HSF1 limits IGF-IIR expression to protect cardiomyocytes and hypertensive ANG II-AT$_1$R-JNK signaling attenuates this protection by impairing SIRT1 deacetylation

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Abstract

Hypertension-induced cardiac hypertrophy and apoptosis are major characteristics of early stage heart failure. Our previous studies found that the activation of insulin-like growth factor receptor II (IGF-IIR) signaling was critical for hypertensive angiotensin II-induced cardiomyocyte apoptosis. However, the detailed mechanism by which angiotensin II (ANG II) regulated IGF-IIR in heart cells remains elusive. In this study, we found that ANG II activated its downstream kinase, JNK, to increase IGF-IIR expression through the ANG II receptor AT$_1$R. The JNK activation subsequently led to SIRT1 degradation via the proteasome, thus preventing SIRT1 from deacetylating heat shock transcription factor 1 (HSF1). The subsequent increase in the acetylation of HSF1 impaired its ability to bind to the IGF-IIR promoter region (nt -748~ -585). HSF1 protected cardiomyocytes by acting as a repressor and protecting against IGF-IIR gene expression, while ANG II diminished the HSF1 repression ability by increasing HSF1 acetylation to activate the IGF-IIR apoptosis pathway. Taken together, these results suggested that HSF1 repressed IGF-IIR gene expression to protect cardiomyocytes. In contrast, ANG II activated JNK to degrade SIRT1, resulting in HSF1 acetylation, which induced IGF-IIR expression and eventually resulted in cardiac hypertrophy and apoptosis. HSF1 could be a valuable target for developing treatments for cardiac diseases in hypertensive patients.

Results

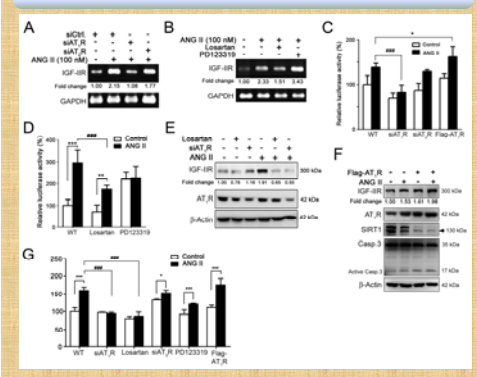

Figure 1. Angiotensin II stimulated IGF-IIR expression to induce apoptosis through the AT$_1$R receptor.
(A-B) Silencing or inhibition of AT$_1$R inhibits ANG II-induced IGF-IIR expression.
(C-D) Knockdown or blockade of AT$_1$R suppressed IGF-IIR promoter
(E) Knockdown or blockade of AT$_1$R also reduced IGF-IIR protein.
(F) Overexpression of Flag-AT$_1$R sensitized H9c2 cells for ANG II-induced IGF-IIR apoptosis.
(G) Knockdown or blockade of AT$_1$R also reduced membrane IGF-IIR protein.

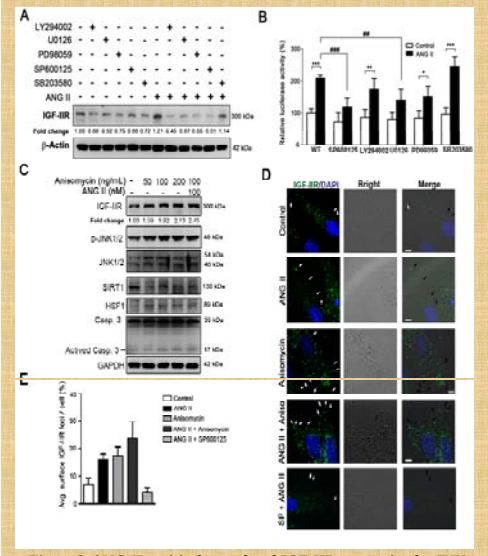

Figure 2. ANG II positively regulated IGF-IIR expression by JNK activation
(A)These kinase inhibitors reduced ANG II-induced IGF-IIR expression.
(B) JNK inhibitor suppressed IGF-IIR expression via negatively regulating its promoter activities
(C) JNK activator also enhanced IGF-IIR expression.
(D-E) ANGII and anisomycin enhanced IGF-IIR membrane translocation, but SP600125 reduced its translocation. White and black arrows indicated IGF-IIR.

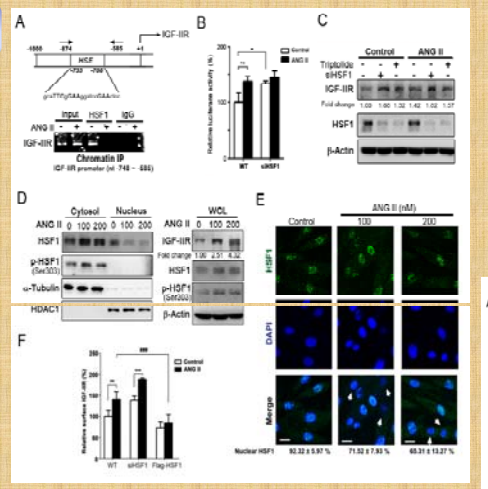

Figure 3. HSF1 suppressed IGF-IIR expression by binding to its promoter region (nt -748~-585), which was diminished by posttranslational modification.
(A)One putative HSF1 binding element located at IGF-IIR promoter (nt -733~-706).Chromate immunoprecipitation (ChIP) showed HSF1 bound to IGF-IIR. Promoter on normal condition.
(B) IGF-IIR promoter activities increased in HSF1-silenced cells.
(C) Increased IGF-IIR expression after HSF1 inhibitor Triptolide and HSF1 siRNA treatment.
(D) Nuclear active HSF1 was decreased, but cytosolic inactive HSF1 was increased after ANG II treatment.
(E)After exposure to ANG II, nuclear HSF1 was decreased
(F) Moreover, membrane IGF-IIR was decreased after silencing or inhibition of HSF1.

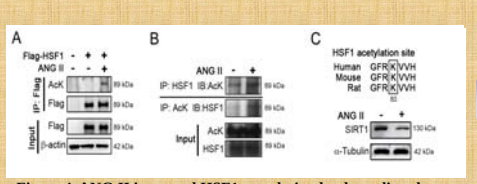

Figure 4. ANG II increased HSF1 acetylation by degrading the HSF1 deacetylase, SIRT1
(A-B) ANG II increased HSF1 acetylation.
(C) HSF1 actylated residue at lysine 80 is conserved in human, mouse and rat. SIRT1 was decreased after ANG II treatment.

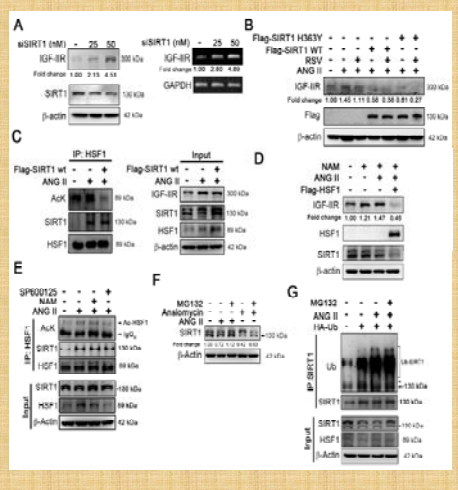

Figure 5. JNK activation increased IGF-IIR expression by impairing SIRT1-deacetylation of HSF1 via proteasome.
(A) Knockdown of SIRT1 enhanced IGF-IIR expression.
(B) Overexpression of SIRT1-wt reduced IGF-IIR expression, but SIRT1-H363Y (deacetylase defected mutant) did not.
(C) Overexpression of SIRT1 impaired ANG II-induced HSF1 acetylation.
(D) SIRT1 inhibitor nicotinamide (NAM) increased IGF-IIR expression; however, overexpression of HSF1 reduced its expression.
(E) SIRT1 inhibitor NAM (10 mM) enhanced ANG II-induced HSF1 acetylation, but SP600125 diminished its acetylation
(F-G) ANG II degraded SIRT1 via ubiquitin-proteasome manner.

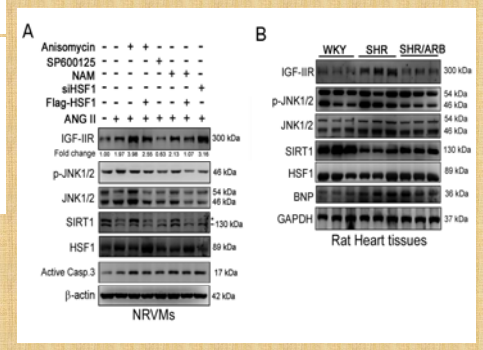

Figure 6. JNK activation resulting in SIRT1 degradation to induce IGF-IIR hypertrophy and apoptosis in Neonatal rat ventricular myocytes (NRVMs) primary cells and spontaneously hypertensive rats (SHR).
(A)JNK1 activation led to SIRT1 degradation to repress HSF1 DNA-binding abilities on IGF-IIR, which in turn activate IGF-IIR expression and induce IGF-IIR apoptosis signaling pathway in neonatal rat ventricular myocytes (NRVMs) primary cells
(B) This phenomenon was also observed on spontaneously hypertensive rats (SHR) heart tissues; however, treatment with with valsartan (SHR/ARB) rescued these damages.

Summary

HSF1 repressed IGF-IIR gene expression to protect cardiomyocyte under normal condition, whereas ANG II activated JNK to degrade SIRT1, followed with HSF1 acetylation, induced IGF-IIR expression, and eventually resulted in cardiac hypertrophy and apoptosis.