII. In our study on H9c2 cardiomyoblast cells we observed IGF2 and IGF2R protein level up-regulated by high glucose treatment. H9c2 cardiomyoblast cells exposed to high glucose not only induced Gαq and calcineurin protein level expression but also increased phosphorylation of PLC-β3 and PKCα in H9c2 cardiomyoblast cells. The IGF2R binding activity to IGF2 and Gαq were increased by high glucose in a time-dependent manner. H9c2 cardiomyoblast cells exposed to high glucose increased the protein level of hypertrophy marker ANP and BNP. Furthermore, inhibition of IGF-IIR or Gαq by RNA interference could block the high glucose-induced cell apoptosis. We also used nuclear factor interleukin-3 (NFIL3), a critical transcriptional repressor, transfect into H9c2 cardiomyoblast cells. The IGF2R signaling pathway were block by NFIL3 transfection in H9c2 cardiomyoblast cells. High glucose-induced cell apoptosis were block by NFIL3 transfection. Together, this study provides a new target therapy for diabetes treatment in myocardial apoptosis. Repression of IGF2R signaling pathway may be a good target for protection diabetes-induced heart failure progression.

## Results

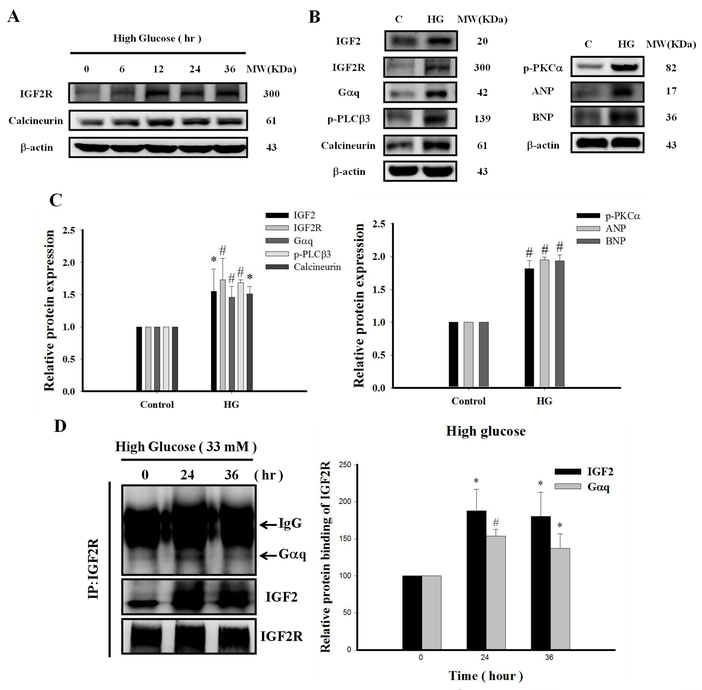


**Fig. 1. Effects of high glucose on insulin-like growth factor-II receptor (IGF-IIR) signaling pathway in H9c2 cardiomyoblast cells.**

(A) Western blot detection of IGF2R and calcineurin protein levels in H9c2 cardiomyoblasts exposed to high glucose (33mM) for 6, 12, 24, and 36 h, with b-actin used as a loading control. (B and C) Western blot analysis of H9c2 cardiomyoblasts exposed to normal glucose (22mM) and high glucose (33mM) for 24 h using indicated antibodies. Using anti-ANP antibody and anti-BNP antibody respectively as the cardiac hypertrophy marker. (D) Western blot analysis of IGF2R binding activity with IGF2 and Gαq by CO-IP in H9c2 cardiomyoblasts exposed to normal glucose (22mM) and high glucose (33mM) for 0, 24 and 36 h. The blots were measured by densitometry. Data are presented as mean±S.D. Bars indicate averages. Statistical significance: *P<0.05; #P<0.01. n=three independent experiments for each data point.

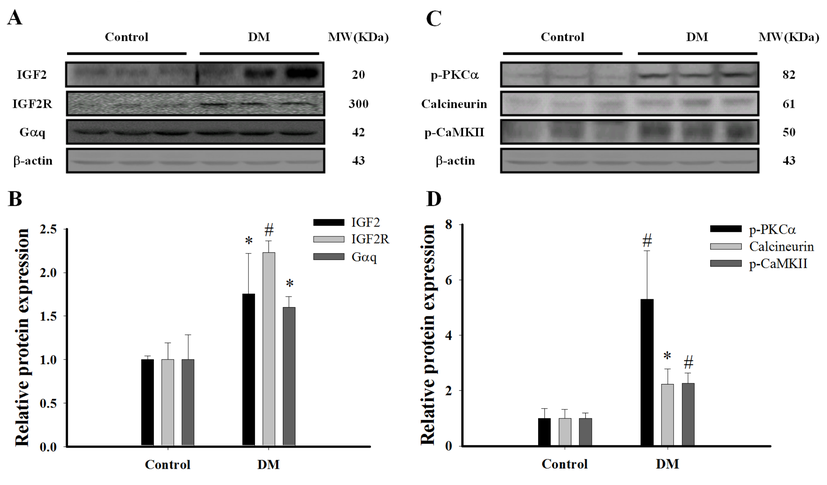


**Fig. 2. Activation of the IGF2R signaling pathway in streptozotocin-induced diabetic rat hearts.**

(A and B) Western blot analysis of IGF2, IGF2R, and Gαq protein levels in streptozotocin-induced diabetic rats. n=3 for each group; Statistical significance: *P<0.05; #P<0.01. (C and D) Western blot analysis of calcineurin protein levels, phosphorylation of PKCa and Calcium/calmodulin-dependent protein kinase II (CaMKII) in streptozotocin-induced diabetic rats. Data are presented as mean±S.D. Bars indicate averages. Statistical significance: *P<0.05; #P<0.01. n=3 for each group.

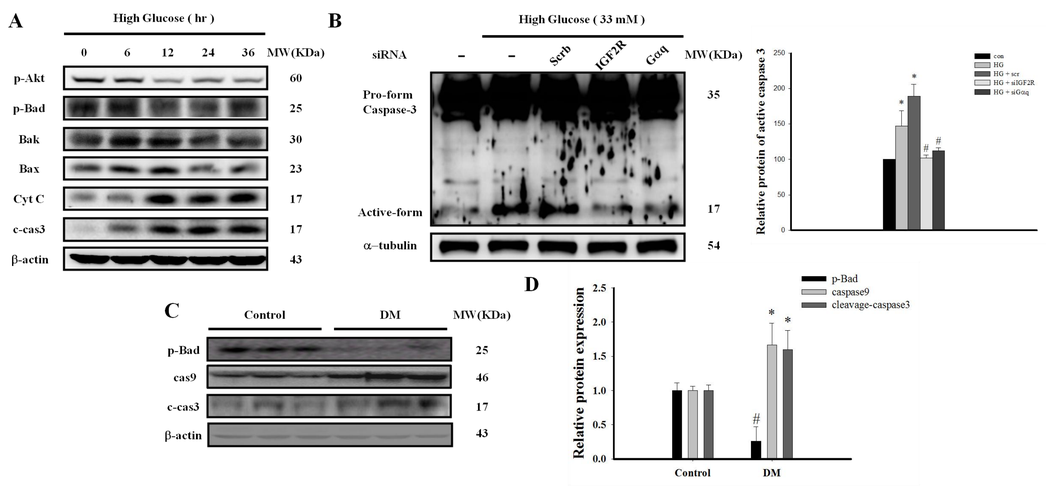


**Fig. 3. High glucose induced apoptosis were block by IGF2R or Gαq RNA interference in H9c2 cardiomyoblast cells and STZ-induced diabetic rat hearts apoptosis.**

(A) Western blot detection of apoptotic proteins in H9c2 cardiomyoblasts exposed to high glucose (33mM) for 6, 12, 24, and 36 h, with b-actin used as a loading control. (B) Western blot detection of caspase-3 in H9c2 cardiomyoblasts exposed to high glucose (33mM) for 36 h and knockdown IGF2R or Gαq RNA interference. Statistical significance: *P<0.01 compared with control; #P<0.01 compared with high glucose treatment. (C and D) Western blot detection of phosphated Bad, caspase-9, and cleavage caspase-3 in streptozotocin-induced diabetic rats. n=3 for each group; Statistical significance: *P<0.05; #P<0.01.

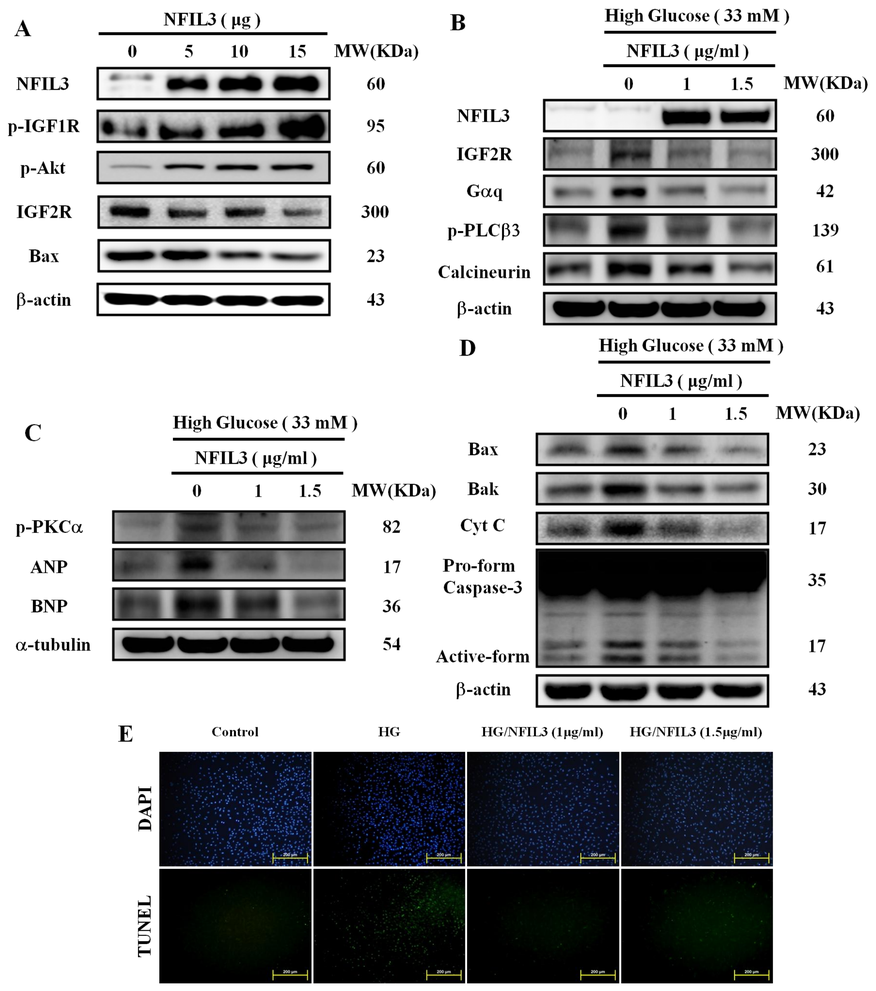


**Fig. 4. NFIL3/E4BP4 enhanced p-IGF1R survival pathway and reduced IGF2R signaling pathway induced-apoptosis in H9c2 cardiomyoblast cells exposed to high glucose.**

(A) Western blot detection of p-IGF1R, p-Akt, IGF2R and Bax protein levels in NFIL3 transfection. (B, C and D) Western blot analysis of H9c2 cardiomyoblasts exposed to high glucose (33mM) for 24 h and transfect NFIL3 using indicated antibodies. (E) Apoptotic cells were detected by TUNEL assay of H9c2 cardiomyoblasts exposed to high glucose (33mM) for 24 h and transfect NFIL3.

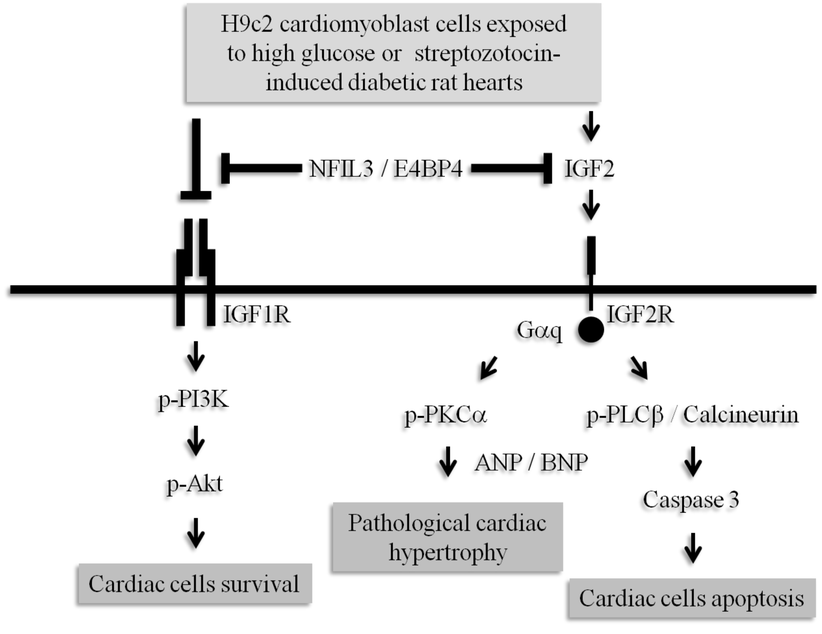


**Fig. 5. A possible role for NFIL3 inhibits IGF2R signaling pathway activation in H9c2 cardiomyoblasts cells by high glucose treatment and STZ- induced diabetic rat hearts.**