Received: 2 September 2019 | Revised: 9 January 2020 | Accepted: 10 January 2020

DOI: 10.1111/cas.14321

ORIGINAL ARTICLE

Cancer Science WILEY

p62 promotes bladder cancer cell growth by activating KEAP1/NRF2-dependent antioxidative response

Tao Li[1,2] | Dali Jiang[1] | Kaijie Wu[1]

[1]Department of Urology, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

[2]Department of Urology, The First Hospital of Xi'an, Xi'an, China

Correspondence
Kaijie Wu, Department of Urology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China.
Email: kaijie_wu@163.com

Abstract

p62 is associated with 2 major cellular defense mechanisms against metabolic and oxidative stress, autophagy and the Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor-E2-related factor 2 (NRF2) system. Recent studies indicate that the p62-KEAP1-NRF2 pathway promotes tumorigenesis and tumor growth mediated by NRF2-dependent antioxidative response. However, whether p62 is involved in bladder cancer (BCa) development remains unknown. Here, we found that p62 is overexpressed in BCa tissue and several BCa cell lines. The knockdown of p62 inhibits BCa cell growth both in vitro and in vivo, with increased intracellular reactive oxygen species level. Mechanically, p62 activates NRF2 signaling by sequestrating KEAP1, which leads to the upregulation of antioxidant genes (*Gclc*, *Gstm5*, and *Gpx2*), thus protecting BCa cells from oxidative stress. Our findings indicate that p62 might be involved in the development of BCa and serve as a potential therapeutic target.

KEYWORDS

bladder cancer, KEAP1, NRF2, oxidative stress, p62

1 | INTRODUCTION

Bladder cancer (BCa) is the most common urinary tract cancer, accounting for 549 000 new cases and 200 000 deaths worldwide in 2018.[1] In the past decade, the incidence and mortality of BCa in China have gradually increased.[2] Currently, the most common treatments for BCa are surgery, radiotherapy, and chemotherapy. Due to the characteristics of easy recurrence, invasion, and metastasis, there has been no technical advancement or innovation in the treatment of BCa. Therefore, an in-depth study of the molecular mechanisms of BCa and search for new drug therapeutic targets have become urgently needed.

p62, encoded by *Sqstm1*, is a multifunctional stress-inducible scaffold protein involved in diverse cellular processes.[3] p62 is associated with 2 major cellular defense mechanisms against metabolic and oxidative stress, autophagy and the Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor (NF)-E2-related factor 2 (NRF2) system. p62 serves as a selective autophagy adaptor and plays a key role in regulating the formation of protein aggregates. In addition, p62 activates the KEAP1-NRF2 system by sequestering KEAP1 through its KEAP1-interacting region (KIR) domain.[4,5] p62 is also linked to other cellular processes through its interaction with many intracellular signalings, including NF-κB[6] and mTOR.[7] Mounting evidence has indicated that p62 is associated with a wide range of diseases, including various cancers, such as liver,[8] lung,[9] breast,[10] pancreatic,[11] prostate,[12] and colon.[13] For example, p62 promotes hepatocellular carcinogenesis by protecting liver cancer-initiating cells from oxidative stress.[14] However, the role of p62 in BCa remains unknown.

Tao Li and Dali Jiang contributed equally to this work.

Nuclear factor-E2-related factor 2 is a master regulator of oxidative response. Under the basal condition, NRF2 is tightly controlled by KEAP1.[15] Intracellular oxidation prevents KEAP1-mediated proteasomal degradation of NRF2. Then NRF2 translocates into the nucleus and induces antioxidant-related genes, which help to protect cells from oxidative stress. Intriguingly, *Sqstm1* is a target gene of NRF2. *Sqstm1* protein, namely p62, interacts with KEAP1 and inhibits KEAP1-mediated NRF2 degradation, leading to the stabilization and activation of NRF2.[4] Accumulating evidence has established the NRF2 pathway as a driver of cancer progression, metastasis, and resistance to therapy.[16]

Here, we observed the overexpression of p62 in human BCa tissue and several BCa cell lines. We found that knockdown of p62 increased intracellular reactive oxygen species (ROS) levels and inhibited BCa cell growth. Mechanically, p62 activates the NRF2-dependent antioxidative pathway by sequestrating KEAP1, thus protecting BCa cells from oxidative stress. Our findings thus indicate that p62 might promote the development of BCa by regulating intracellular antioxidant response.

2 | MATERIALS AND METHODS

2.1 | Cell culture and transfection

T24, RT4, 5637, TCCSUP, 253J, SV-HUC-1, and SW780 cell lines were purchased from ATCC, and all BCa cells were cultured in DMEM or RPMI-1640 medium supplemented with 10% FBS. The single guide RNAs (sgRNAs) targeting p62 (sgp62) were constructed in this study. The upstream sequence of sgp62 was 5′-CACCGCATTGTCAATTCCTCGTCAC-3′, and the downstream sequence was: 5′-AAACGTGACGAGGAATTGACAATGC-3′ (sequence #1). The upstream sequence of sgp62 was 5′-CACCGAATGGCCATGTCCTACGTGA-3′,andthedownstreamsequencewas:5′-AAACTCACGTAGGACATGGCCATTC-3′ (sequence #2). Single guide RNA transfections were applied to silence the expression of p62 in BCa cells. p62 cDNA was cloned into the pcDNA3.1 vector. The sgRNAs or plasmids, Lipofectamine 2000 (Invitrogen) and FBS-free medium, were mixed according to the manufacturer's protocol for transfection. 293FT cells were applied for the packaging of lentivirus. Real-time PCR and western blot analyses were used to monitor transfection efficiency.

2.2 | Reagents and Abs

Cycloheximide (CHX) and bafilomycin A1 (Baf A1) were purchased from Sigma-Aldrich. Rabbit primary Abs against p62, B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), and KEAP1 were purchased from Cell Signaling Technology. Mouse primary Abs against Bcl-2 and β-actin were purchased from Cell Signaling Technology. The rabbit primary Abs against NRF2 and glutamate-cysteine ligase catalytic subunit (GCLC) were purchased from Abcam.

2.3 | Colony formation assay

After sgRNA infection and selection, the cells were plated in 6-well plates at a density of 1000 cells/well. After 10 days of culture, the cells were washed with PBS, fixed with 4% paraformaldehyde, and stained with crystal purple for 10 minutes. The stained colonies were then photographed and counted.

2.4 | Cell proliferation assay

The BCa cells were inoculated into 96-well plates at a cell density of 1×10^4 cells per well and incubated at 37°C, 5% CO_2 atmosphere overnight. Each well was added with MTT at 0, 24, 48, and 72 hours. After incubating for another 4 hours, DMSO was added to each well. The crystals were sufficiently dissolved by shaking for 10 minutes, and the absorbance was measured at a wavelength of 490 nm to calculate the cell survival rate.

2.5 | Western blot analysis

Bladder cancer cell protein extracts were prepared according to standard protocols. Cell lysates were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were incubated with specific primary Abs at 4°C overnight. The protein immunoreactive signals were tested by the ECL detection system (Thermo Fisher Scientific).

2.6 | Immunofluorescence

5637 Cells were plated on coverslips, fixed with 4% paraformaldehyde for 20 minutes, and permeabilized in PBS containing 0.3% Triton X-100 for 10 minutes. Cells were then incubated in the blocking buffer (5% BSA in PBS) at room temperature for 1 hour followed by staining with primary Abs (p62 and KEAP1). Coverslips were washed and incubated for 1 hour with the secondary Abs. Next, the cells were stained with DAPI and blocked with glycerol. Fluorescence microscopy was applied to detect the fluorescence of the cells.

2.7 | Immunohistochemistry

Tissue specimens were fixed with 4% paraformaldehyde and embedded with paraffin for further analysis. The sections were stained with the specific primary Abs as described above. Images of tumor slides were obtained on a Nanozoomer Slide Scanner (Hamamatsu). Bladder cancer tissues were obtained from patients undergoing resection at the First Affiliated Hospital of Xi'an Jiaotong University. Written informed consent was obtained from the patients, and the protocol was approved by the Ethical Review Board of Xi'an Jiaotong University.

2.8 | Quantitative RT-PCR

Total RNA was extracted using the RNeasy Kit (Qiagen) according to the manufacturer's protocol. Then, 1 μg total RNA was reverse transcribed into cDNA using a reverse transcription kit (Takara). The cDNA was used for quantitative PCR analysis on an iCycler iQ5 Real-Time PCR detection system (Bio Rad) following the manufacturer's instructions. The expression of the target gene was normalized to the expression of *Gapdh*. Relative gene expression was calculated using the standard $2^{-\Delta\Delta Ct}$ method. The following primer sequences are used: *Gclc* F, 5′-GGGGTGACGAGGTGGAGTA-3′; R, 5′-GTTGGGGTTTGTCCTCTCCC-3′; *Gstm5* F, 5′-TCATCCAAGTCTATGGTTCTGGG-3′; R, 5′-CCACAGATGTACCGTTTCTCCT-3′; *Gpx2* F, 5′-GCCTCAAGTATGTCCGACATG-3′; R, 5′-GGAGAACGGGTCATCATAAGGG-3′; and *Gapdh* F, 5′-GCTAAGCAGTTGGTGGTGCA-3′; R, 5′-TCACCACCATGGAGAAGGC-3′.

2.9 | Intracellular ROS and glutathione detection

Intracellular ROS were detected by 2′,7′-dichlorofluorescin diacetate (DCFDA; Applygen). Briefly, BCa cells were stained with 5 μmol/L DCFDA in an incubator at 37°C for 30 minutes then washed twice in PBS before FACS analysis. Intracellular glutathione (GSH) was measured with a colorimetric GSH detection kit (Solarbio). At least 10^6 cells were washed with ice-cold PBS and lysed in lysis buffer. After 2-3 freeze-thaw cycles in liquid nitrogen, the supernatant was collected for measurement of absorbance at 412 nm. The GSH standard curve was generated for determining the sample GSH concentrations.

2.10 | Bladder cancer xenografts

5637 Cells were stably infected with sgp62 and control (sgCon). Male Balb/c nude mice at the age of 5-6 weeks were purchased from the Laboratory Animal Center of Xi'an Jiaotong University. The sgp62 5637 or sgCon 5637 cells (1×10^6) were injected s.c. into the right flank. Tumor volumes were calculated using the formula $0.5 \times ab^2$ ("*a*" = length, "*b*" = width). All animal experiments were carried out according to the guidelines approved by the Animal Care and Use Committee of the Laboratory Animal Center of Xi'an Jiaotong University.

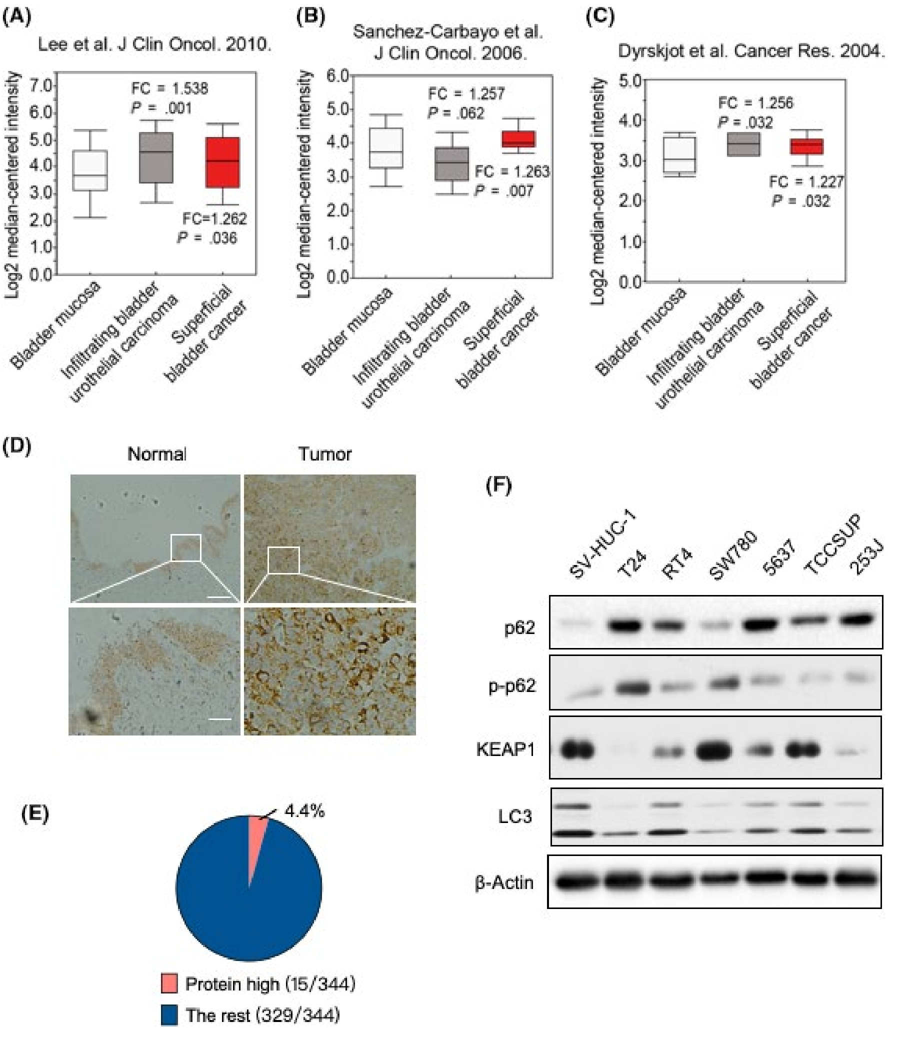

FIGURE 1 p62 is overexpressed in human bladder cancer (BCa) and cell lines. A-C, p62 expression in human BCa in 3 different studies. FC, fold change. D, Representative immunohistochemical images of p62 in normal and tumor tissue from patients with BCa. E, 4.4% (15/344) of BCa patients showed upregulated p62 protein level in The Cancer Genome Atlas (>2-fold). F, Expression of p62, phosphor-p62, Kelch-like ECH-associated protein 1 (KEAP1), and microtubule-associated protein 1A/1B-light chain 3 (LC3) in the indicated BCa cell lines

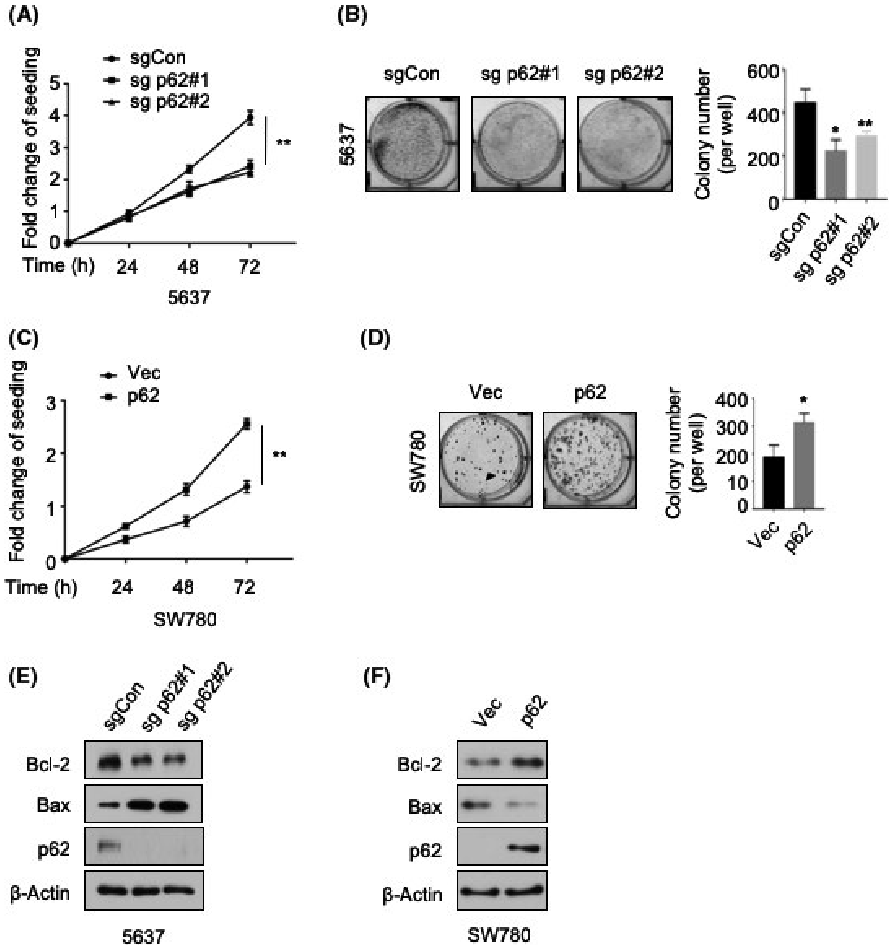

FIGURE 2 p62 promotes bladder cancer cell proliferation and inhibits its apoptosis in vitro. A-D, MTT cell proliferation and colony formation assay after p62 knockdown and overexpression. E, F, Immunoblots of B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) after p62 knockdown and overexpression. Data are shown as the mean ± SD, Student's *t* test, **P* < .05, ***P* < .01. Con, control; sg, single guide; Vec, vector

2.11 | Statistical analysis

Statistical analysis was undertaken with GraphPad Prism version 5 software. Data are presented as mean ± SEM. Student's *t* test was used for data normally distributed. A *P* value of less than .05 was considered statistically significant.

3 | RESULTS

3.1 | p62 is overexpressed in human BCa and cell lines

To explore the role of p62 in BCa, we first analyzed the p62 expression in human BCa. p62 was found to be overexpressed in 3 different BCa studies (Figure 1A-C).[17-19] These 3 studies obtained gene expression data from 165,[17] 109,[18] and 41[19] BCa samples, respectively, by microarray analysis. Furthermore, analysis of clinical tissue specimens confirmed the upregulation of p62 in BCa tissues (Figure 1D). We also noticed that the protein level of p62 in BCa patients upregulated (more than twofold) at a frequency of 4.4% (15 of 344 cases) in The Cancer Genome Atlas database (Figure 1E).[20] We then determined the expression of p62 in several human BCa cell lines by western blots. Among 7 cell lines tested, T24, RT4, 5637, TCCSUP, and 253J showed high p62 baseline expression, whereas p62 was barely detected in SV-HUC-1 and SW780 (Figure 1F). These data suggest that p62 was upregulated in human BCa, at least partially.

3.2 | p62 promotes BCa cell proliferation and inhibits its apoptosis in vitro

To study the biological function of p62 in BCa, we applied the CRISPR/Cas9-mediated gene knockdown strategy in 5637 cells, which showed a high p62 baseline expression (Figure 1F). Both MTT cell proliferation study and colony formation assay showed that p62 deletion strongly inhibited cell growth of 5637 (Figure 2A,B). In addition, we took advantage of the SW780 cell line, which expressed a low level of p62 (Figure 1F), to overexpress p62. We observed that p62 overexpression significantly promoted the growth of SW780 cells (Figure 2C,D). Furthermore, p62 knockdown decreased the level of Bcl-2 while it increased the level of Bax, indicating a proapoptotic phenotype of p62-deficient BCa cells (Figure 2E). In contrast, p62 overexpression induced the opposite effect (Figure 2F). Together, these data indicate a requirement for p62 in BCa cell growth.

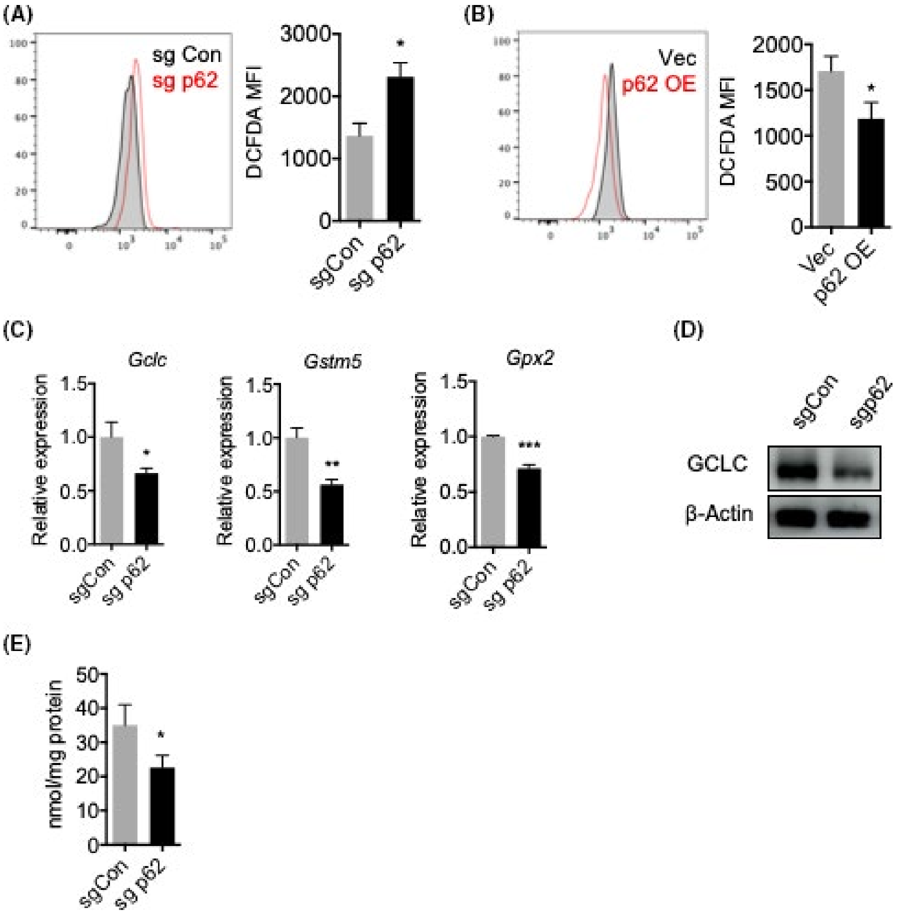

FIGURE 3 p62 is required for oxidative resistance in bladder cancer cells. A, B, Intracellular reactive oxygen species level in p62-deleted 5637 cells and p62-overexpressed (p62 OE) SW780 cells. Mean fluorescence intensity (MFI) was quantified. C, Expression of nuclear factor-E2-related factor 2-targeted genes. D, Immunoblots of glutamate-cysteine ligase catalytic subunit (GCLC) after p62 knockdown. E, Intracellular level of glutathione in control and p62-deficient 5637 cells. Data are shown as the mean ± SD, Student's *t* test, **P* < .05, ***P* < .01, ****P* < .001. Con, control; DCFDA, 2′,7′-dichlorofluorescin diacetate; sg, single guide; Vec, vector

3.3 | p62 is required for oxidative resistance in BCa cells

As p62-NRF2 signaling is the major intracellular antioxidative response,[3] we speculated that p62 is related to the redox homeostasis within BCa cells. To that end, we detected intracellular ROS levels by a DCFDA probe. Indeed, we found that p62 deletion resulted in an increased level of ROS (Figure 3A), whereas p62 overexpression rescued this phenotype (Figure 3B). We then investigated the activation of NRF2 by determining the expression of NRF2 targeted genes, among which we observed a significant downregulation of *Gclc*, *Gstm5*, and *Gpx2* after p62 knockdown (Figure 3C,D). More importantly, p62 knockdown led to a decrease in GSH, one of the major antioxidants to prevent oxidative stress (Figure 3E). These results suggest that p62 activates the NRF2-dependent antioxidative response in BCa cells, thus protecting them from oxidative stress and promoting their growth.

3.4 | p62 activates NRF2 by sequestering KEAP1

We then attempted to obtain the molecular mechanism by which p62 activates NRF2. As mounting evidence indicates that p62 is a crucial regulator of the KEAP1/NRF2-dependent antioxidative response,[3] we asked whether p62 participates in this pathway in BCa cells. p62 deletion in 5637 cells resulted in an elevated level of KEAP1 and, at the same time, a decreased level of NRF2. Overexpression of p62 in SW780 cells led to the opposite effects (Figure 4A). This is consistent with previous studies that reported that KEAP1 is degraded through p62-mediated autophagy.[21,22] We then confirmed the interaction between p62 and KEAP1 by coimmunoprecipitation in 293T cells (Figure 4B). Moreover, p62 and KEAP1 were colocated in 5637 cells (Figure 4C). To evaluate the role of p62 in regulating KEAP1 protein abundance, we undertook the CHX chase assay in 5637 cells. We found that p62 deletion slowed down the KEAP1 degradation rate (Figure 4D,E). Furthermore, p62 inhibited the ubiquitylation of NRF2 (Figure 4F). As p62 is degraded through autophagy, we used Baf A1 to induce autophagy impairment, which led to the accumulation of p62 (Figure 4G). Consistent with p62 overexpression (Figure 4A), autophagy inhibition increased the level of KEAP1 while it decreased the level of NRF2 in 5637 cells (Figure 4G). These data indicate that p62 in BCa cells activates NRF2 by regulating KEAP1 abundance.

It has been reported that p62 activates NRF2 signaling by sequestering KEAP1 through its KIR domain.[5] To determine whether the KIR domain mediates the interaction with KEAP1, we generated a KIR-deleted mutant of p62, then examined the interaction of the mutant with KEAP1 by assessing their immunoprecipitation. We observed that the KIR-deleted p62 failed to precipitate together with KEAP1 (Figure 5A). Moreover, the KIR-deleted mutant restored the KEAP1 level and decreased NRF2 abundance (Figure 5B) by

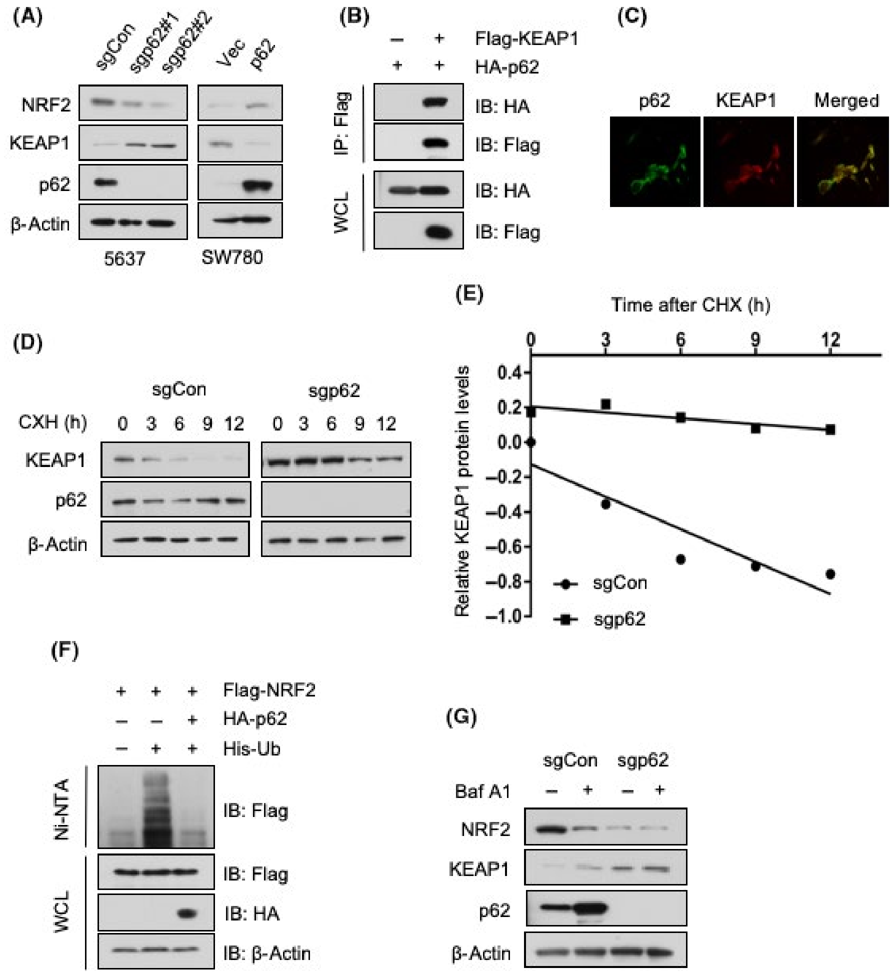

FIGURE 4 p62 activates nuclear factor-E2-related factor 2 (NRF2) by regulating Kelch-like ECH-associated protein 1 (KEAP1) abundance. A, Immunoblots of NRF2 and KEAP1 after p62 knockdown and overexpression in 5637 and SW780 bladder cancer cells, respectively. B, Immunoassay of lysates of 293T cells cotransfected with vectors expressing hemagglutinin-tagged (HA-) p62 and Flag-tagged (Flag-) KEAP1, immunoprecipitated (IP) with anti-Flag and analyzed by immunoblot (IB). WCL, whole-cell lysates. C, Immunofluorescence of p62 and KEAP1 in 5637 cells. D. E, 5637 cells were harvested at the indicated time points after treatment with cycloheximide (CHX). Immunoblots of p62 and relative KEAP1 protein levels were quantified. F, Ubiquitylation of NRF2 in the presence or absence of p62 in 293T cells. G, Immunoblots of NRF2, KEAP1, and p62 after bafilomycin A1 (Baf A1) treatment in 5637 cells. Con, control; sg, single guide; Vec, vector

increasing the ubiquitination of NRF2 (Figure 5C). In addition, the CHX chase assay showed a KIR-dependent degradation of KEAP1 (Figure 5D,E). Consistently, the KIR-deleted p62 failed to reduce ROS in BCa cells, indicating the deficiency of a potent intracellular antioxidant response (Figure 5F). It has been reported that the phosphorylation of p62 at serine 349 is important for the p62-mediated NRF2 activation.[4] We confirmed this mechanism in the BCa cells by using a p62 S349A mutant, which failed to interact with KEAP1 (Figure 5G) and induce NRF2 activation (Figure 5H). In fact, we observed a basal level of phospho-p62 in all 7 cell lines (Figure 1F). These results collectively suggest that the p62 KIR domain and p62 phosphorylation at S349 are both important to p62-KEAP1 interaction, which disrupts the KEAP1-NRF2 association and provokes NRF2 stabilization and activation.

3.5 | Knockdown of p62 inhibits BCa growth in vivo

Finally, we examined the role of p62 in BCa cell growth in vivo. p62-depleted 5637 cells were inoculated into nude mice. Tumor volume (Figure 6A,B) and weight (Figure 6C) were dramatically reduced when p62 was deleted. Moreover, the deficiency of p62 led to the upregulation of KEAP1 and downregulation of NRF2 (Figure 6D), thus blunting KEAP1-NRF2 signaling, which was consistent with the in vitro data (Figure 4A). Accordingly, one of the NRF2-targeted genes, *GCLC*, was downregulated (Figure 6D). In addition, the level of Bcl-2 was decreased in p62-depleted 5637 cells (Figure 6D). Consistently, immunohistochemistry results showed that NRF2 and GCLC were also reduced in p62-depleted 5637 cells (Figure 6E). Together, our data suggest that p62 promotes BCa cell proliferation by activating NRF2-dependent antioxidative response in vivo.

4 | DISCUSSION

Although mounting evidence suggests that p62 is involved in tumor initiation and progression,[3] little is known about its role in BCa. Our study showed that p62 is overexpressed in a portion of BCa patients and BCa cell lines. The in vivo and in vitro data indicated that p62 promotes BCa cell growth by activating KEAP1-NRF2 signaling and protecting cancer cells from oxidative stress.

It is well appreciated that p62 is involved in tumor development. Some of the first evidence is that accumulation of p62 during tumorigenesis is associated with the upregulation of NF-κB, a critical survival regulator induced by the oncogene *Ras*.[23] In addition, p62 facilitates the tumor growth through the activation mTORC1 and induction of c-Myc.[14] Dysfunction of the p62-KEAP1-NRF2

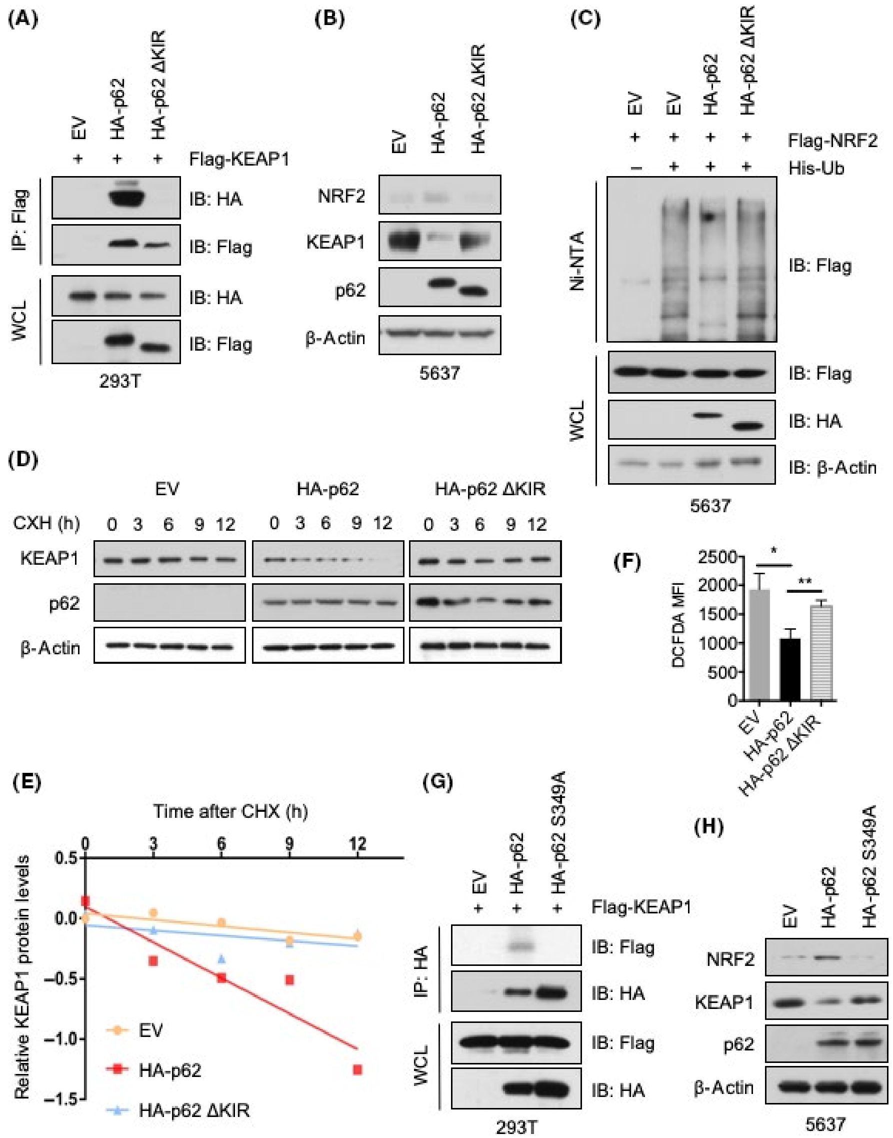

FIGURE 5 p62 in bladder cancer cells decreases Kelch-like ECH-associated protein 1 (KEAP1) abundance through its KEAP1-interacting region (KIR) domain, thus activating nuclear factor-E2-related factor 2 (NRF2) signaling. A, Immunoassay of lysates of 293T cells cotransfected with vectors expressing Flag-KEAP1 and HA-p62 or HA-p62 ΔKIR (KIR-deleted p62), immunoprecipitated (IP) with anti-Flag and analyzed by immunoblot (IB). B, 5637 cells were transfected with HA-p62 or HA-p62 ΔKIR following by immunoblots of NRF2, KEAP1, and p62. C, 5637 cells were transfected with Flag-NRF2, His-Ub and HAp62, or HA-p62 ΔKIR, following by IB of anti-Flag. D, E, 5637 cells were harvested at indicated time points after cycloheximide (CHX) treatment in 5637 cells transfected with HA-p62 or HA-p62 ΔKIR and relative KEAP1 protein levels were quantified. F, Intracellular reactive oxygen species level of 5637 cells transfected with p62 or p62 ΔKIR. G, Immunoassay of lysates of 293T cells cotransfected with vectors expressing Flag-KEAP1 and HA-p62 or HA-p62 S349A, IP with anti-Flag and analyzed by IB. H, 5637 cells were transfected with HA-p62 or HA-p62 S349A following by immunoblots of NRF2, KEAP1, and p62. DCFDA, 2′,7′-dichlorofluorescin diacetate; EV, empty vector; MFI, mean fluorescence intensity; WCL, whole-cell lysates. *$P < .05$, **$P < .01$.

axis has been observed in several cancer types.[3] For instance, persistent phosphorylation of p62 at serine 349 has been found in hepatoma,[8,24] which results in the activation of NRF2.[4] Knockout of p62 in a liver cancer cell line markedly abrogates tumor growth.[24] We found that upregulation of p62 promotes NRF2 activation in BCa cells, in which the phosphorylation of p62 at serine 349 might play a crucial role. In addition, p62 is a selective receptor of autophagy, which has been reported to be associated with genomic damage, metabolic stress, and tumorigenesis.[25] Autophagy plays dual roles in cancer biology. The basal level of autophagy operates as a mechanism for tumor suppression through the reduction of damaged cellular components.[26] However, autophagy also helps cancer cells to overcome stressful conditions (such as hypoxia and nutrient deprivation) by recycling intracellular components and supplying metabolic substrates.[27] We found that all 7 BCa cell lines showed a certain level of autophagy (Figure 1F). Future studies are needed to elucidate the role of p62-mediated autophagy in BCa.

The impact of NRF2 on cancer is complex. An emerging concept is that NRF2 has its yin and yang side in tumor development.[16,28]

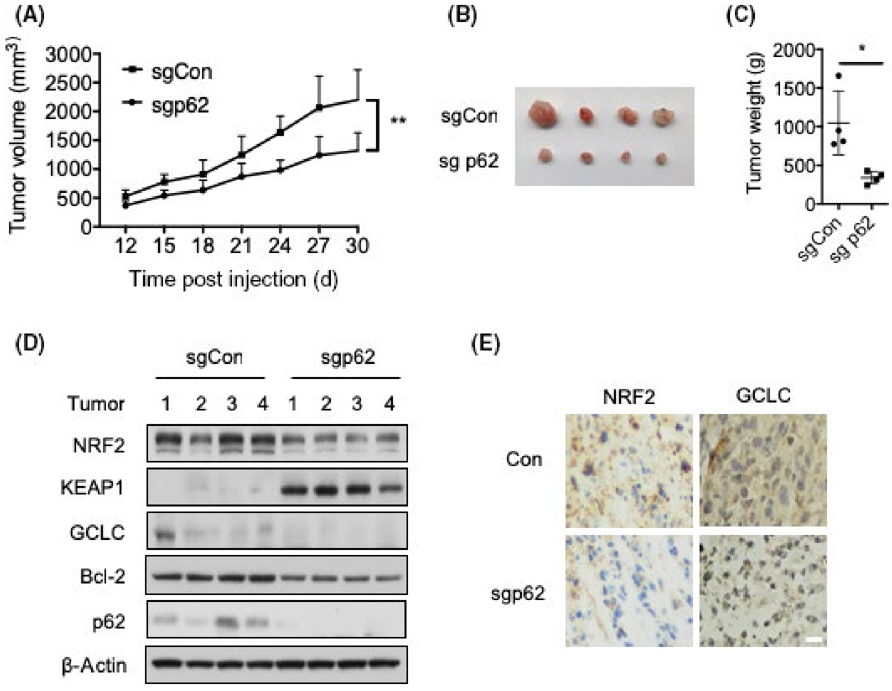

FIGURE 6 Knockdown of p62 inhibits bladder cancer growth in vivo. A, 5637 cells were infected with single guide (sg) p62 and cultured for 48 h and then inoculated into nude mice. Tumor volume was calculated at indicated time points. B, C, Tumor appearance and weight of control and p62-depleted 5637 cells are shown. D, Immunoblots of indicated proteins of tumors from control and p62-depleted 5637 cell-induced tumor. E, Representative immunohistochemistry images of nuclear factor-E2-related factor 2 (NRF2) and glutamate-cysteine ligase catalytic subunit (GCLC). Scale bar, 50 μm. Data are shown as the mean ± SD, Student's *t* test, *$P < .05$, **$P < .01$. Bcl-2, B-cell lymphoma-2; Con, control; KEAP1, Kelch-like ECH-associated protein 1

On one hand, NRF2 is required for the prevention of chemical and radiation-induced carcinogenesis by quenching ROS and repairing oxidative damage through the expression of its target genes.[29-32] On the other hand, recent studies have described that NRF2 activation promotes cancer progression[14,33-35] and metastasis.[36] In addition to the genes related to antioxidant response, NRF2 also targets the genes that control proliferation (such as *Notch1*, *Igf1*, and *Itgb2*) and angiogenesis (such as *Pdgfc* and *Vegfc*).[37] In addition, NRF2 is the key transcription factor that mediates metabolic reprogramming in cancer cells by increasing glucose uptake and directing it to the pentose phosphate pathway.[38] Many studies have shown that NRF2 also regulates the tumor microenvironment by reducing the expression of proinflammatory cytokines.[16,39]

In summary, we showed that p62 is often overexpressed in human BCa tissue and cell lines. Functionally, p62 promotes BCa cell growth in vitro and in vivo. Mechanically, p62 activates the NRF2-dependent antioxidative response and protects tumor cells from oxidative stress. Our study thus indicated that p62 might serve as a potential therapeutic target in BCa.

DISCLOSURE

The authors declare that they have no conflict of interests.

ORCID

Tao Li https://orcid.org/0000-0002-0300-6454

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66:115-132.
3. Moscat J, Karin M, Diaz-Meco MT. p62 in Cancer: signaling adaptor beyond autophagy. *Cell*. 2016;167:606-609.
4. Ichimura Y, Waguri S, Sou Y-S, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol Cell*. 2013;51:618-631.
5. Komatsu M, Kurokawa H, Waguri S, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol*. 2010;12:213-223.
6. Sanchez P, De Carcer G, Sandoval IV, Moscat J, Diaz-Meco MT. Localization of atypical protein kinase C isoforms into lysosome-targeted endosomes through interaction with p62. *Mol Cell Biol*. 1998;18:3069-3080.
7. Duran A, Amanchy R, Linares J, et al. p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Mol Cell*. 2011;44:134-146.
8. Saito T, Ichimura Y, Taguchi K, et al. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. *Nat Commun*. 2016;7:12030.
9. Inoue D, Suzuki T, Mitsuishi Y, et al. Accumulation of p62/SQSTM1 is associated with poor prognosis in patients with lung adenocarcinoma. *Cancer Sci*. 2012;103:760-766.
10. Luo RZ, Yuan ZY, Li M, Xi SY, Fu J, He J. Accumulation of p62 is associated with poor prognosis in patients with triple-negative breast cancer. *Onco Targets Ther*. 2013;6:883-888.
11. Todoric J, Antonucci L, Di Caro G, et al. Stress-activated NRF2-MDM2 cascade controls neoplastic progression in pancreas. *Cancer Cell*. 2017;32(6):824-839 e8.
12. Giatromanolaki A, Sivridis E, Mendrinos S, Koutsopoulos AV, Koukourakis MI. Autophagy proteins in prostate cancer: relation with anaerobic metabolism and Gleason score. *Urol Oncol*. 2014;32(39):e11-e18.
13. Niklaus M, Adams O, Berezowska S, et al. Expression analysis of LC3B and p62 indicates intact activated autophagy is associated with an unfavorable prognosis in colon cancer. *Oncotarget*. 2017;8:54604-54615.
14. Umemura A, He F, Taniguchi K, et al. p62, upregulated during preneoplasia, induces hepatocellular carcinogenesis by

maintaining survival of stressed HCC-initiating cells. *Cancer Cell*. 2016;29:935-948.
15. Suzuki T, Yamamoto M. Stress-sensing mechanisms and the physiological roles of the Keap1-Nrf2 system during cellular stress. *J Biol Chem*. 2017;292:16817-16824.
16. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the hallmarks of cancer. *Cancer Cell*. 2018;34:21-43.
17. Lee JS, Leem SH, Lee SY, et al. Expression signature of E2F1 and its associated genes predict superficial to invasive progression of bladder tumors. *J Clin Oncol*. 2010;28:2660-2667.
18. Sanchez-Carbayo M, Socci ND, Lozano J, Saint F, Cordon-Cardo C. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. *J Clin Oncol*. 2006;24:778-789.
19. Dyrskjøt L, Kruhøffer M, Thykjaer T, et al. Gene expression in the urinary bladder: a common carcinoma in situ gene expression signature exists disregarding histopathological classification. *Cancer Res*. 2004;64:4040-4048.
20. Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell*. 2018;174:1033.
21. Taguchi K, Fujikawa N, Komatsu M, et al. Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc Natl Acad Sci USA*. 2012;109:13561-13566.
22. Bae S, Sung S, Oh S, et al. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. *Cell Metab*. 2013;17:73-84.
23. Duran A, Linares JF, Galvez AS, et al. The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell*. 2008;13:343-354.
24. Inami Y, Waguri S, Sakamoto A, et al. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol*. 2011;193:275-284.
25. Yun CW, Lee SH. The roles of autophagy in cancer. *Int J Mol Sci*. 2018;19:3466.
26. Tang D, Kang R, Livesey KM, et al. Endogenous HMGB1 regulates autophagy. *J Cell Biol*. 2010;190:881-892.
27. Rabinowitz JD, White E. Autophagy and metabolism. *Science*. 2010;330:1344-1348.
28. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol*. 2013;53:401-426.
29. Tao S, Justiniano R, Zhang DD, Wondrak GT. The Nrf2-inducers tanshinone I and dihydrotanshinone protect human skin cells and reconstructed human skin against solar simulated UV. *Redox Biol*. 2013;1:532-541.
30. Tao S, Park SL, de la Vega MR, Zhang DD, Wondrak GT. Systemic administration of the apocarotenoid bixin protects skin against solar UV-induced damage through activation of NRF2. *Free Radic Biol Med*. 2015;89:690-700.
31. Knatko EV, Ibbotson SH, Zhang Y, et al. Nrf2 activation protects against solar-simulated ultraviolet radiation in mice and humans. *Cancer Prev Res (Phila)*. 2015;8:475-486.
32. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev*. 2013;27:2179-2191.
33. Tao S, Rojo de la Vega M, Chapman E, Ooi A, Zhang DD. The effects of NRF2 modulation on the initiation and progression of chemically and genetically induced lung cancer. *Mol Carcinog*. 2018;57:182-192.
34. Satoh H, Moriguchi T, Takai J, Ebina M, Yamamoto M. Nrf2 prevents initiation but accelerates progression through the Kras signaling pathway during lung carcinogenesis. *Cancer Res*. 2013;73:4158-4168.
35. DeNicola GM, Karreth FA, Humpton TJ, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*. 2011;475:106-109.
36. Wang H, Liu X, Long M, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. *Sci Transl Med*. 2016;8:334ra51.
37. Malhotra D, Portales-Casamar E, Singh A, et al. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res*. 2010;38:5718-5734.
38. Lee SB, Sellers BN, DeNicola GM. The regulation of NRF2 by nutrient-responsive signaling and its role in anabolic cancer metabolism. *Antioxid Redox Signal*. 2018;29:1774-1791.
39. Long M, Tao S, Rojo de la Vega M, et al. Nrf2-dependent suppression of azoxymethane/dextran sulfate sodium-induced colon carcinogenesis by the cinnamon-derived dietary factor cinnamaldehyde. *Cancer Prev Res (Phila)*. 2015;8:444-454.

How to cite this article: Li T, Jiang D, Wu K. p62 promotes bladder cancer cell growth by activating KEAP1/NRF2-dependent antioxidative response. *Cancer Sci*. 2020;111:1156–1164. https://doi.org/10.1111/cas.14321