Endoplasmic Reticulum Stress-Mediated p62 Downregulation Inhibits Apoptosis via c-Jun Upregulation

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Abstract
Cereblon (CRBN), a substrate receptor of cullin 4-RING E3 ligase (CRL4) regulates the ubiquitination and degradation of c-Jun, mediating the lipopolysaccharide-induced cellular response. However, the upstream signaling pathway that regulates this process is unknown. In this study, we describe how endoplasmic reticulum (ER) stress reversely regulates sequestosome-1 (p62) and c-Jun protein levels. Furthermore, our study reveals that expression of p62 attenuates c-Jun protein levels through the ubiquitin-proteasome system. Conversely, siRNA knockdown of p62 elevates c-Jun protein levels. Immunoprecipitation and immunoblotting experiments demonstrate that p62 interacts with c-Jun and CRBN to form a ternary protein complex. Moreover, we find that CRBN knockdown completely abolishes the inhibitory effect of p62 on c-Jun. Using brefeldin A as an inducer of ER stress, we demonstrate that the p62/c-Jun axis participates in the regulation of ER stress-induced apoptosis, and that CRBN is required for this regulation. In summary, we have identified an upstream signaling pathway, which regulates p62-mediated c-Jun degradation. Our findings elucidate the underlying molecular mechanism by which p62/c-Jun axis regulates the ER stress-induced apoptosis, and provide a new molecular connection between ER stress and apoptosis.

Key Words: p62, c-Jun, CRBN, Endoplasmic reticulum stress, Apoptosis, Ubiquitination

INTRODUCTION
The ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP) are two major cellular pathways responsible for protein degradation (Ciechanover, 2005; Rubinsztein, 2006; Dikic, 2017). In the UPS, proteins are first modified by ubiquitin to form specific types of ubiquitinated proteins that are then targeted for degradation by the 26S proteasome (Grice and Nathan, 2016). In the ALP, autophagic substrates including soluble proteins, protein complexes and aggregates, are engulfed by a double-layered membrane to form autophagosomes, which are further directed to lysosomes for degradation by lysosomal hydrolases (Korolchuk et al., 2010). UPS inhibitors lead to the activation of ALP (Zhu et al., 2016; Wang et al., 2019) and ALP deficiency attenuates the UPS (Korolchuk et al., 2010). Therefore, there is crosstalk and interplay between these two pathways (Jian et al., 2017), and the autophagy receptor, sequestosome 1 (p62), plays important roles in both processes (Liu et al., 2016). On the one hand, p62 is a substrate of autophagy and can be degraded by the ALP by binding to microtubule-associated protein 1A/1B-light chain 3 (LC3) using its LC3-interacting region (Pankiv et al., 2007; Shvets et al., 2008). It can also be ubiquitinated and degraded through the UPS; a process that is regulated by E3 ligases including Parkin (Song et al., 2016) and X-linked inhibitor of apoptosis protein (Huang et al., 2018). On the other hand, p62 functions as a scaffold protein through its ubiquitin-associated (UBA) domain, and mediates the ubiquitination of proteins including NF-κB essential modulator (NEMO) in the presence of E3 ubiquitin ligase TRAF6 (Zotti et al., 2014). p62 is also responsible for shuttling the ubiquitinated proteins to the proteasome for degradation (Geetha et al., 2008), p62 may also inhibit the ubiquitination of proteins including NF-E2-related factor 2 (Nrf2) through binding to the E3 ligase complex component Keap1, thereby activating Nrf2 (Komatsu et al., 2010). In addition, p62 promotes the aggregation of ubiquitinated substrates for autophagic degradation (Pankiv et al., 2007; Zaffagnini et al., 2018).

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For example, in neurodegenerative diseases, p62 promotes the aggregation of misfolded proteins, including pathogenic ataxin-3 with expanded polyglutamine in Machado-Joseph disease (Zhou et al., 2014) and a mutant form of superoxide dismutase 1 (SOD1) in amyotrophic lateral sclerosis (Gal et al., 2007). Collectively, these studies indicate that p62 may regulate protein degradation by modulating E3 ligase-associated UPS and autophagy-associated ALP (Lippai and Lőw, 2014; Shin et al., 2020). These studies demonstrated the versatile roles played by p62 in the regulation of protein degradation in the UPS and ALP.

The UPS and ALP can regulate cell death and survival through the degradation of different substrates. However, the regulation of cell death and survival by p62 is somewhat controversial (Jin et al., 2009; Jain et al., 2010). It has been reported that p62 protects oxidative stress-induced cell death via the translocation of the transcription factor Nrf2 to the nucleus, thereby inducing the expression of its cytoprotective targets in a neuroblastoma cell line IMR-32 (Liu et al., 2007) and primary mouse hepatocytes (Ichimura et al., 2013). In contrast, p62 promotes HAMLET (a complex of oleic acids and decalcified α-lactalbumin)-induced apoptosis by activating caspase-8 in a glioma cell line U87MG (Zhang et al., 2013). These studies indicate that p62 regulates cell death and cell survival by modulating different signaling pathways.

Activator protein-1 (AP-1) transcription factor complex (Halazonetis et al., 1988; Kouzarides and Ziff, 1988) responds to a variety of cellular signals, including inflammatory stimulation (Mackman et al., 1991) and endoplasmic reticulum (ER) stress (Fuest et al., 2012), and participates in the regulation of cell death (Shaulian and Karin, 2002; Meng and Xia, 2011). It is understood that the major subunit of the AP-1 transcription factor complex, the proto-oncogene c-Jun, is responsible for its role in the regulation of cell death. c-Jun protects cells from ER stress-induced death by upregulating Down syndrome critical region 1 (DSCR1) (Zhao et al., 2008) or by downregulating tumor suppressor PTEN (Hettinger et al., 2006). It has also been reported that c-Jun promotes apoptotic cell death in NIH 3T3 fibroblasts (Bossy-Wetzel et al., 1997) and CRBN-mediated ubiquitination and degradation of c-Jun protects THP-1 cells from apoptosis induced by lipopolysaccharide (Yang et al., 2018), although the upstream signaling pathway triggering this regulation is unknown. Our previous study indicated that CRBN interacts with p62 and protects cells from death induced by pathogenic protein aggregates through its competitive binding to ubiquitinated proteins (Zhou et al., 2018). CRBN exhibits the non-enzymatic function in this regulation, while at the same time acting as a substrate receptor of the E3 ligase CRBN using immunoprecipitation and immunoblotting techniques. We further investigated the regulation of p62 on c-Jun protein levels, and the role of CRBN in this regulation. Finally, we comment on the potential function of CRBN in ER stress-induced apoptosis and the underlying molecular mechanism.

**MATERIALS AND METHODS**

**Materials**

The primary antibodies used in this work were obtained from the following sources: anti-HA (sc-7392) and anti-ubiquitin (sc-8017) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-c-Jun (CPA1634) antibody was from Cohesion Biosciences (London, UK); anti-FLAG (0912-1) and anti-GFP (EM30501) antibodies were from HuaAn Biotechnolgy (Hangzhou, China); mouse anti-CRBN antibody was a gift from Dr. Xiu-Bao Chang (Mayo Clinic College of Medicine, Scottsdale, AZ, USA); rabbit anti-CRBN antibody (11435-1-AP) and anti-GAPDH (60004-1) antibody were from ProteinTech Group (Rosemont, IL, USA); anti-cleaved caspase 3 (9661S) and anti-PARP1 (9532S) antibodies were from Cell Signaling Technology (Danvers, MA, USA); anti-p62 (P0067) antibody was from Sigma (Saint Louis, MO, USA); anti-LC3 (NB100-2220) antibody was from Novus Biologicals (Centennial, CO, USA); anti-FLAG affinity gel (B23102) and anti-HA magnetic beads (B26301) were from Bimake (Houston,TX, USA); rabbit IgG (A7016) for control immunoprecipitation was from Beyotime Biotechnology (Haimen, Jiangsu, China); and HRP-labeled secondary antibodies were from Beyotime Biotechnology and Thermo Fisher (Waltham, MA, USA).

Chemicals were from the following companies: MG132 (CC2775) was from ChemCatch (Suwanee, GA, USA); brefeldin A (s7046) was from Selleck (Houston, TX, USA); puromycin (P8230) was from Solarbio (Beijing, China); Hoechst (C1022) was from Beyotime Biotechnology; and propidium iodide (PI, KGA214-50) was from Nanjing KeyGen BioTech (Nanjing, Jiangsu, China).

Strep-FLAG (SF)-c-Jun, FLAG-p62, HA-CRBN, and GFP-p62 plasmids were from our previous work (Yang et al., 2018; Zhou et al., 2018). The control siRNAs (ID: 35164) and sip62 were synthesized by GenePharma (Shanghai, China) or RiboBio Co (Guangzhou, Guangdong, China). The siRNA sequences for human sip62 are sense #1: CAUGUGCUUAC GUGAGGAUGAT, antisense #1: UCAUCCUUACAGU GACAUUUG; sense #2: GCAUGUAAUGUUAUCGUUAGT; antisense #2: AUCGAUAUCAACUUCAUUGCTT.

**Cell culture**

Human embryonic kidney (HEK) 293T and HeLa cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (PAN Biotech, Aidenbach, Germany and Lonsera, Ciudad de la Costa, Uruguay) and 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco, Waltham, MA, USA). Cells were passaged every two or three days.

**Transfection**

Plasmids were transfected with lipofectamine 3000 (Life Technologies, Carlsbad, CA, USA) or polyethyleneimine (PEI, Sigma) transfection reagent and siRNAs were transfected with lipofectamine 2000 transfection reagent (Life Technologies) or riboFECT™ CP reagent (RiboBio Co) according to the manufacturer’s instructions. Culture medium was changed 6 h following transfection for subsequent experiments.

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Construction of plasmids and stable cell lines

The 21-nucleotide sequence for shRNA targeting human CRBN was CCCAGACACTGAGGATGAAAT and the corresponding sequence was inserted into the pLKO.1-TRC lentiviral vector to construct the pLKO.1-shCRBN plasmid. In order to produce lentiviral particles, pLKO.1-TRC or pLKO.1-shCRBN plasmid was transfected into HEK293T cells together with the packaging plasmids psPAX2 and pMD2G at a ratio of 3:2:1. At 48 and 72 h following transfection, the culture medium containing the lentiviral particles was collected and filtered by a 0.45 μm filter (Merck Millipore, Billerica, MA, USA). The lentiviral particles were then used to infect HEK293T cells in a six-well plate. In order to obtain stable transduced cell lines, the infected cells were selected using puromycin (2 μg/mL) for 2 weeks.

Preparation of cell lysate

Following transfection or drug treatment, cells were lysed in modified RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1 mM EDTA) on ice with brief sonication. Cell lysates were obtained after centrifugation (13,000 g) for 15 min at 4°C.

Immunoprecipitation

Flag-tagged proteins were purified with anti-Flag affinity gel according to a previously reported method (Zhu et al., 2018). The affinity gel was first washed three times with TBST (TBS with 0.1% Tween 20) and incubated with cell lysates at 4°C overnight. The gel was centrifuged at 500 g for 1 min, washed three times with the modified RIPA buffer and three times with RIPA buffer containing 0.3 M NaCl. SF-c-Jun and its interacting proteins were eluted twice with RIPA buffer containing 200 μg/mL Flag peptide (DYKDDDDK, ChinaPeptides, Shanghai, China). The eluate was combined and used for Western blotting analyses.

HA-tagged CRBN was purified with anti-HA magnetic beads according to a method described previously (Zhu et al., 2018). Briefly, anti-HA magnetic beads were prewashed three times with TBS. Beads were further blocked with 5% BSA in TBS and then incubated with cell lysates at 4°C overnight. The magnetic beads were separated from cell lysates using a magnetic separation stand, and then washed ten times with the modified RIPA buffer containing 0.6 M NaCl to remove the nonspecific binding proteins. Finally, the beads were boiled twice with 50 μL SDS sample loading buffer and the eluate was combined for immunoblotting analysis.

Immunoprecipitation of endogenous c-Jun was carried out using a procedure described previously (Yang et al., 2018). Briefly, cell lysates were pre-incubated with protein A/G agarose beads to remove nonspecific binding proteins, incubated with 1 μg of control IgG or c-Jun antibody at 4°C overnight, and further incubated with prewashed protein A/G agarose beads (40 μL) at 4°C for 5 h. The protein A/G agarose beads were washed four times with PBS and proteins were eluted by heating with 60 μL of 2× SDS sample loading buffer.

Western blotting

Western blotting analysis was carried out based on a previously used method (Yang et al., 2018). Briefly, cell lysates or affinity-purified samples were separated by SDS-PAGE electrophoresis and proteins were transferred to PVDF membranes. Membranes were further blocked with 5% non-fat milk in TBST at room temperature for 1 h, incubated with the indicated primary antibodies at 4°C for overnight, and washed three times with TBST at room temperature (each for 10 min). After the washing steps, membranes were incubated with secondary antibodies at room temperature for 1 h, and washed three times with TBST at room temperature (each for 10 min). Proteins were visualized on membranes using Western blotting chemiluminescent horseradish peroxidase substrate (NCM Biotech, Suzhou, China), and signals were recorded using a Tanon 5200 imaging system (Tanon, Shanghai, China).

Propidium iodide (PI) staining

PI staining was described in a previous study (Hou et al., 2015). Briefly, HEK293T cells stably expressing shNC or shCRBN in 24-well plates were transfected with FLAG or FLAG-p62 plasmids for 24 h. Cells were stained with PI for 5 min, and then washed once with PBS. Cells were further stained with Hoechst for 7 min, and images were captured using an inverted microscope (IX71, Olympus, Tokyo, Japan).

TdT-mediated dUTP Nick-End Labeling (TUNEL) staining

The colorimetric TUNEL apoptosis assay kit was purchased from Beyotime Biotechnology. HEK293T cells stably expressing shNC or shCRBN were used to perform the TUNEL assay according to the manufacturer’s instructions. The cells were washed once with PBS, fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.3% Triton X-100 for 5 min, washed three times with PBS, and then stained with the TUNEL solution at room temperature for 1 h. The cells were further incubated with Hoechst for 5 min to visualize the cellular nuclei. The images were captured using an inverted microscope (IX71, Olympus).

Statistics

Statistical analysis was performed using two-tailed Student’s t-test for the number of biological replicates indicated in the figure legends. *p<0.05; **p<0.01; ***p<0.001.

RESULTS

Autophagic degradation of p62 leads to c-Jun upregulation

Activation of autophagy may result in a decrease of p62 protein levels (Wu et al., 2018). Since c-Jun responds to a variety of cellular signals, we examined whether c-Jun is affected by the activation of autophagy. Consistent with previous studies (Ding et al., 2007; Lin et al., 2017), our results showed an increase in LC3II and the LC3II/LC3I ratio upon brefeldin A and cisplatin treatment, indicating the activation of autophagy in HEK293T and HeLa cells (Fig. 1). Furthermore, we found that c-Jun protein levels were increased after cells were treated with brefeldin A, HBSS, and cisplatin (Fig. 1A, Supplementary Fig. 1). These results suggest that p62 may participate in the regulation of c-Jun in ER stress-induced autophagy.

p62 downregulates c-Jun protein levels through the UPS

It has been reported that p62 may regulate the cellular degradation pathway (Pankiv et al., 2007; Geetha et al., 2008; Zotti et al., 2014; Zaffagnini et al., 2018). Since ER stress-induced autophagy reversely regulates p62 and c-Jun (Fig. 1), we investigated the possible regulation of c-Jun protein levels by p62. Ectopic expression of p62 in HEK293T cells resulted...
in a decrease in c-Jun protein levels (Fig. 2A), while siRNA knockdown of p62 increased c-Jun protein levels (Fig. 2B). These experiments demonstrate that p62 may indeed regulate c-Jun protein levels. Given the crosstalk between the ALP and the UPS, the fact that blockade of the UPS activates the ALP (Wang and Wang, 2015), and deficiency in the ALP leads to the accumulation of p62 (which in turn binds to ubiquitinated proteins, delaying their degradation by the UPS) (Korolchuk et al., 2009), we examined the degradation of c-Jun following overexpression of p62. Since the UPS may degrade c-Jun, we considered the UPS. Therefore, we transfected FLAG or FLAG-p62 plasmids into HEK293T cells and then divided the transfected cells into two plates for further treatment with DMSO, or with the proteasome inhibitor MG132. The data clearly show that MG132 inhibits the reduction in c-Jun protein following p62 expression (Fig. 2C). This suggests that p62 downregulates c-Jun protein levels, most probably through the UPS. Furthermore, we found that p62 could promote the ubiquitination of c-Jun (Fig. 2D). Collectively, these data demonstrate that p62 may promote the ubiquitination and degradation of c-Jun.

p62 interacts with c-Jun

In our previous work, we discovered that CRBN interacts with p62 (also shown in Supplementary Fig. 2) and prevents the formation of pathogenic protein aggregates in cell lines and primary neuronal cells (Zhou et al., 2018). We have also demonstrated that CRBN interacts with c-Jun and reduces c-Jun protein levels through the UPS, leading to the attenuation of the transcription activity of the associated transcription factor complex AP-1 and suppression of the inflammatory response (Yang et al., 2018). Since p62 downregulates c-Jun (Fig. 2), we sought to examine whether p62 also interacts with c-Jun. First, we co-expressed Strep-FLAG-(SF)-c-Jun with GFP or GFP-p62 in HEK293T cells and immunoprecipitated SF-c-Jun and its interacting proteins. Immunoblotting images unambiguously indicated the presence of p62 in the c-Jun immunoprecipitate (Fig. 3A). We also expressed FLAG or FLAG-p62 in HEK293T cells and immunoprecipitated FLAG-p62 and its interacting proteins. The results demonstrate that FLAG-p62 binds to c-Jun (Fig. 3B). We further used an anti-c-Jun antibody to immunoprecipitate the endogenous c-Jun for immunoblotting analysis, and confirmed the endogenous interaction between p62 and c-Jun (Fig. 3C).

p62, CRBN, and c-Jun form a ternary protein complex

Our previous work (Yang et al., 2018) and the data described above indicates that c-Jun interacts with both CRBN and p62. We then asked whether p62, CRBN, and c-Jun form a ternary complex. Therefore, we co-expressed GFP-p62 with HA-CRBN and SF-c-Jun in HEK293T cells and performed two rounds of immunoprecipitation. We slightly adjusted the amount of transfected plasmids to obtain similar levels of protein expression in these samples (Fig. 4A, left panel). In the first round of immunoprecipitation, we purified SF-c-Jun with anti-FLAG affinity gel. Immunoblotting of the purified samples shows that both CRBN and p62 are detected in the c-Jun immunoprecipitate but not in the mock immunoprecipitate (Fig.
p62/c-Jun axis regulates ER stress-induced apoptosis

The experiments described above indicate that p62 may participate in the regulation of c-Jun under ER stress. Since c-Jun protected cells against ER stress-induced apoptosis (Supplementary Fig. 3), we examined the role of p62, CRBN, and c-Jun in ER stress. We first constructed the stable control or CRBN knockdown HEK293T cell lines using shRNA. We then used these cell lines to express FLAG or FLAG-p62 to investigate the effect of p62 on apoptosis under ER stress. Using brefeldin A as an ER stress inducer (Moon et al., 2012; Zhu et al., 2017), we found that overexpression of p62 reduced PARP1 protein levels and increased cleaved caspase-3 protein levels, indicating the role of p62 in brefeldin A-induced apoptosis activation in HEK293T cells. Furthermore, CRBN knockdown eliminated this regulation, indicating the essential role of CRBN in this process (Fig. 5A). We also used prop-
Conjugated primary antibody was added to prevent the degradation of c-Jun upon p62 expression, which resulted in approximately equal c-Jun protein levels in two cell lysates. p62 interacts with c-Jun. (A) HEK293T cells were transfected with GFP-p62 and pcDNA3.1 or Strep-FLAG (SF)-c-Jun plasmids using PEI transfection reagent. At 36 h post-transfection, cells were treated with MG132 (10 μM) for 12 h and lysed in RIPA buffer. SF-c-Jun was purified with anti-FLAG affinity gel. Cell lysates and purified samples were immunoblotted using the antibodies indicated. (B) HEK293T cells were transfected with FLAG or FLAG-p62 for 48 h. The resulting cell lysates were subjected to anti-FLAG immunoprecipitation. The immunoprecipitates and cell lysates were subjected to immunoblot with the antibodies indicated. (C) Untransfected-HEK293T cells were treated with MG132 (10 μM) for 12 h and lysed in RIPA buffer. IgG or anti-c-Jun antibodies were used for immunoprecipitation of endogenous proteins. Endogenous proteins in cell lysates and the immunoprecipitates were immunoblotted with the antibodies indicated.

Fig. 4. c-Jun, p62, and CRBN form a ternary complex and CRBN is required for p62-mediated c-Jun degradation. (A) HEK293T cells were first transfected with GFP-p62 and HA-CRBN, GFP-p62 and SF-c-Jun, or GFP-p62, HA-CRBN, and SF-c-Jun plasmids using PEI transfection reagent. The amount of plasmids was slightly adjusted to express proteins at similar levels in different samples and pcDNA3.1 was used to balance the total amount of plasmids used. At 36 h post-transfection, cells were treated with MG132 (10 μM) for 12 h and lysed. In the first round of immunoprecipitation, SF-c-Jun and its interacting proteins were purified with anti-FLAG affinity gel and eluted with FLAG peptides. The eluate was further purified with HA magnetic beads in the second round of immunoprecipitation. The cell lysates and the first and second round affinity-purified samples were immunoblotted with the antibodies indicated. (B) HEK293T cells stably expressing shNC or shCRBN were transiently transfected with FLAG or FLAG-p62 for 48 h. The cell lysates were subjected to immunoblotting with the antibodies indicated. The CRBN antibody was used to examine the knockdown efficiency.

demonstrate that the p62/c-Jun axis participates in the regulation of ER stress-induced apoptosis (Fig. 6).
DISCUSSION

Activation of autophagy causes the autophagic degradation of p62, leading to reduced apoptosis (Levine and Kroemer, 2008; Lamark et al., 2009). The molecular mechanisms underlying autophagy-regulated apoptosis are largely unknown (Tang et al., 2019; Tilija Pun et al., 2020). In the present study, we found that activation of autophagy leads to the downregulation of p62 and upregulation of c-Jun (Fig. 1, 4), and that p62 promotes the proteasomal degradation of c-Jun in a CRBN-dependent manner (Fig. 2, 4). This suggests crosstalk between the UPS and ALP cellular degradation pathways (Hewitt et al., 2016; Bustamante et al., 2018). Indeed, it has been shown that p62 functions as a scaffolding protein and binds to ubiquitinated proteins through its UBA domain (Seibenhener et al., 2004) and LC3 through its LC3-interacting region (Pankiv et al., 2007).

In this study, we confirmed that p62-mediated apoptosis under brefeldin treatment is due to the proteasomal degradation of c-Jun, which may occur following the recruitment of the substrate receptor CRBN of the CRL4 E3 ligase leading to the ubiquitination of c-Jun and its degradation. This finding is consistent with a previous study which found that autophagic degradation of p62 protected cells from ER-stress induced apoptosis (Ogata et al., 2006). Furthermore, we identified a novel molecular mechanism by which p62 upregulates c-Jun, and thereby protects cells from apoptosis. The mitochondrial pathway may be activated upon induction of ER stress, which causes the release of cytochrome c, and eventually activates the executioner caspases including caspase-3, leading to PARP1 cleavage (Iuruaro and Muñoz-Pinedo, 2016). ER stress also activates the extrinsic or death receptor pathway, which may activate caspase-8, and thus caspase-3 (Yamaguchi and Wang, 2004; Sano and Reed, 2013). Since the activation of caspase-3 is important in ER stress-induced apoptosis (Masud et al., 2007), we confirmed caspase-3-associated apoptosis in the p62/c-Jun axis (Fig. 5, 6). However, it has also been reported that p62 could enhance bortezomib resistance in multiple myeloma cells, and then promote their survival (Milan et al., 2015; Marino et al., 2017), indicating that p62 may regulate survival or death in different cell lines.

Our previous studies showed that CRBN suppresses the formation of p62 bodies (Zhou et al., 2018) and promotes the ubiquitination and proteasomal degradation of c-Jun (Yang et
In the present study, we found that p62, c-Jun, and CRBN form a ternary protein complex (Fig. 3), suggesting that p62 promotes the ubiquitination and degradation of c-Jun in the presence of CRBN. The present study also indicates that p62 may act as a scaffold protein to promote the function of the CRL4<sup>CRBN</sup> E3 ligase. Indeed, it has been reported that p62 may enhance the TRAF6-mediated ubiquitination of NEMO (Zotti <em>et al.</em>, 2014) and increase Parkin-induced mitochondrial clustering (Narendra <em>et al.</em>, 2010). This is in accordance with the discovery that the amyloid precursor protein may act as a substrate recognition unit of CRL-4<sup>CRBN</sup> E3 ligase to regulate the ubiquitination of cytosolic and membrane-bound proteins leading to their subsequent degradation (Del Prete <em>et al.</em>, 2016).

In summary, we have identified that p62 forms a ternary complex with c-Jun and CRBN and thus promotes the ubiquitin-mediated c-Jun degradation. Downregulation of p62 induced by ER stress exhibits the protective role against apoptosis by reducing the c-Jun degradation. This work revealed the essential role of CRBN in the regulation of c-Jun by p62 and elucidated a new molecular mechanism by which the p62/c-Jun axis regulates the ER stress-induced apoptosis.

**CONFLICT OF INTEREST**

None.

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