RESEARCH ARTICLE

Autophagic Degradation of Caspase-8 Protects U87MG Cells Against H_2O_2-induced Oxidative Stress

Yi-Bo Zhang[1]*, Wei Zhao[2], Rui-Xia Zeng[3]

Abstract

Oxidative stress induces apoptosis in many cellular systems including glioblastoma cells, with caspase-8 activation was regarded as a major contribution to H_2O_2-induced cell death. This study focused on the role of the autophagic protein p62 in H_2O_2-induced apoptosis in U87MG cells. Oxidative stress was applied with H_2O_2, and cell apoptosis and viability were measured with use of caspase inhibitors or autophagic mediators or siRNA p62, GFP-p62 and GFP-p62-UBA (del) transfection. We found that H_2O_2 -induced U87MG cell death was correlated with caspase-8. To understand the role of p62 in MG132-induced cell death, the levels of p62/SQSTM1 or autophagy in U87MG cells were modulated with biochemical or genetic methods. The results showed that the over-expression of wild type p62/SQSTM1 significantly reduced H_2O_2 induced cell death, but knockdown of p62 aggravated the process. In addition, inhibition of autophagy promoted p62 and active caspase-8 increasing H_2O_2 -induced apoptosis while induction of autophagy manifested the opposite effect. We further demonstrated that the function of p62/SQSTM1 required its C-terminus UBA domain to attenuate H_2O_2 cytotoxity by inhibition of caspase-8 activity. Our results indicated that p62/SQSTM1 was a potential contributor to mediate caspase-8 activation by autophagy in oxidative stress process.

Keywords: p62 - caspase-8 - U87MG - hydrogen peroxide - autophagy

Asian Pac J Cancer Prev, **14 (7)**, 4095-4099

Introduction

Reactive oxygen species (ROS), which leads to oxidative stress (OS) at excess amount, positively contributes to carcinogenesis and to malignant progression of tumor cells (Gerald et al., 2004). In contrast to normal cells, cancer cells, which exhibit an accelerated metabolism, demand higher ROS concentrations to maintain their high proliferation rate (Sosa et al., 2013). Importantly, several studies showed that OS production promotes cell apoptosis in cancer treatment with various radio (Mitsuishi et al., 2012) and chemotherapeutic drugs (Saha, 2012). However, the mechanism of apoptosis induced by OS is not clear.

H_2O_2, which mimics OS by producing excess ROS in cells, is applied for exploring the OS-induced cell death. Numerous studies have provided evidence that unwanted protein aggregates, such as β-amyloid, ubiquitinated proteins, which are involved in ROS generation process, bring out apoptosis ultimately (Pan et al., 2013; Vernon and Tang, 2013). Moreover, H_2O_2-induced apoptosis is directly through caspase-8 and is not through the mitochondria-dependent caspase-9 activation (Wu et al., 2011). In addition, it also induced autophagy in H_2O_2-treated cells, accompanied with a decreased level of p62 and the formation of autophagic vacuoles (Seo et al., 2011).

p62 is a central regulator of tumorigenesis due to its abilities to modulate autophagy, to control the levels of ROS and misfolded proteins, and to ensure a timely transit of cell through mitosis; all crucial factors in cancer (Moscat and Diaz-Meco, 2012). Previous studies have shown that p62 binds ubiquitin at its C-terminus, suggesting that it may serve as a storage compartment for ubiquitinated proteins (Yip et al., 2006). In addition, p62 binds specifically to ubiquitinated proteins, acting as a putative ubiquitin chain-targeting factor that shuttles these substrates for proteasome and autophagy degradation (Korolchuk et al., 2009). In particular, the UBA domain played a potential role in the p62 function as an adaptor or cargo receptor for degradation of ubiquitinated proteins (Long et al., 2010). In this study, we attempted to explored the role of caspase-8 in H_2O_2-induced associated apoptosis in U87MG cells, to evaluated the function of p62 in caspase-8 autophagic degradation response to oxidative insult by using the UBA domain deletion and knockdown of p62.

Materials and Methods

Plasmids, chemical reagents and antibodies

The GFP-p62 plasmid was kindly provided by Dr. Terje Johansen (University of Tromsø, Tromsø, Norway).

*[1]Department of Pathogen Biology, [2]Department of Laboratory Animal, [3]Department of Human Anatomy, School of Basic Medical Sciences, Liaoning Medical University, Jinzhou, China *For correspondence: jyzyb2009@163.com*

The GFP-LC3 plasmid was provided by professor Ying-Yu Chen (Peking University, Heath Science Center, Beijing, China). A GFP-p62-UBA(del) plasmid was constructed by using CCGctcgag ATGGCGTCG CTCACCGTGAAGGCCT and CCGgaattc TCATGGCGG GAGATGTGG GTACAAG as PCR primers, and Xhol and EcoRI the restriction sites. All plasmids used in this study were confirmed by DNA sequencing.

3-methyladenine (3MA), rapamycin (RAPA) were purchased from Sigma-Aldrich (st Louis, MO, USA) was purchased from HyClone. AC-IETD-CHO, z-VAD-FMK, FAM-LETD-FMK, and FAM-LEHD-FMK was purchased from Biomol (Biomol. Hamburg, Germany). Antibodies of rabbit anti-LC3 or p62 (Sigma), mouse anti-actin (ZhongShan GoldenBridge Biotechnology, Beijing, China), mouse anti-Ub and anti-caspase-8 (p18) (P4D1) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were applied following the vendors recommendation.

Cell culture and transfection

U87MG cells were purchased from the cell bank at Peking Union Medical University. U87MG cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose (Hyclone, Beijing, China) supplemented with 10% fetal bovine serum (FBS, Sijiqing, Zhejiang, China). Fugene HD reagent (Roche, Penzberg, Germany) was used for cell transfection following the manufacture's standard protocols. The cells were observed and photographed under a DMIRB inverted fluorescent microscope (Leica, Solms, Germany).

Cell viability

The cell viability after H_2O_2 treatment was determined using the CellTiter-96® Aqueous - One Solution Cell Proliferation (MTS) Assay kit (Promega, Madison, WI, USA). The cells were seeded in 96-well plates at 0.5~1x104 cells per well for 24 h and then treated with H_2O_2 of necessary conditions according to the experimental design. The MTS reagents were applied for 1 h at 37 °C, and the plates were subjected to measures at 490 nm with a Synergy HT Multi-Mode Microplate Reader (BioTek, Winooski, VT USA).

The cell apoptosis index was measured using Calcein–AM/PI method. The cells after H_2O_2 treatments were incubated with 1 μg/ml Calcein–AM (Invitrogen, Eugene, OR, USA) and 10 μg/ml propidium iodide (PI, Invitrogen) for 30 min at 37°C. A double-blinded cell counting was performed for live (green) and dead cells under fluorescent microscope. At lease three non-overlapped fields were acquired from each well under different treatment conditions, the number of stained cells was counted using ImageJ software and the percentage of PI-positive cells/ total (both Calcein and PI positive cells) was calculated.

Caspase activity assay

Cells treated with H_2O_2 for 3, 6, 9, 12 h were harvested and incubated in culture media with 10 μmol/L of FAM-LETD-FMK (caspase-8 fluorescent substrate) or FAM-LEHD-FMK (caspase-9 fluorescent substrate) for 1 h at 37°C. After washed 3 times with Apoptosis Wash Buffer, the cells were suspended in 300 μL buffers and analyzed with a fluorescence microscope in three independent experiments.

RNA interference

The targeted fragment of siRNAs against p62 was 5'-GCATTGAAGTTGATATCGAT-3 as previously published (Pankiv et al., 2010) . Cells were grown in 6-well plates and transfected using Fugene HD reagents with siRNA or the scrambled control (Augct, Beijing, China) at a final concentration 20 nmol/L. After 72 h, the p62 protein levels were examined by western blot and cells in parallel conditions were used for H_2O_2 experiments.

Western blots

Cells in 6-well plates were lysed in 80-100 uL modified RIPA buffer (Thermo, Rockford, IL,USA) containing the full cocktail of protease inhibitors (Thermo). The protein concentrations were determined with the BCA protein assay kit (Novagen, San Diego CA, USA).Then proteins were separated by 10 or 15% SDS-PAGE and transferred to nitrocellulose filters, the blotted with accorded antibodies, secondary antibodies pre-labeled with IRDye800CW or IRDye700CW were used for the scanning by the Odyssey (LI-COR Biosciences, Lincoln, NE, USA)

Statistical analysis

The experimental data presented in this study were from at least 3 independent experiments. The data values in cell viability are presented as means with SEM Following one-way ANOVA and student T tests, the *p* value less than 0.05 was considered as statistical significance.

Results

p62 attenuated H_2O_2-induced caspase-8 dependent apoptosis in U87MG cells

In H_2O_2-treated U87MG cells, we observed that the amount of apoptotic cells were time and dose dependent on H_2O_2 (Figure 1A and 1B). To explore the mechanism of apoptosis, we employed caspase-8 inhibitor AC-LETD-CHO or pan-caspase inhibitor z-VAD-FMK, and found that the caspase-8 inhibitor effectively attenuated H_2O_2 induced cell death to the similar level as that of the pan capspase inhibitor (Figure 1C). Subsequencely, as expected, caspase-8 activity and caspase-8 active component (p18) were correlative with H_2O_2 treatment time course (Figure 1D).This suggested that caspase-8 appeared to be the predominant type of caspase in mediating H_2O_2-induced apoptosis. Interestingly, p62, which is autophagic relative protein, significantly increased with H_2O_2 treatment (Figure 1E). Since p62 was thought to recruit poly-ubiquitinated caspase-8 and subsequently allow its full activation in TRAIL-treated cells (Jin et al., 2009), it indicated that p62 was a crucial molecule in H_2O_2-induced apoptosis.

To assess the function of p62, we modulated the p62 protein level in U87MG cell with the transfection of siRNA p62 and GFP-p62 (Figure 1F and 1G). As shown in Figure 1H, p62 deficiency promoted the significantly decrease of H_2O_2-treated cell viability from 49 % reduced

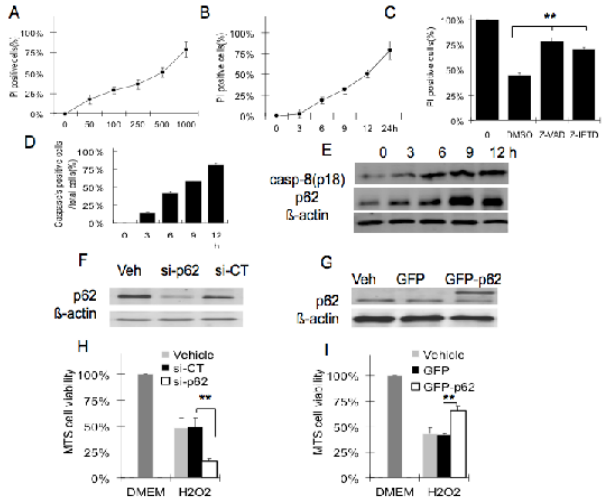

Figure 1. Oxidative Stress Induced Caspase-8 Dependent Cell Death Modulated by p62 Protein Levelin U87MG Cells. (A) and (B) The dose and time course of H_2O_2-induced cell death in U87MG cells were charactered by calcein-AM/PI staining. Data was shown as the averaged percent of PI positive cells ±SEM from three independent experiments by counting a total of 200 cells. (C) The cell death rate of U87MG cells treated with 500 μmol/L H_2O_2 for 12 h with pretreatments of pan-caspase (z-VAD-FMK) or caspase-8 (AC-IETD-CHO) inhibitors for 30 min. (D) The activities of caspase-8 were evaluated following H_2O_2 treatment for 0, 3, 6, 9, 12 h. The cells were preloaded with FAM-LETD-FMK (caspase-8 fluorescent substrate) at 37 °C for 60 min. The numbers of positively stained cells were counted and shown as the averaged percentage ±SEM from three independent experiments. (E) The western blot of caspase-8 activation in U87MG cells treated with 500 μmol/L H_2O_2 for 0, 3, 6, 9, 12 h. The protein levels of p62 and caspase-8 p18 were detected. (F) and (G) Transfection of siRNA targeted to p62/SQSTM1 for 72 h increase the cell death in U87MG cells after 500 μmol/L H_2O_2 treatment for 12 h. (H) and (I) H_2O_2-induced cell death in U87MG cell with overexpression of p62/SQSTM1 for 24 h. The cell viability was assayed by MTS at 12 h. The results were normalized and presented as mean±SEM from five independent experiments. $*P < 0.05$, $**P < 0.01$

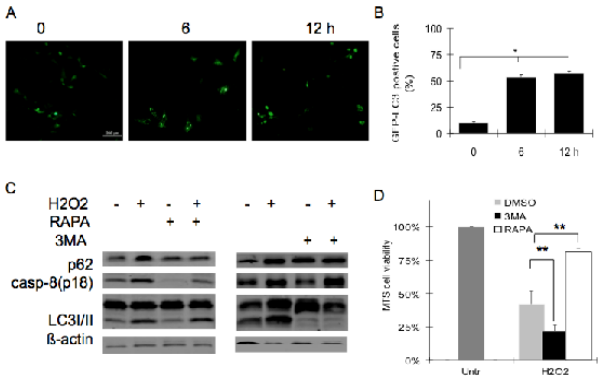

Figure 2. Activation or Inhibition of Autophagy Influenced H_2O_2-induced Cell Death and Associated with Changes of p62 Protein Levels and Caspases-8 Activity. (A) H_2O_2 treatments (500 μmol/ml) for 0, 6, 12 h induced GFP-LC3 aggregation in transfected U87MG cells. (B) From the confocol images in each sample, a random 50 GFP positive cells were analyzed for the percentage with at least three aggregated GFP-LC3 spectacles as positive for autophagy. U87MG cells were treated with autophagy inhibitor of 3MA at 5 mmol/L or autophagy activator rapamycin at 2.5 μmol/L for 3 h. (C) The protein levels of LC3 I/II, p62 and caspase-8 were determined by western blot at 12 h. (D)The cell viability was assayed by MTS at 12 h. The results were normalized and presented as mean±SEM from five independent experiments. $*P<0.05$. $**P < 0.01$

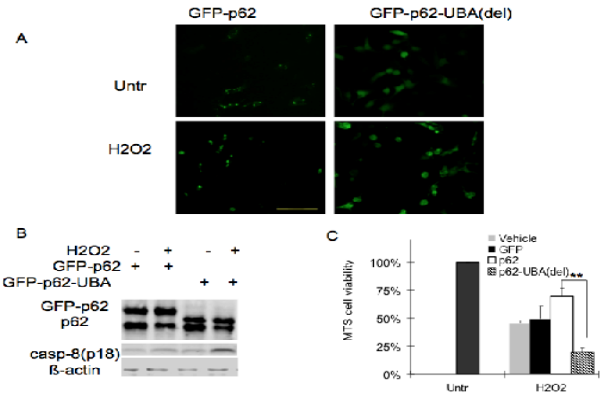

Figure 3. H_2O_2 Induced Cell Death was Modulated by p62 Aggregation and Dependent on the UBA Domain. (A) The p62/SQSTM1 UBA-deletion mutant was unable to form aggregated fluorescent speckles in U87MG cells. Cells were transfected for 24 h, and then exposed to H_2O_2 for 12 h before photographed. (Bar=100 μm) (B) Western blot for the comparison of caspase-8 activation following GFP-p62 and GFP-p62-UBA(del) transfection. After transfected for 24 h, the protein levels of caspase-8 p18 and p62 from 12 h H_2O_2-treated U87MG cells were detected by western blot. (C) Transfection of p62/SQSTM1 mutant attenuated the Mg132-induced cytotoxicity. U87MG cells were transfected with GFP-p62 or GFP-p62-UBA(del) for 24 h and assayed by MTS after 12 h exposure in 0.5 μmol/L H_2O_2. The results were presented as mean ±SEM from three independent experiments. $**P<0.01$.

to 16 %. Meanwhile, overexpression of p62 t significantly increased the survival rate of U87MG from 42% to 65% (Figure 1I). The results suggested p62 inhibited the cytotoxicity of H_2O_2 in U87MG cells.

Modulation of autophagy in U87MG cells altered H_2O_2-induced cell death with changes of p62/SQSTM1 and caspase-8 at protein levels

The caspase-8 and p62 protein degradation were mediated by autophagy. To explore whether autophagy occurred in H_2O_2-induced cell death, we tested autophagy using the GFP-LC3 conversion as the marker of the autophagosomes.in U87MG cells. The cells with more than 3 fluorescent puncta were counted as positive for autophagy activation (Young et al., 2009). We found that approximately 53 % and 57% of GFP-LC3 transfected U87MG cells were observed with autophagy activation after treated with 500 μmol/ml H_2O_2 for 6 h and 12 h (Figure2A and 2B). To investigate whether autophagy is functionally involved in H_2O_2-induced cytotoxicity, we used the reagents to either activate or inhibit the autophagy process in U87MG cells. The results showed that the inhibition of autophagy accelerated the H_2O_2-induced cell death, whereas the activation of autophagy with rapamycin exposure attenuated the cytotoxicity (Figure 2C and 2D).

UBA domain was key structure of p62/SQSTM1 which attenuated H_2O_2-induced cell death

UBA domain at the C-terminus of p62/SQSTM1

was suggested to be a key domain to bind and recruit ubiquitinated proteins, including polyubiquitinated caspase-8 (Laussmann et al., 2011). We used an overexpression plasmid of p62 mutant with the deletion of its UBA domain and tagged with GFP(Zhang et al., 2013). When transfected into the U87MG cells and compared with the wild type p62, we found that the UBA-deleted p62 largely abolished the ability to form intracellular aggregates (Figure 3A). The H_2O_2-induced cell death could be significantly aggravated by transfection of GFP-p62-UBA (del) (Figure 3C), as well as the caspase-8 cleavage and p18 production (Figure 3B).

Discussion

Oxidative stress, which is a state with excess ROS induced by H_2O_2, lead to the production of peroxides and free radicals that damage mitochondria (Sun et al., 2012; Wu et al., 2012). However, numerous studies showed that H_2O_2-induced apoptosis was dependent on caspase-8 activation(Tartier et al., 2000; Yamakawa et al., 2000; Wu et al., 2011), not but on caspase-9 activation which is induced by mitochondria dysfunction. In this study, it also showed that caspase-8 activation was major to contribute to H_2O_2 induced cell death in U87MG cells.

Recent studies suggested that caspase-8 activation was associated with p62/SQSTM1, an autophagic protein(Jin et al., 2009; Young et al., 2012; Zhang et al., 2013). The ubiquitin-binding protein p62 is required to complete the final activation by promoting aggregation of polyubiquitinated caspase-8. Meanwhile, p62 was an essential mocule to mediates the clearance of polyubiquitinated proteins in some diseases, such as Morbus Huntington (Kaniuk et al., 2007; Tung et al., 2010; Myeku and Figueiredo-Pereira,2011). The ubiquitined unwanted protein, caspase-8, which produced in oxidative stress process, was cleared by p62 adoption to autophagy (Bjorkoy et al., 2005; Pan et al., 2011). Moreover, p62-mediated autophagic degradation of active caspase-8 was a novel mechanism of cancer cell restraint to apoptosis(Hou et al., 2010). It prompted that p62 aggravation in cell plasma binding with active caspase-8 or other ubiquitinated proteins to neutralize their cytotoxicity and to degrade them by autophagy pathway. Therefore, it is controversial or complex to explain the role of p62 in caspase-8 activation. In H_2O_2-treated U87MG cells, we found that active caspase-8 level and/or cell viability was relative to the p62 protein level. Simultaneously, autophagy occurrence was together with p62 decreasing. In addition, UBA domain deletion leads to that p62 has no the ability to covalently bind ubiquitinated proteins (Ciani et al., 2003), which inhibited autophagic degradation of caspase-8 due to the loss of binding function. It suggested that harmful active caspase-8 was mediated by autophagy for the survival of cancer cells.

Autophagy and apoptosis are same important for regulating cell fate in response to cytotoxic stress(Levine and Kroemer,2008). Recent reports indicated that autophagy could be a adaptive cytoprotection response before cell death(Hung et al., 2009; Ishida and Nagata,2009; Han et al., 2012). The ability of autophagy even determined the fate of different cell under same stress(Liang et al., 2013). The association between caspase-8 and p62(Korolchuk et al., 2009; Ullman et al., 2011; Puissant et al., 2012) provide more evidences for the link of autophagy and apoptosis, which help us to better understand the complicated cell death processes. Therefore, H_2O_2 mediated apoptosis could be a suitable cell model to explore for the role of autophagy in cell death process. The detailed molecular mechanism on how capspase 8 activation modulated by p62 needs further investigation.

Acknowledgements

We thank Dr. Terje Johansen for providing the GFP-p62 plasmid and professor Ying-Yu Chen for the GFP-LC3 plasmid.

References

Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T.2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol.171, 603-14.

Ciani B, Layfield R, Cavey JR, Sheppard PW, Searle MS.2003. Structure of the ubiquitin-associated domain of p62 (SQSTM1) and implications for mutations that cause Paget's disease of bone. J Biol Chem.278, 37409-12.

Gerald D, Berra E, Frapart YM, Chan DA, Giaccia AJ, Mansuy D, Pouyssegur J, Yaniv M, Mechta-Grigoriou F.2004. JunD reduces tumor angiogenesis by protecting cells from oxidative stress. Cell.118, 781-94.

Han J, Pan XY, Xu Y, Xiao Y, An Y, Tie L, Pan Y, Li XJ.2012. Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative stress damage. Autophagy.8, 812-25.

Hou W, Han J, Lu C, Goldstein LA, Rabinowich H.2010. Autophagic degradation of active caspase-8: a crosstalk mechanism between autophagy and apoptosis. Autophagy.6, 891-900.

Hung SY, Huang WP, Liou HC, Fu WM.2009. Autophagy protects neuron from Abeta-induced cytotoxicity. Autophagy.5, 502-10.

Ishida Y, Nagata K.2009. Autophagy eliminates a specific species of misfolded procollagen and plays a protective role in cell survival against ER stress. Autophagy.5, 1217-9.

Jin Z, Li Y, Pitti R, Lawrence D, Pham VC, Lill JR, Ashkenazi A.2009. Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. Cell.137, 721-35.

Kaniuk NA, Kiraly M, Bates H, Vranic M, Volchuk A, Brumell JH.2007. Ubiquitinated-protein aggregates form in pancreatic beta-cells during diabetes-induced oxidative stress and are regulated by autophagy. Diabetes.56, 930-9.

Korolchuk VI, Menzies FM, Rubinsztein DC.2009. A novel link between autophagy and the ubiquitin-proteasome system. Autophagy.5, 862-3.

Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC.2009. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol Cell.33, 517-27.

Laussmann MA, Passante E, Dussmann H, Rauen JA, Wurstle ML, Delgado ME, Devocelle M, Prehn JH, Rehm M.2011. Proteasome inhibition can induce an autophagy-dependent apical activation of caspase-8. Cell Death Differ.18, 1584-97.

Levine B, Kroemer G.2008. Autophagy in the pathogenesis of disease. Cell.132, 27-42.

Liang X, Wei SQ, Lee SJ, Fung JK, Zhang M, Tanaka A, Choi AM, Jin Y.2013. p62 SQSTM1 /LC3b Complex Confers Cytoprotection on Lung Epithelial Cells After Hyperoxia. Am J Respir Cell Mol Biol.

Long J, Garner TP, Pandya MJ, Craven CJ, Chen P, Shaw B, Williamson MP, Layfield R, Searle MS.2010. Dimerisation of the UBA domain of p62 inhibits ubiquitin binding and regulates NF-kappaB signalling. J Mol Biol.396, 178-94.

Mitsuishi Y, Motohashi H, Yamamoto M.2012. The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. Front Oncol.2, 200.

Moscat J, Diaz-Meco MT.2012. p62: a versatile multitasker takes on cancer. Trends Biochem Sci.37, 230-6.

Myeku N, Figueiredo-Pereira ME.2011. Dynamics of the degradation of ubiquitinated proteins by proteasomes and autophagy: association with sequestosome 1/p62. J Biol Chem.286, 22426-40.

Pan JA, Ullman E, Dou Z, Zong WX.2011. Inhibition of protein degradation induces apoptosis through a microtubule-associated protein 1 light chain 3-mediated activation of caspase-8 at intracellular membranes. Mol Cell Biol.31, 3158-70.

Pan JA, Fan Y, Gandhirajan RK, Madesh M, Zong WX.2013. Hyperactivation of the mammalian degenerin MDEG promotes caspase-8 activation and apoptosis. J Biol Chem.288, 2952-63.

Pankiv S, Lamark T, Bruun JA, Overvatn A, Bjorkoy G, Johansen T.2010. Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to promyelocytic leukemia bodies. J Biol Chem.285, 5941-53.

Puissant A, Fenouille N, Auberger P.2012. When autophagy meets cancer through p62/SQSTM1. Am J Cancer Res.2, 397-413.

Saha T.2012. LAMP2A overexpression in breast tumors promotes cancer cell survival via chaperone-mediated autophagy. Autophagy.8, 1643-56.

Seo G, Kim SK, Byun YJ, Oh E, Jeong SW, Chae GT, Lee SB.2011. Hydrogen peroxide induces Beclin 1-independent autophagic cell death by suppressing the mTOR pathway via promoting the ubiquitination and degradation of Rheb in GSH-depleted RAW 264.7 cells. Free Radic Res.45, 389-99.

Sosa V, Moline T, Somoza R, Paciucci R, Kondoh H, ME LL.2013. Oxidative stress and cancer: an overview. Ageing Res Rev.12, 376-90.

Sun B, Sun GB, Xiao J, Chen RC, Wang X, Wu Y, Cao L, Yang ZH, Sun XB.2012. Isorhamnetin inhibits H(2)O(2)-induced activation of the intrinsic apoptotic pathway in H9c2 cardiomyocytes through scavenging reactive oxygen species and ERK inactivation. J Cell Biochem.113, 473-85.

Tartier L, McCarey YL, Biaglow JE, Kochevar IE, Held KD.2000. Apoptosis induced by dithiothreitol in HL-60 cells shows early activation of caspase 3 and is independent of mitochondria. Cell Death Differ.7, 1002-10.

Tung YT, Hsu WM, Lee H, Huang WP, Liao YF.2010. The evolutionarily conserved interaction between LC3 and p62 selectively mediates autophagy-dependent degradation of mutant huntingtin. Cell Mol Neurobiol.30, 795-806.

Ullman E, Pan JA, Zong WX.2011. Squamous cell carcinoma antigen 1 promotes caspase-8-mediated apoptosis in response to endoplasmic reticulum stress while inhibiting necrosis induced by lysosomal injury. Mol Cell Biol.31, 2902-19.

Vernon PJ, Tang D.2013. Eat-me: autophagy, phagocytosis, and reactive oxygen species signaling. Antioxid Redox Signal.18, 677-91.

Wu PF, Long LH, Zeng JH, Guan XL, Zhou J, Jin Y, Ni L, Wang F, Chen JG, Xie N.2012. Protection of L-methionine against H(2)O(2)-induced oxidative damage in mitochondria. Food Chem Toxicol.50, 2729-35.

Wu Y, Wang D, Wang X, Wang Y, Ren F, Chang D, Chang Z, Jia B.2011. Caspase 3 is activated through caspase 8 instead of caspase 9 during H_2O_2-induced apoptosis in HeLa cells. Cell Physiol Biochem.27, 539-46.

Yamakawa H, Ito Y, Naganawa T, Banno Y, Nakashima S, Yoshimura S, Sawada M, Nishimura Y, Nozawa Y, Sakai N.2000. Activation of caspase-9 and -3 during H_2O_2-induced apoptosis of PC12 cells independent of ceramide formation. Neurol Res.22, 556-64.

Yip KH, Feng H, Pavlos NJ, Zheng MH, Xu J.2006. p62 ubiquitin binding-associated domain mediated the receptor activator of nuclear factor-kappaB ligand-induced osteoclast formation: a new insight into the pathogenesis of Paget's disease of bone. Am J Pathol.169, 503-14.

Young JE, Martinez RA, La Spada AR.2009. Nutrient deprivation induces neuronal autophagy and implicates reduced insulin signaling in neuroprotective autophagy activation. J Biol Chem.284, 2363-73.

Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG.2012. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. J Biol Chem.287, 12455-68.

Zhang YB, Gong JL, Xing TY, Zheng SP, Ding W.2013. Autophagy protein p62/SQSTM1 is involved in HAMLET-induced cell death by modulating apotosis in U87MG cells. Cell Death Dis.4, e550.

Zhang YB, Gong JL, Xing TY, Zheng SP, Ding W.2013. Autophagy protein p62/SQSTM1 is involved in HAMLET-induced cell death by modulating apotosis in U87MG cells. Cell Death Dis. doi: 10.1038/cddis.2013.77.