Biochemical and Biophysical Research Communications 456 (2015) 122–128

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Crosstalk between JNK and NF-κB signaling pathways via HSP27 phosphorylation in HepG2 cells

Jie Ruan [a,1], Zhilin Qi [b,1], Lei Shen [a], Yi Jiang [a], Yimiao Xu [a], Lei Lan [a], Lan Luo [c,*], Zhimin Yin [a,*]

[a] Jiangsu Province Key Laboratory for Molecular and Medical Biotechnology, College of Life Science, Nanjing Normal University, Nanjing, Jiangsu, PR China
[b] Department of Biochemistry, Wannan Medical College, Wuhu, Anhui, PR China
[c] State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, Jiangsu, PR China

ARTICLE INFO

Article history:
Received 2 November 2014
Available online 21 November 2014

Keywords:
JNK
HSP27 phosphorylation
NF-κB
Apoptosis

ABSTRACT

The crosstalk of intracellular signaling pathways is extremely complex. Previous studies have shown that there is a potential crosstalk between MAPKs and NF-κB signaling pathways. It has been reported that JNK regulates cell survival under some conditions. But the molecular mechanism through which JNK regulates cell survival is still unclear. In the present study, we hypothesized that there was a crosstalk between JNK and NF-κB signaling pathway regulating cell survival and HSP27 phosphorylation mediates such a crosstalk. Our data showed that in HepG2 cells, suppression of JNK activation by a specific inhibitor or overexpression of JNK inactive mutant enhanced TNF-α-induced apoptosis. In addition, reduction of JNK activation attenuated HSP27 phosphorylation envoked by TNF-α, especially the phosphorylation of HSP27 at serine 78 residue. Our results also showed that suppression of JNK activation reduced the degradation of IκB-α, but did not affect IKK phosphorylation upon TNF-α stimulation. Co-immunoprecipitation experiments demonstrated that JNK regulated the degradation of IκB-α through promoting the formation of HSP27/IKK/IκB-α ternary complex in response to TNF-α. Suppression of JNK activation hindered HSP27 phosphorylation at Ser78 residue and subsequently reduced the interaction between IKK and IκB-α. Taken together, our study suggests that through modulation the phosphorylation of HSP27, JNK plays an important roles in cell survival via regulating NF-κB signaling pathway.

1. Introduction

Jun N-terminal kinases (JNKs), one class of MAPKs, which are involved in the regulation of cell proliferation, differentiation and apoptosis. JNKs have been proposed to stimulate apoptosis in response to UV radiation, hyperosmolarity, ischemia–reperfusion, heat shock, or oxidative stress [1]. Although accumulating evidences have suggested that JNK signaling is a proapoptotic cause in cells [2], the precise role of JNK signaling in apoptosis remains controversial. Moreover, a recent study indicates that JNK signaling is related with cell survival [3].

Heat shock proteins (HSPs) are family of molecular chaperones which have been implicated to play an anti-apoptotic role in response to proapoptotic stimuli [4]. HSP27 acts as an ATP-independent holdase chaperone that targets stress-induced misfolded polypeptides and protects cells against various cytotoxic conditions such as oxidative stress, cancer chemotherapeutic agent treatment, Fas stimulation [5]. It has been demonstrated that the specific changes in phosphorylation may allow HSP27 to function various of activities [6]. HSP27 is phosphorylated at several distinct serine residues (Ser15, Ser78, and Ser82) depend on the stimulators [7]. We notice that wild-type HSP27 increases cellular resistance against different stressful stimulation [8], but the mutant HSP27 lacking phosphorylation sites no longer has such function [9]. HSP27 can phosphorylated by various upstream kinases including MAPK-activated protein kinase-2 (MK-2), AKT, protein kinase C (PKC), as well as cyclic adenosine monophosphate (cAMP)-dependent kinase [10,11]. Although JNK is a member of MAPK family, it is unknown if JNK could lead to HSP27 phosphorylation.

NF-κB, a transcription factor, plays a critical role in many physiological processes including immune responses, inflammation, and cell growth and death. Previous study has demonstrated that HSP27 associates with the IκB kinase (IKK) complex to regulate TNF-α -induced NF-κB activation [12]. HSP27 negatively regulates

* Corresponding authors at: College of Life Sciences, Nanjing Normal University, No. 1 Wenyuan Road, Nanjing 210046, PR China. Fax: +86 25 85891305 (Z. Yin).
E-mail addresses: lanluo@nju.edu.cn (L. Luo), yinzhimin@njnu.edu.cn (Z. Yin).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbrc.2014.11.045
0006-291X/

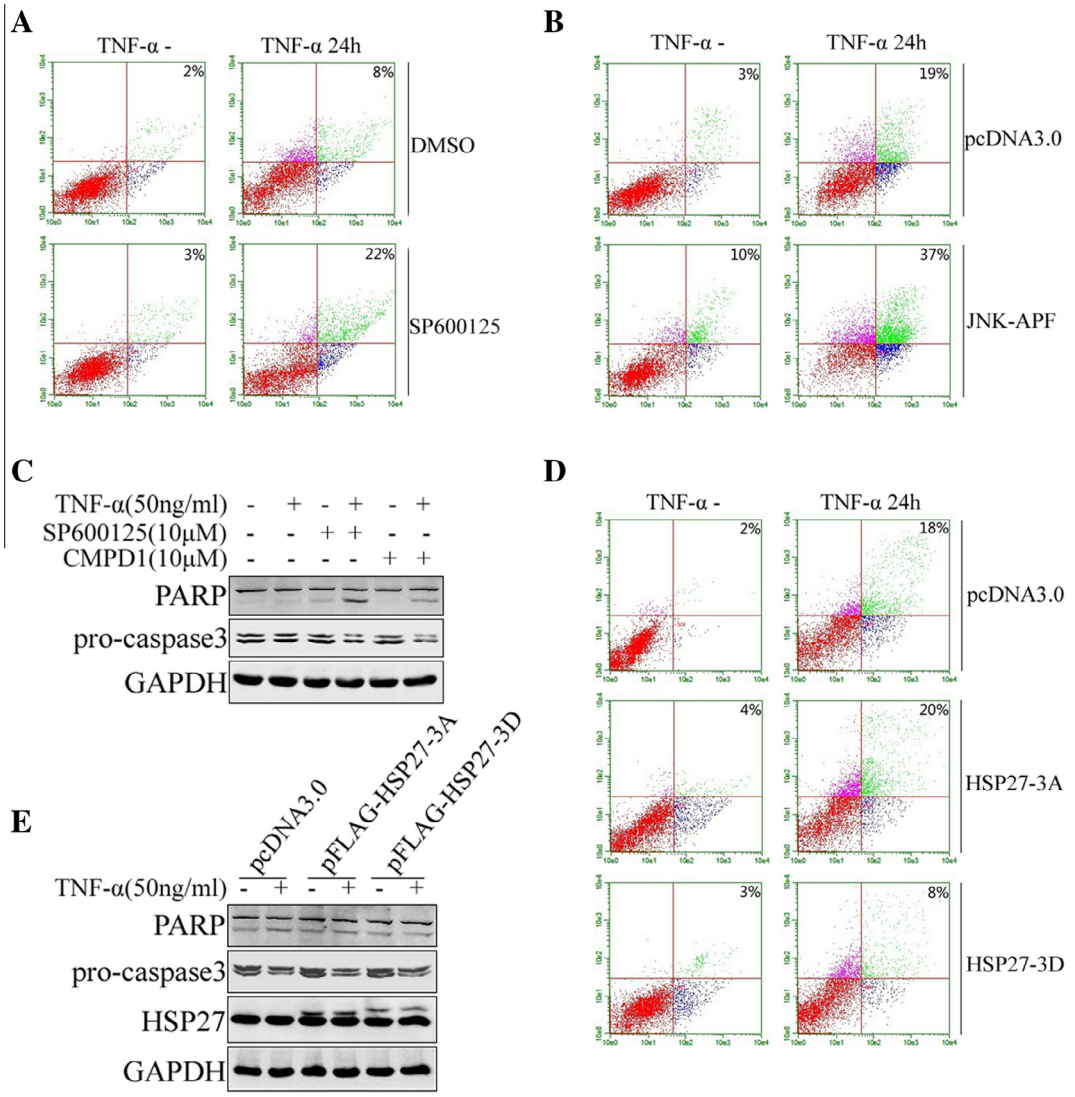

Fig. 1. Suppression of JNK activation and HSP27 phosphorylation facilitates TNF-α induced apoptosis. (A) HepG2 cells were pretreated with or without SP600125 (20 μM) for 2 h, and then were incubated with TNF-α (50 ng/ml) for 24 h, apoptotic ratios were detected by flow cytometry. (B and D) Cells were transiently transfected with JNK-APF (4 μg) plasmid, or transfected with pFLAG-HSP27-3A or pFLAG-HSP27-3D. Forty-eight hours after transfection, cells were treated with TNF-α (50 ng/ml) for 24 h. Apoptotic ratios were detected by flow cytometry. (C) HepG2 cells were pretreated with SP600125 and CMPD1 for 2 h, or (E) transfected with pFLAG-HSP27-3A, pFLAG-HSP27-3D and control plasmid pcDNA3.0 for 48 h, and then were treated with TNF-α (50 ng/ml) for 24 h. PARP and pro-caspase3 were detected by Western blot analysis. In the blot probed with anti-HSP27, the upper band is overexpressed HSP27 with FLAG tag, the lower band is endogenous HSP27. The experiments were conducted in triplicate and data were shown as mean ± SD.

TNF-α triggered IKK activation [12]. However, the role of phosphorylation of HSP27 in TNF-α triggered NF-κB signaling is still unclear.

In this study, we investigated the role of HSP27 phosphorylation in protecting HepG2 cells against TNF-α-induced apoptosis and the effect of HSP27 on mediating the crosstalk between JNK and NF-κB pathway under TNF-α stimulation. Our findings highlight a novel role of JNK in cell survival via affecting HSP27 phosphorylation and subsequently regulating NF-κB signaling pathway.

2. Materials and methods

2.1. Cell culture and transfection

HepG2 cells were obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, P.R. China). The cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal calf serum (Hyclone) and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin) in an atmosphere of 5% CO_2 at 37 °C. Transient transfection was performed by using the Fugene HP reagent (Roche Applied Science, Basel, Switzerland) according to the manufacturer's instructions. In all cases, the total amount of DNA was normalized by the empty control plasmids.

2.2. Antibodies and reagents

Mouse monoclonal antibody and rabbit polyclonal antibody against Flag-tag were purchased from Sigma. Rabbit polyclonal antibodies against GAPDH, and phospho-HSP27 (S15, S78 and S82) were obtained from Bioworld Technology, Inc. Rabbit monoclonal antibodies against ERK, phospho-ERK (Thr202/Tyr204), p38, p-p38 (Thr180/Tyr182), JNK/SAPK, phospho-JNK/SAPK (Thr183/Tyr185), phospho-IKKα (Ser180)/IKKβ (Ser181), IKKβ,

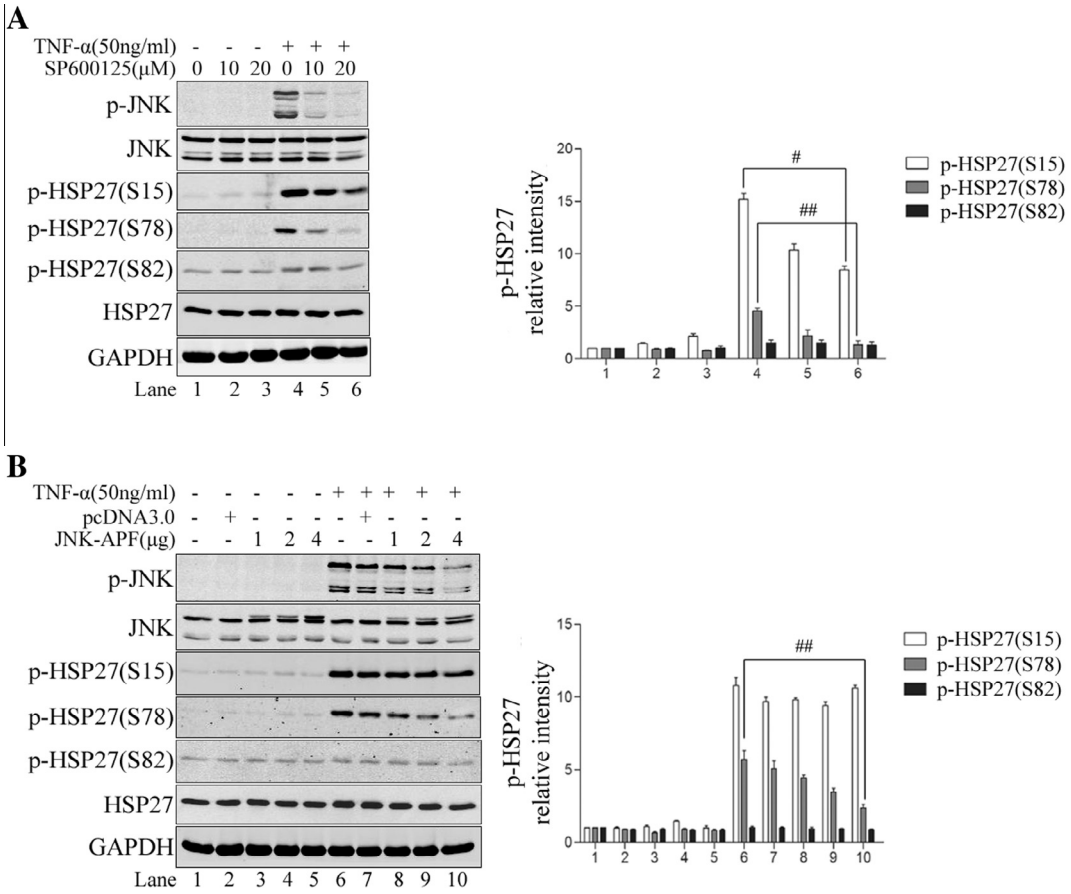

Fig. 2. Inhibition of JNK decreases the phosphorylation of HSP27. (A) HepG2 cells were pretreated with or without different concentrations of SP600125 (10 and 20 μM) for 2 h followed by stimulation with TNF-α (50 ng/ml) for 15 min, and the levels of phosphorylated HSP27(Ser15, Ser78, Ser82) and total HSP27 were detected by Western blotting. (B) HepG2 cells were transfected with JNK-APF (1–4 μg) and control vector, 48 hours after transfection, cells were treated with TNF-α (50 ng/ml) for 15 min. Cell lysates were prepared and subjected to Western blotting. The experiment was done in triplicate and data were shown as mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to TNF-α.

phospho-IκB-α, PARP, Caspase-3, and mouse monoclonal antibodies against HSP27 and IκB-α were obtained from Cell Signaling Technology. Secondary antibodies coupled to IRDye800 flurophore for use with the Odyssey Infrared Imaging System were purchased from Rockland. SP600125 and CMPD1 were from Cell Signaling Technology. Apoptotic detecting regent (FITC-Annexin V and propidium iodide buffer) were from Molecular ProbesTM (Invitrogen). Recombinant human TNF-α was from BD Pharmingen or BD Transduction Laboratories (San Diego, CA, USA).

2.3. Plasmids

HSP27 phosphorylation mutants including pFLAG-HSP27-3A, pFLAG-HSP27-3D and JNK-APF were constructed by using standard techniques. In brief, DNA fragment encoding FLAG-tag was generated by high-fidelity PCR and cloned into pcDNA3.0 vector. HSP27 phosphorylation mutants were generated using over-lap PCR. All of the constructs were confirmed by DNA sequencing.

2.4. Co-immunoprecipitation and immunoblot analysis

Cell lysates were centrifuged (15,000×*g*) at 4 °C for 15 min. Proteins were immunoprecipitated with indicated antibodies respectively. The precleared Protein A/G PLUS-agarose beads (Santa Cruz Biotechnology) were incubated with immunocomplexes for 2 h and washed four times with lysis buffer. Immunoblotting analysis was performed as previously described [13]. The antibody–antigen complexes were visualized by using IRDye800 flurophore-conjugated antibody (LI-COR Biosciences, Lincoln, NE) and the LI-COR Odyssey Infrared Imaging System according to the manufacturer's instruction. Quantification was directly performed on the blot using the LI-COR Odyssey Analysis software. Aliquots of whole cell lysates were subjected to immunoblotting to confirm appropriate expression of proteins.

2.5. Annexin V/PI assay

Floating and adherent cells were collected, washed twice with PBS (pH 7.4), resuspended in 150 μl of Annexin-binding buffer and incubated with 0.4 μl of Annexin VFITC. After 20 min in the dark at room temperature, 150 μl of Annexin-binding buffer containing 3 μl of PI (5 μg/ml) was added, the flow cytometric analysis was carried out using the Guava EasyCyte™ System. For each sample 5000 cells were analyzed. Data were analyzed by using Guava TUNEL Software (Guava Technologies, Hayward, CA, USA) and both early apoptotic cells and late apoptotic cells were considered as apoptotic cells.

2.6. Statistical analysis

Data were represented as means ± SD. We performed statistical comparisons with the unpaired two-tailed Student's *t* test. In all analysis, a value of $p < 0.05$ was considered statistically significant.

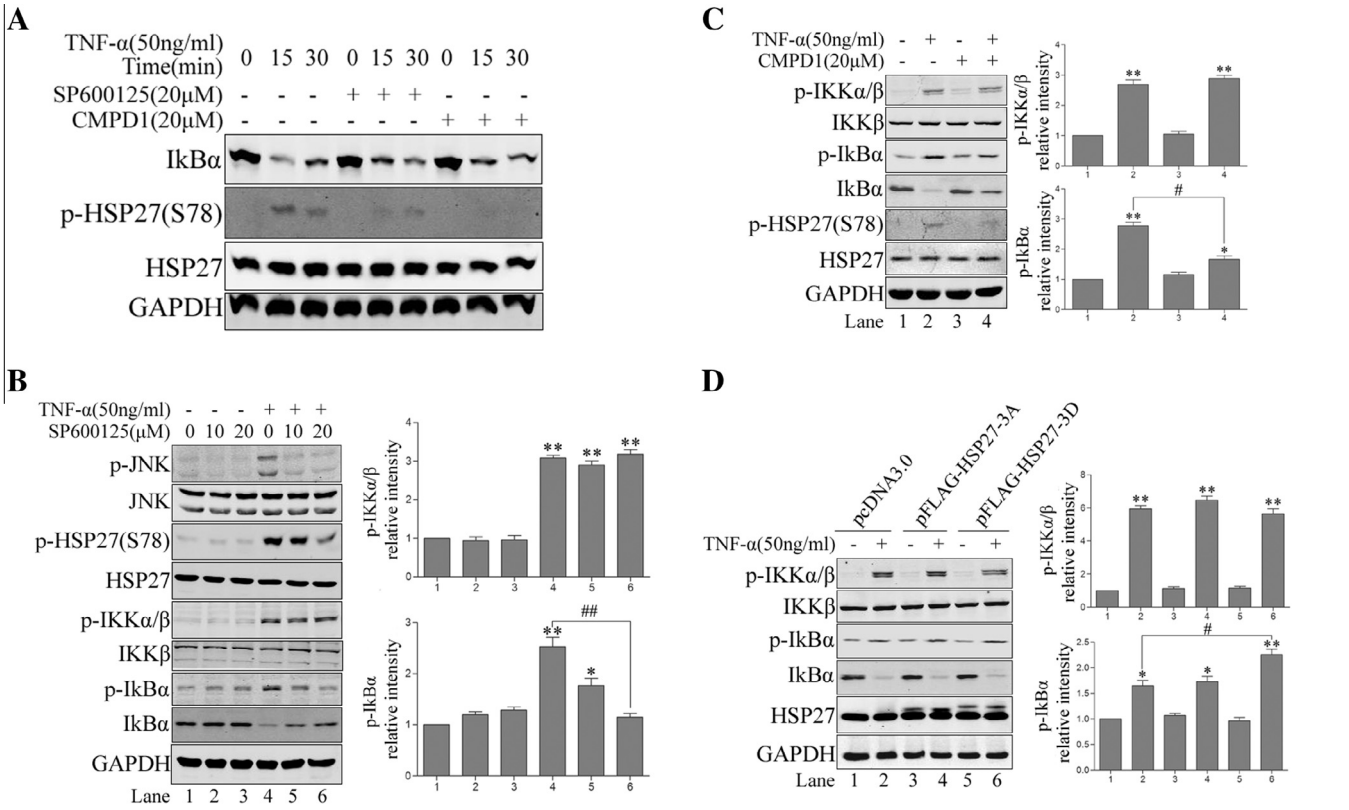

Fig. 3. JNK induces the degradation of IκB-α through phosphorylating HSP27. (A) HepG2 cells were pretreated with SP600125 and CMPD1 for 2 h, and then were treated with TNF-α (50 ng/ml) for indicated time followed by detection of IκBα through Western blot analysis. (B) HepG2 cells were pretreated with SP600125 (0, 10 and 20 μM) for 2 h and then treated with TNF-α (50 ng/ml, Lane 4–6) or not (Lane 1–3) for 15 min. (C) HepG2 cells were pretreated with 20 μM CMPD1(Lane 3 and 4) or not(Lane 1 and 2) for 2 h and then treated with TNF-α (50 ng/ml, Lane 2 and 4) or not (Lane 1and 3) for 15 min. (B and C) Cell lysates were subjected to Western blot analysis by using indicated antibodies. (D) HepG2 cells were transfected with pFLAG-HSP27-3A, pFLAG-HSP27-3D or control vector, and 48 hours after transfection cells were treated with TNF-α (50 ng/ml) for 15 min. Cell lysates were subjected to Western blot analysis with p-IKKα/β, IKKβ, p-IκBα, IκBα, HSP27 and GAPDH respectively. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to TNF-α.

3. Results

3.1. JNK activation and HSP27 phosphorylation negatively regulates TNF-α induced apoptosis

Since it is controversial that if JNK promotes apoptosis or survival of cells [1–3], we observed the effects of JNK on TNF-α-induced apoptosis of HepG2 cells by treating cells with a JNK inhibitor SP600125 or by transfecting cells with JNK-APF, a dominant negative type of JNK, to suppress JNK activation. Results from flow cytometry assay using Annexin V-FITC/PI double staining indicated that the apoptotic ratio increased significantly in the group of cells in which JNK activation was prevented (Fig. 1A and B). Western Blot assay showed that cleavage of poly (ADP-ribose) polymerase (PARP) also increased in JNK activity suppressed cells (Fig. 1C). CMPD1, an inhibitor of p38, played a similar role in PARP cleavage, but the efficiency of CMPD1 was weaker than SP600125. These data strongly suggested that JNK, as similar as p38, prevented TNF-α-induced apoptosis. HSP27 has been implicated to play an anti-apoptotic role in response to various proapoptotic stimuli [4], we subsequently evaluated the relationship between HSP27 phosphorylation and TNF-α-induced HepG2 cell apoptosis. We transfected HepG2 cells with HSP27-3A (a phosphorylation-resistant mutant of HSP27, where all three MK2 phosphorylated serine residues were mutated to alanine residues), HSP27-3D (a phosphorylation mimicking mutant of HSP27, where all three serine residues were mutated to aspartic acid residues) or empty vectors, respectively. The results showed that the apoptotic ratio was higher in HSP27-3A transfected cells than that of in pcDNA3.0 transfected cells. By contrast, transfection of HSP27-3D protected cells from apoptosis (Fig. 1D). Meanwhile, the cleavage of PARP induced by TNF-α were attenuated by over-expression of HSP27-3D but slightly enhanced by introduction of HSP27-3A (Fig. 1E). Altogether, above data suggested that both JNK and HSP27 phosphorylation negatively regulated TNF-α induced apoptosis.

3.2. Inhibition of JNK activity prevents the phosphorylation of HSP27

As above data showed that both JNK and HSP27 played a similar role in TNF-α-induced apoptosis of HepG2 cells suggesting the interaction between JNK and HSP27. On the other hand, it has been reported that MK2, which can be activated by p38 is an upstream phosphorylation kinase of HSP27 and we found CMPD1 also enhanced TNF-α-induced apoptosis [14], we thus examined the effects of JNK, a member of MAPKs, on HSP27 phosphorylation. As shown in Fig. 2A, inhibition of JNK activity by using JNK inhibitor SP600125 suppressed HSP27 phosphorylation, especially the phosphorylation of Ser78, in a dose-dependent manner. The same results were obtained when we transfected HepG2 cells with JNK-APF plasmid (Fig. 2B). These results demonstrated that suppression of JNK activity attenuated the phosphorylation of HSP27 (Ser78) suggesting that JNK might be an upstream kinase of HSP27 and suppression of JNK activity increased TNF-α-induced apoptosis of HepG2 cells through attenuating phosphorylating HSP27.

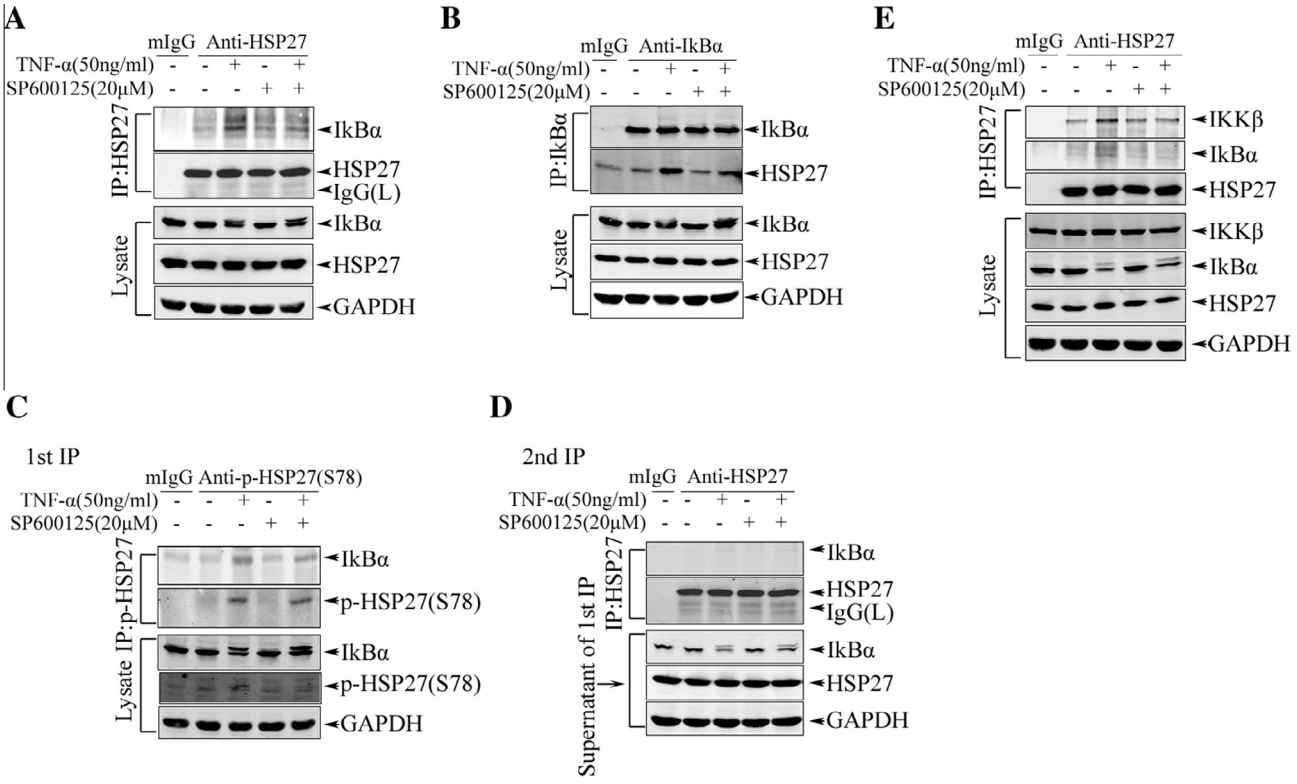

Fig. 4. Phospho-HSP27 interacts with IκB-α and IKKα/β and promotes the binding of IKKα/β with IκB-α. (A) HepG2 cells were pretreated with SP600125 (20 μM) for 2 h (Lane 4 and 5) and lysed after TNF-α (50 ng/ml) challenge for 15 min. The lysates were immunoprecipitated with anti-HSP27 monoclonal antibody followed by analyzing the immunopellets with immunoblotting by using IκBα and HSP27 antibodies. The whole lysates were subjected to immunoblot analysis with HSP27, IκBα and GAPDH antibodies respectively. (B) HepG2 cells were treated with SP600125 and TNF-α as same as (A). The lysates were immunoprecipitated with anti-IκBα monoclonal antibody and then the immunopellets were subjected to immunoblotting with HSP27and IκBα antibodies. The whole lysates were analyzed by immunoblotting with HSP27, IκBα and GAPDH antibodies respectively. (C) HepG2 cells were treated with SP600125 and TNF-α as same as (A). The lysates were immunoprecipitated with p-HSP27 antibody followed by immunoblotting with IκBα and p-HSP27 antibodies in the immunoprecipitations. The whole lysates were subjected to immunoblot analysis with p-HSP27, IκBα and GAPDH antibodies respectively. (D) Supernatants of first IP were immunoprecipitated with anti-HSP27 monoclonal antibody followed by immunoblotting with IκBα and HSP27 antibodies. The whole supernatants of first IP were subjected to immunoblot analysis with HSP27, IκBα and GAPDH antibodies respectively. (E) HepG2 cells were treated with SP600125 and TNF-α as same as (A). The lysates were immunoprecipitated with anti-HSP27 and the immunopellets were subjected to immunoblotting with anti-IKK and anti-IκBα monoclonal antibodies. The whole lysates were analyzed by Western blot analysis with HSP27, IκBα, IKK, GAPDH antibodies.

3.3. JNK induces the degradation of IκB-α through phosphorylating HSP27

We subsequently observed how JNK regulated TNF-α-induced apoptosis via HSP27 phosphorylation. Since NF-κB is a critical regulator of genes involved in cellular growth and death, we treated HepG2 cells with TNF-α (50 ng/ml) for 15 min, and detected the level of IκB-α in cells. As shown in Fig. 3A, TNF-α led to IκB-α level decline, but when cells were pretreated with SP600125 or CMPD1 respectively, the decrease of IκB-α was prevented significantly. These results showed that the suppression of HSP27 phosphorylation by JNK or p38 inhibitor could attenuate the degradation of IκB-α. To further investigate the correlation between IκB-α degradation and HSP27 phosphorylation, HepG2 cells were pretreatment with indicated concentration of SP600125 for 2 h, and then stimulated with 50 ng/ml TNF-α or not for 15 min followed by detection of p-IκB-α and p-IKKα/β level. Western blot assay demonstrated that suppression of HSP27(Ser78) phosphorylation by the JNK inhibitor attenuated the level of p-IκB-α but not p-IKKα/β (Fig. 3B) The similar results were obtained when we pretreated HepG2 cells with CMPD1 for 2 h followed by stimulation with 50 ng/ml TNF-α (Fig. 3C). We then transfected HSP27-3A or HSP27-3D mutants to HepG2 cells respectively. As expected, HSP27-3A, HSP27-3D and pcDNA did not alter p-IKKα/β level, but HSP27-3D significantly increased p-IκB-α level compared with pcDNA (Fig. 3D). Taken together, above results strongly suggested phosphorylation(Ser78) could enhance TNF-α-induced increase of p-IκB-α and degradation of IκB-α, but could not affect the level of p-IKKα/β. JNK, as well as p38 might play its role in degradation of IκB-α through phosphorylating HSP27.

3.4. Phospho-HSP27 interacts with IκB-α and IKKα/β and promotes the binding of IKKα/β with IκB-α

It has been reported that the ability of HSP27 binding with other proteins depends on its phosphorylation levels [15]. Above data indicated that suppression of HSP27 phosphorylation reduced TNF-α-induced IκB-α phosphorylation significantly (Fig. 3C), but did not affect IKK phosphorylation. We thus observed the interaction between phospho-HSP27, IκB-α and IKK. As shown in Fig. 4A and B, TNF-α stimulation increased the amount of complex between HSP27 and IκB-α, and SP600125 treatment resulted in the reduction of the binding between HSP27 and IκB-α (Fig. 4A), suggesting that JNK could increase TNF-α-stimulated HSP27 and IκB-α association. We further designed a secondary immune precipitated innovative approach to detect accurately whether the phospho-HSP27 bound with IκB-α. We used the p-HSP27 (Ser78) monoclonal antibody to immunoprecipitate IκB-α in cell lysates and then used the HSP27 monoclonal antibody to immunoprecipitate IκB-α in supernatant of the first immunoprecipitation. As shown in Fig. 4C, TNF-α stimulation induced the complex formation between phosphorylated HSP27 and IκB-α, and SP600125 attenuated the amount of the complex. It is interested that, in supernatant of the first immunoprecipitation, no complex of

HSP27 and IκB-α was detected (Fig. 4D). These data indicated that phosphorylated HSP27 but not unphosphorylated HSP27 interacted with IκB-α.

As above results suggested that phosphorylation of HSP27 did not affect IKK activation, we further investigated whether Phospho-HSP27 regulated the binding ability of IKK with IκB-α. Co-immunoprecipitation showed that phospho-HSP27 interacted with IκB-α and IKKα/β simultaneously in HepG2 cells after 15 min stimulation of TNF-α and treatment of SP600125 resulted in the reduction of the complex Phospho-HSP27 with IKK and IκB-α (Fig. 4E) suggesting that JNK promoted the integration of IKK with IκB-α through inducing HSP27 phosphorylation.

4. Discussion

In the present study, we explored the effects of JNK TNF-α-induced apoptosis in HepG2 cells and found that suppression of JNK activity potentiated TNF-α-induced apoptosis. Mechanismly, JNK induced HSP27 phosphorylation and then regulated NF-κB pathway. Our investigation indicated the crosstalk between JNK and NF-κB pathways and the bridge linkage role of phosphor-HSP27 in this crosstalk.

It has been documented that HSP27 can be phosphorylated by MK-2 and AKT [10], and is also a substrate of PKCα, PKCδ and cAMP-dependent kinase [11]. In the present study, we found that JNK was also involved in phosphorylation of HSP27. Interestingly, we noticed that JNK mainly phosphorylated Ser78 of HSP27 but not Ser 15 and Ser 82. It has been reported that Ser78 of HSP27 is phosphorylated by a number of kinases including MK2/3/5, PKA, PKG [16]. In our study, JNK inhibitor SP600125 significantly inhibited Ser78 phosphorylation in HSP27, but the direct interaction between JNK and HSP27 was not detected (data not shown). Thus we speculate that JNK maybe regulate phosphorylation of HSP27 through intermediating above HSP27 upstream kinases.

Accumulating evidence has indicated that JNK works as a regulator of proapoptotic death signaling events [17]. For instance, JNK-specific inhibitors attenuate the apoptosis of hepatocytes and sinusoidal endothelial cells during hepatic I/R injury [18]. However, there was still some reports suggested that JNK promotes survival under adverse conditions [19]. It has been shown that the sustained activation of JNK was related with apoptosis, whereas the acute and transient activation of JNK was involved in cell proliferative or survival pathway [20,21]. In the present study, we found that suppression of JNK activation enhanced TNF-α-induced apoptosis. We further found that JNK acted as an upstream kinase of HSP27 phosphorylation and inhibition of HSP27 phosphorylation obviously enhanced TNF-α-induced apoptosis in HepG2 cells. Thus our study demonstrated that phosphorylating HSP27 is a novel mechanism by which JNK protected cells against TNF-α-induced apoptosis.

The crosstalk of intracellular signal pathways is extremely complex, which is the reaction of a variety of cellular signal pathways. Since both NF-κB and JNK share several common upstream signaling molecules, there are several levels of crosstalk between them [22], but the mechanism of their crosstalk is still unclear. It has been reported that LPS and extracellular nucleotides lead to the coordinate control of Ras/MEK/ERK and NF-κB signaling pathways in Raw 264.7 cells, suggesting the possibility of crosstalk between these two pathways [23]. In the present study, we found that inhibiting JNK activation decreased TNF-α-stimulated the association between phosphorylated HSP27 and IκB-α. It is well known IκB-α protein was phosphorylated by IKK complex upon TNF-α stimulation. In our observation, phosphorylated HSP27 regulated IκB-α phosphorylation and degradation, but did not affect the activation of IKK. Further co-immunoprecipitation experiments suggested that phosphorylated HSP27 could form a complex with IKK and IκB, and promoted the combination of IKK and IκB under TNF-α stimulation. Thus, these data indicated that JNK intereacted with NF-κB through phosphorylating HSP27 in HepG2 cells to regulate TNF-α-induced apoptosis.

In conclusion, our work demonstrates that TNF-α stimulation leads to JNK activation and in turn phosphorylates HSP27. Phosphorylated HSP27 then binds to IKK and facilitates the activation and degradation of IκB. These findings strongly suggest a crosstalk between JNK and NF-κB pathways in TNF-α-induced apoptosis in HepG2 cells. And phosphorylated HSP27 is the mediator conjugated these two pathways.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Nos. 81072433, 81172798 and 31071000), Jiangsu Major Nature Science Foundation of High Education (No. 12KJA180006), Research Fund for the Doctoral Program of Higher Education of China (20123207120012), the Priority Academic Program Development of Jiangsu Higher Education Institutions (No. 164320H106), the Jiangsu Provincial Nature Science Foundation (No. BK2012452), and the Natural Science Research Project of Anhui Provincial Education Department (No. KJ2013B311).

References

[1] A. Lin, B. Dibling, The true face of JNK activation in apoptosis, Aging Cell 1 (2002) 112–116.
[2] A.S. Ghosh, B. Wang, C.D. Pozniak, M. Chen, R.J. Watts, J.W. Lewcock, DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity, J. Cell Biol. 194 (2011) 751–764.
[3] J. Liu, A. Lin, Role of JNK activation in apoptosis: a double-edged sword, Cell Res. 15 (2005) 36–42.
[4] H.M. Beere, Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways, J. Clin. Invest. 115 (2005) 2633–2639.
[5] E.V. Mymrikov, A.S. Seit-Nebi, N.B. Gusev, Large potentials of small heat shock proteins, Physiol. Rev. 91 (2011) 1123–1159.
[6] Y. Xu, Y. Diao, S. Qi, X. Pan, Q. Wang, Y. Xin, X. Cao, J. Ruan, Z. Zhao, L. Luo, C. Liu, Z. Yin, Phosphorylated Hsp27 activates ATM-dependent p53 signaling and mediates the resistance of MCF-7 cells to doxorubicin-induced apoptosis, Cell. Signal. 25 (2013) 1176–1185.
[7] J. Landry, H. Lambert, M. Zhou, J.N. Lavoie, E. Hickey, L.A. Weber, C.W. Anderson, Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II, J. Biol. Chem. 267 (1992) 794–803.
[8] Y. Wu, J. Liu, Z. Zhang, H. Huang, J. Shen, S. Zhang, Y. Jiang, L. Luo, Z. Yin, HSP27 regulates IL-1 stimulated IKK activation through interacting with TRAF6 and affecting its ubiquitination, Cell. Signal. 21 (2009) 143–150.
[9] J.N. Lavoie, H. Lambert, E. Hickey, L.A. Weber, J. Landry, Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27, Mol. Cell. Biol. 15 (1995) 505–516.
[10] M.J. Rane, Y. Pan, S. Singh, D.W. Powell, R. Wu, T. Cummins, Q. Chen, K.R. McLeish, J.B. Klein, Heat shock protein 27 controls apoptosis by regulating Akt activation, J. Biol. Chem. 278 (2003) 27828–27835.
[11] M. Meier, G.L. King, A. Clermont, A. Perez, M. Hayashi, E.P. Feener, Angiotensin AT(1) receptor stimulates heat shock protein 27 phosphorylation in vitro and in vivo, Hypertension 38 (2001) 1260–1265.
[12] K.J. Park, R.B. Gaynor, Y.T. Kwak, Heat shock protein 27 association with the I kappa B kinase complex regulates tumor necrosis factor alpha-induced NF-kappa B activation, J. Biol. Chem. 278 (2003) 35272–35278.
[13] V. Adler, Z. Yin, S.Y. Fuchs, M. Benezra, L. Rosario, K.D. Tew, M.R. Pincus, M. Sardana, C.J. Henderson, C.R. Wolf, R.J. Davis, Z. Ronai, Regulation of JNK signaling by GSTp, EMBO J. 18 (1999) 1321–1334.
[14] J. Rouse, P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, A.R. Nebreda, A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins, Cell 78 (1994) 1027–1037.
[15] L. Rojanathammanee, E.B. Harmon, L.A. Grisanti, P. Govitrapong, M. Ebadi, B.D. Grove, M. Miyagi, J.E. Porter, The 27-kDa heat shock protein confers cytoprotective effects through a beta 2-adrenergic receptor agonist-initiated complex with beta-arrestin, Mol. Pharmacol. 75 (2009) 855–865.
[16] M. Haslbeck, T. Franzmann, D. Weinfurtner, J. Buchner, Some like it hot: the structure and function of small heat-shock proteins, Nat. Struct. Mol. Biol. 12 (2005) 842–846.

[17] C. Tournier, P. Hess, D.D. Yang, J. Xu, T.K. Turner, A. Nimnual, D. Bar-Sagi, S.N. Jones, R.A. Flavell, R.J. Davis, Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway, Science 288 (2000) 870–874.
[18] T. Uehara, B. Bennett, S.T. Sakata, Y. Satoh, G.K. Bilter, J.K. Westwick, JNK mediates hepatic ischemia reperfusion injury, J. Hepatol. 46 (2005) 850–859.
[19] C.J. Dougherty, L.A. Kubasiak, H. Prentice, P. Andreka, N.H. Bishorpic, K.A. Webster, Activation of c-Jun N-terminal kinase promotes survival of cardiac myocytes after oxidative stress, J. Biol. Chem. 362 (2002) 561–571.
[20] I. Sanchez-Perez, J.R. Murguıa, R. Perona, Cisplatin induces a persistent activation of JNK that is related to cell death, Oncogene 16 (1998) 533–540.
[21] Y.R. Chen, T.H. Tan, The c-Jun N-terminal kinase pathway and apoptotic signaling (review), Int. J. Oncol. 16 (2000) 651–662.
[22] Y. Zhang, F. Chen, Reactive oxygen species (ROS), troublemakers between nuclear factor-kappaB (NF-kappaB) and c-Jun NH(2)-terminal kinase (JNK), Cancer Res. 64 (2004) 1902–1905.
[23] M. Aga, J.J. Watters, Z.A. Pfeiffer, G.J. Wiepz, J.A. Sommer, P.J. Bertics, Evidence for nucleotide receptor modulation of cross talk between MAP kinase and NF-kappa B signaling pathways in murine RAW 264.7 macrophages, Am. J. Physiol. Cell Physiol. 286 (2004) C923–C930.