FEBS Letters 587 (2013) 170–177

journal homepage: www.FEBSLetters.org

AMPK mediates a pro-survival autophagy downstream of PARP-1 activation in response to DNA alkylating agents

Jing Zhou [a,b], Shukie Ng [a,b], Qing Huang [b], You-Tong Wu [b], Zhengqiu Li [c], Shao Q. Yao [c], Han-Ming Shen [a,b,d,*]

[a] Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Republic of Singapore
[b] Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117597, Republic of Singapore
[c] Department of Chemistry, Faculty of Science, National University of Singapore, Singapore 117543, Republic of Singapore
[d] Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117597, Republic of Singapore

ARTICLE INFO

Article history:
Received 30 August 2012
Revised 16 November 2012
Accepted 16 November 2012
Available online 29 November 2012

Edited by Noboru Mizushima

Keywords:
Autophagy
AMPK
Alkylating agent
DNA damage
PARP-1
Necrosis

ABSTRACT

In this study we aim to elucidate the signaling pathway and biological function of autophagy induced by MNNG, a commonly used DNA alkylating agent. We first observed that MNNG is able to induce necrotic cell death and autophagy in *Bax*−/− *Bak*−/− double knockout MEFs. We analyzed the critical role of PARP-1 activation and ATP depletion in MNNG-mediated cell death and autophagy via AMPK activation and mTOR suppression. We provide evidence that suppression of AMPK blocks MNNG-induced autophagy and enhances cell death, suggesting the pro-survival function of autophagy in MNNG-treated cells. Taken together, data from this study reveal a novel mechanism in controlling MNNG-mediated autophagy via AMPK activation downstream of PARP-1 activation and ATP depletion.

1. Introduction

DNA alkylating agents are capable of inducing DNA damage by attaching an alkyl group to DNA, and have been widely used as chemotherapeutic drugs in cancer chemotherapy [1,2]. On the other hand, PARP-1 is the founding member of the PARP family, a group of nuclear enzymes that play a critical role in DNA damage repair through poly(ADP-ribosyl)ation [3]. It is known that poly-(ADP-ribosyl)ation is an energetically expensive process, causing rapid depletion of cellular β-nicotinamide adenine dinucleotide (NAD+), failure in ATP production, and eventually necrotic cell death [4]. It has been reported that *N*-methyl-*N*′-nitro-*N*-nitroso-guanidine (MNNG), a commonly used alkylating agent, kills cells by necrosis through massive production of DNA strand breaks, over-activation of PARP and ATP depletion [5,6].

Macroautophagy (referred as autophagy hereafter in this study) is a cellular catabolic degradation process in response to starvation or other stress conditions whereby cellular proteins and organelles are engulfed to autophagosomes and eventually digested via lysosomes to sustain cellular homeostasis [7,8]. Although it is still controversial regarding the role of autophagy in cell death or cell survival, accumulating evidence tends to suggest that autophagy generally serves as a pro-survival mechanisms, especially under stress conditions, such starvation, oxidative stress, metabolic stress and DNA damage [9–11]. So far, there are limited reports regarding the involvement of autophagy in the response to DNA alkylating agents. An early study showed that MNNG induced obvious increase of autophagic vacuoles in syrian golden hamster pancreatic organ explants [12]. Moreover, MNNG treatment was reported to induce autophagy in Myc/p53ERTAM lymphoma cells in vivo and knockdown of ATG5 significantly sensitized MNNG-induced cytotoxic effect [13], suggesting that autophagy serves as a survival pathway in tumor cells treated with alkylating agents. At present the signaling pathways involved in alkylating agent-induced autophagy are still elusive. In this study we have identified that PARP-1 activation, ATP depletion and subsequent activation of AMP-activated protein kinase (AMPK) play an important role in the induction of autophagy and such autophagy serves as a pro-survival function in MNNG-induced necrotic cell death.

* Corresponding author. Address: Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, 16 Medical Drive, Singapore 117597, Republic of Singapore. Fax: +65 6779 1489.
E-mail address: han-ming_shen@nuhs.edu.sg (H.-M. Shen).

0014-5793/$36.00 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.febslet.2012.11.018

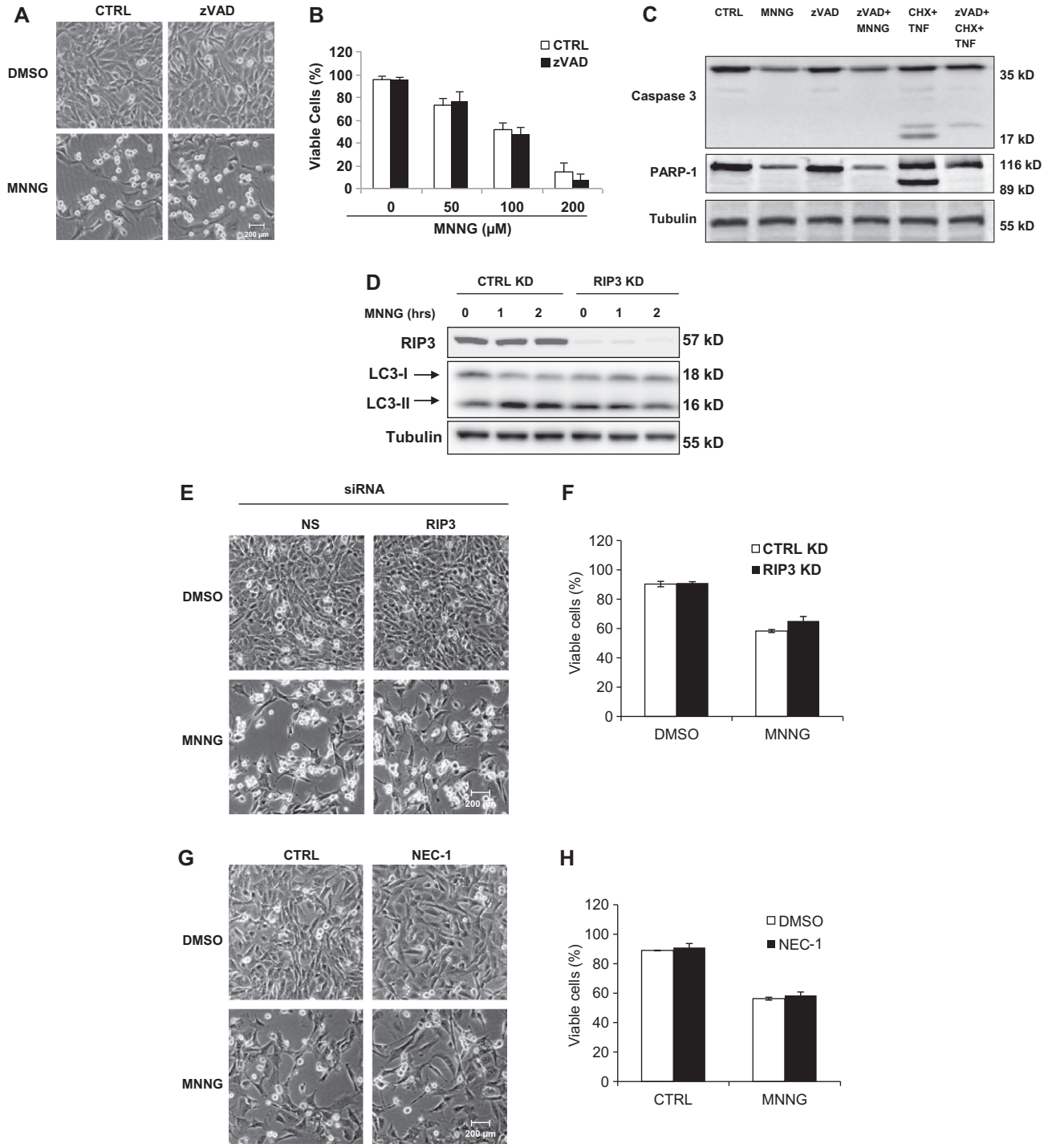

Fig. 1. MNNG induces caspase-independent necrotic cell death and autophagy. (A) DKO MEFs were pretreated with or without zVAD (50 μM) and exposed to MNNG (100 μM) for 24 h. Cell death was examined under microscope for morphological changes. (B) DKO MEFs were treated designated concentration of MNNG for 24 h, with or without zVAD. Cell viability was quantified by the PI exclusion assay coupled with flow cytometry. (C) DKO MEFs were exposed to MNNG (100 μM) for 24 h or TNFα (25 ng/ml) plus CHX (10 μg/ml) for 12 h in the presence or absence of zVAD (50 μM) and caspase-3 and PARP cleavage were detected by Western blot. (D) DKO MEFs were transfected with either control or RIP3 siRNA for 48 h, and cells were subsequently treated with MNNG (100 μM) for 24 h. (E) Morphological changes were examined under the microscope in cells as treated in (D). (F) Cell viability was measured using the same method as in (B) after the same treatment as in (D). (G) DKO MEF cells were treated with DMSO (control) or MNNG (100 μM), with or without necrostatin-1 (30 μM) for 24 h. Morphological pictures were captured under the microscope. (H) Cell viability was measured using the same method as in (B) after the same treatment as in (G). Data in panels B, F and H were presented as mean ± S.D. from three independent experiments.

2. Materials and methods

2.1. Reagents

MNNG, rapamycin, chloroquine (CQ), cycloheximide (CHX), propidium iodide (PI), ATP detection kit, and anti-tubulin antibody were purchased from Sigma (St. Louis, MO, USA). 3,4-Dihydro-5[4-(1-piperindinyl)butoxy]-1(2*H*)-isoquinoline (DPQ), necrostatin-1 and Compound C were from Calbiochem (San Diego, CA, USA). Pan-caspase inhibitor z-VAD-FMK was purchased from BioMol (Plymouth meeting, PA, USA). Antibodies against PARP-1, caspase-3, phospho-AMPK, AMPK, and phospho-p70S6K were

purchased from Cell Signaling (Beverly, MA, USA); anti-PAR antibody was from Trevigen (Gaithersburg, MD) and anti-RIP3 was from ProSci (Poway, CA, USA). Antibody against LC3 was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Mouse recombinant TNFα was obtained from R&D (Minneapolis, MN, USA). Mouse AMPKα siRNA was purchased from Santa Cruz (Santa Cruz, CA, USA). The ON-TARGETplus SMARTpool mouse Atg7 (#L-049953-00-0005), PARP-1 (#L-040023-00-0005), RIPK3 (#L-049919-00-0005), and a non-specific targeting ON-TARGETplus (#D-001810-10-05) siRNA were from Dharmacon Inc. (Lafayette, CO, USA).

2.2. Cell culture and MNNG treatment

Bax−/− Bak−/− double knockout (DKO) MEFs were kindly provided by Dr. Emily H.-Y. Cheng (Washington University, St. Louis, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Hyclone) in a 5% CO_2 atmosphere at 37 °C. For MNNG treatment, cells were treated in full medium with MNNG for 15 min. Cells were then washed and fed with fresh medium without MNNG and cultured for the indicated incubation periods.

2.3. Detection of cell death

Cell death was measured and quantified by (i) morphological changes examined under the light microscope, and (ii) live cell PI exclusion test coupled with flow cytometry, following established methods in our laboratory [14].

2.4. Western blotting

After designated treatments, cell were collected and washed once in PBS. Cell pellets were lysed in lysis buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF) with protease inhibitor cocktail (Roche). Western blotting was then performed following the established methods in our laboratory [15].

2.5. ATP measurement

Intracellular ATP was extracted from cells in the exponential phase of growth and measured by the luciferin/luciferase method using a bioluminescent somatic cell assay kit (Sigma) in accordance with the manufacturer's protocol. In brief, at the end of experiments, cells were collected using ice-cold cell dissociation solution (Sigma) and resuspended in ice-cold PBS (pH = 7.8). Cells were incubated with freshly prepared ATP assay mix and ATP releasing reagent and subjected to bioluminescent detection. The ATP level was presented as percentage to the untreated control group.

2.6. Transient siRNA transfection

Cells were transfected with 100 nM smartpool siRNA of Atg7, PARP-1, AMPKα, and RIPK3, together with a non-targeting siRNA control (from Dharmacon) using DharmaFECT™ 4 siRNA Transfection Reagent (Dharmacon) according to the supplier's protocol.

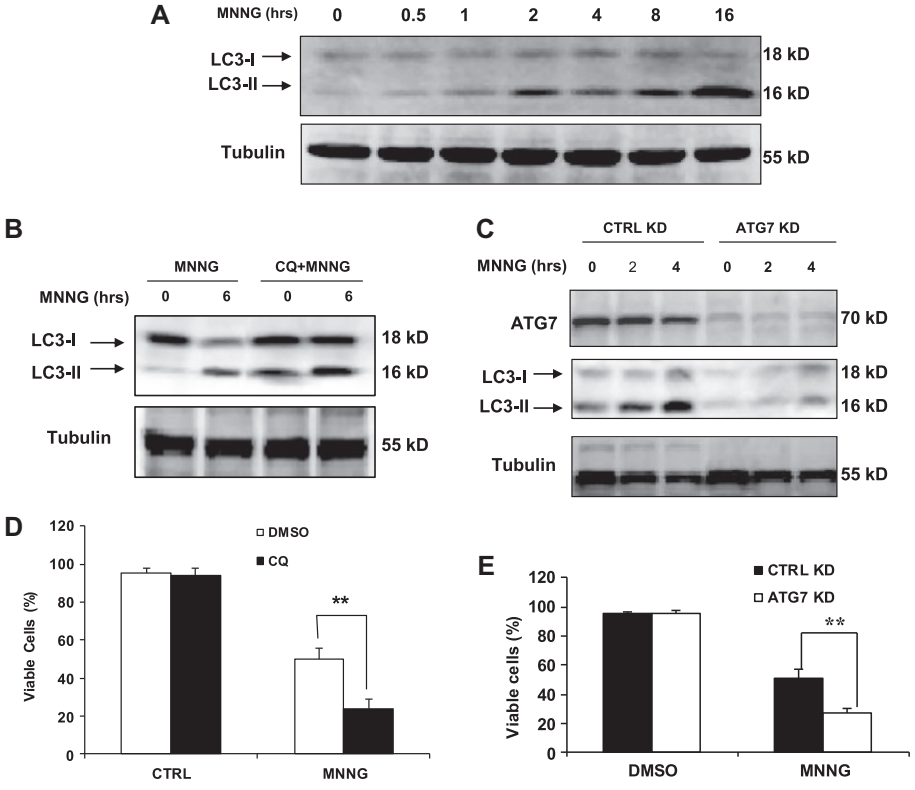

Fig. 2. Autophagy plays a pro-survival role in MNNG-induced cell death (A) DKO MEFs were treated with MNNG (100 μM) as designated and the LC3-II level was detected by Western blot. (B) DKO MEFs were treated with MNNG (100 μM) with or without the presence of CQ (40 μM) for detection of autophagic flux. (C) DKO MEFs were transfected with either control or ATG7 siRNA. Cells were then treated with MNNG (100 μM), and cell lysates were subjected to Western blot. (D) Cells were treated as described in panel (B) and cell death was quantified using the PI exclusion test coupled with flow cytometry when treated with MNNG for 24 h. (E) Cells were treated as described in panel (C) and cell death was quantified using the PI exclusion test coupled with flow cytometry when treated with MNNG for 24 h. Data in both panel D and E were presented as mean ± S.D. from three independent experiments (**$P < 0.01$, *t*-test).

2.7. Statistics

The results obtained from each experiment are expressed as mean ± standard deviation (SD) from at least three independent experiments. The significance level was set at $P < 0.05$ for each analysis using student's *t*-test.

3. Results

3.1. MNNG induces autophagy to protect against non-apoptotic cell death

Here we first examined MNNG-induced cell death in DKO MEFs, a cell line that is deficient of mitochondria-dependent apoptosis pathway and widely used in study of necrosis [16]. MNNG induced significant cell death concentration-dependently, based on morphological changes (Fig. 1A) and cell viability assay (Fig. 1B). Notably, no caspase 3 and PARP cleavage was observed in cells treated with MNNG (Fig. 1C). zVAD, a pan-caspase inhibitor, failed to block MNNG-induced cell death (Fig. 1A). To confirm the effectiveness of zVAD as a pan-caspase inhibitor, we tested cells treated with CHX + TNFα as a positive control of apoptosis and found that zVAD completely blocked TNFα-induced caspase 3 and PARP cleavage (Fig. 1C). These findings indicate that MNNG induces caspase-independent necrotic cell death in DKO MEFs, which are consistent with the previous findings [17]. Similar results were also observed in MNNG-treated L929 mouse sarcoma cells and *p53*−/− HCT116 colon cancer cells (data not shown). Necroptosis is a form of non-apoptotic cell death involving RIP1-RIP3 signaling pathway and could be blocked by necrostin-1 [18,19]. Here we performed RIP3 knockdown assay (Fig. 1D) and found that RIP3 knockdown failed to have any marked effect on MNNG-mediated cell death, based on both cell morphology and cell viability measured by the PI-exclusion test (Fig. 1E and F). Similar negative results were also found in cells treated with necrostatin-1 (Fig. 1G and H). Therefore, MNNG is likely to induce a form of necrotic cell death belong to either Parthanatos" based on the new recommendation by the Nomenclature Committee on Cell Death (NCCD) [19] or "PARP-mediated necrotic cell death" based on the previous classification [18].

3.2. Autophagy is a protective mechanism in MNNG-induced cell death

Next we examined the autophagic response in cells treated with MNNG. As shown in Fig. 2A, there was significant increase of LC3-II in DKO MEFs treated with MNNG, from 2 h onwards. Similar increase of LC3-II was also observed in *p53*−/− HCT116 colon cancer cells (data not shown). More importantly, the presence of CQ further increased the LC3-II level (Fig. 2B), suggesting that MNNG is capable of promoting autophagy flux. Finally, we attempted to determine the role of autophagy in MNNG-induced necrotic cell death by suppression of autophagy. First, autophagy was blocked by knockdown of ATG7. As shown in Fig. 2C, knockdown of ATG7 almost completely abolished the formation of LC3-II. Notably, knockdown of ATG7 significantly sensitized MNNG-induced cell death (Fig. 2E). Second, we tested the effect of CQ on MNNG-induced cell death and as shown in Fig. 2D, CQ markedly reduced the percentage of viable cells as determined by the PI live cell exclusion test. Taken together, these results indicate that autophagy acts as a cell survival mechanism in MNNG-induced necrotic cell death.

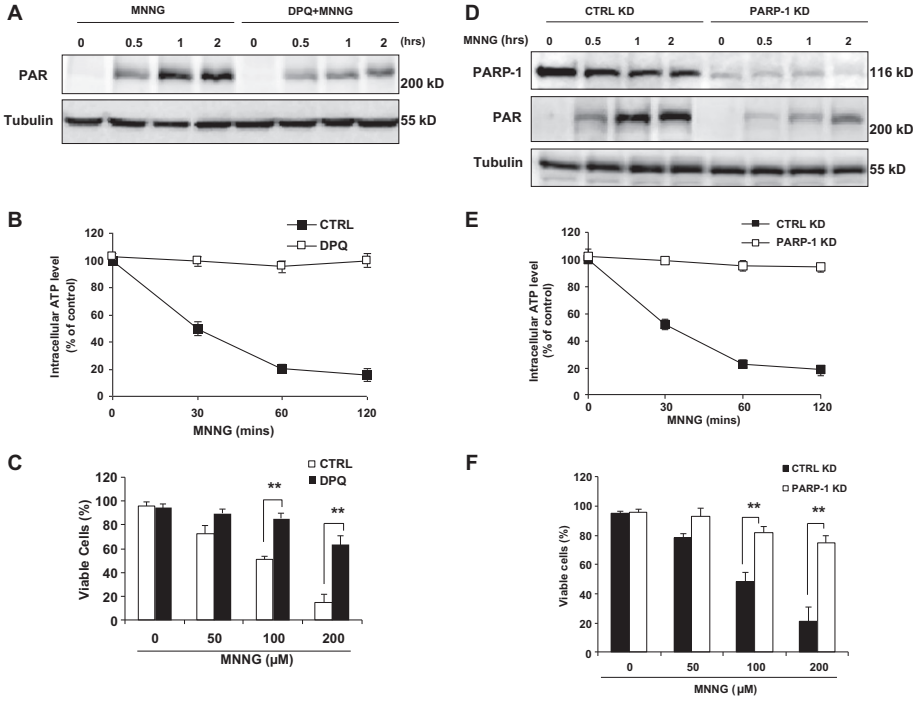

Fig. 3. Suppression of PARP-1 rescues MNNG-induced cell death. (A) MNNG-induced PARP-1 activation. DKO MEFs were exposed to MNNG (100 μM), with or without 1 h pretreatment of DPQ (10 μM), and the formation of PAR polymer was detected using Western blot. (B) DKO MEFs were treated as in panel A for measurement of intracellular ATP level. Data were shown as means ± S.D. of three independent experiments (**C**) Cells were exposed to MNNG (100 μM) for 24 h, with or without 1 h pretreatment of DPQ (10 μM). Cell viability was measured using the PI exclusion test coupled with flow cytometry. Data were shown as means ± S.D. of three independent experiments (**$P < 0.01$, *t*-test). (D) DKO MEFs were first subjected to PARP-1 siRNA knockdown, and then treated as in panel A. (E) DKO MEFs were treated as in panel D and the cellular ATP level was measured as in panel B. (F) Cells with PARP-1 knockdown were treated with MNNG (100 μM) for 24 h and cell viability was measured as in panel C. Data in panel C and F were shown as means ± S.D. of three independent experiments (**$P < 0.01$, *t*-test).

3.3. PARP-1 activation and ATP depletion lead to MNNG-meditated necrotic cell death

After establishing the pro-survival role of autophagy in MNNG-induced necrotic cell death, here we set to investigate the upstream signaling pathways controlling MNNG-mediated cell death. PARP-1 activation has been implicated in the cell death process induced by alkylating agent including MNNG [17,20]. Such cell death is believed to be the result of PARP-mediated rapid ATP depletion [21,22]. As expected, MNNG caused a strong formation of poly (ADP-ribose) (PAR) polymer (Fig. 3A) and dramatic decrease of intracellular ATP level (Fig. 3B). To test the involvement of PARP

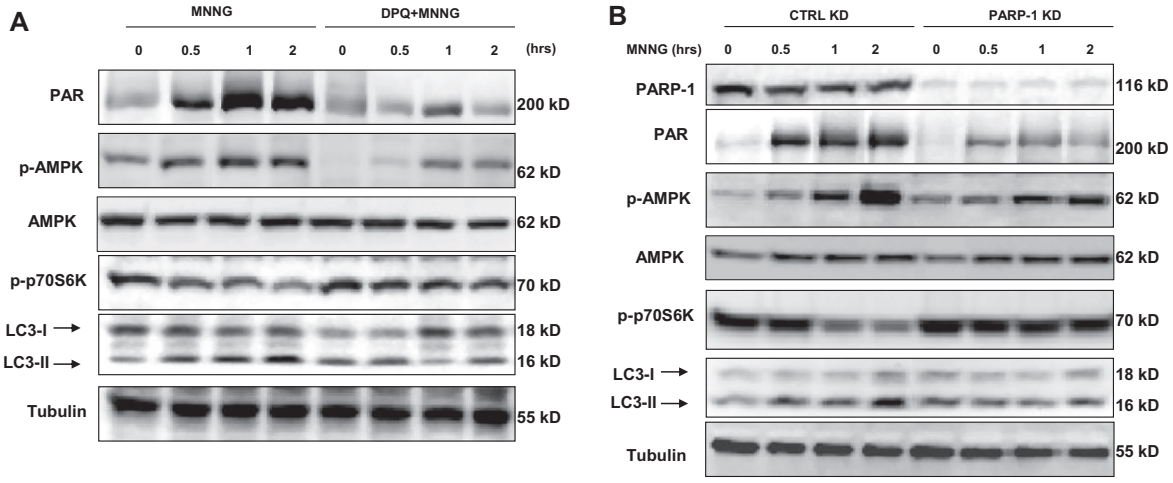

Fig. 4. PARP-1 activation leads to AMPK activation, mOTR suppression and autophagy. (A) DKO MEFs were treated as described in Fig. 3A and subjected to Western blot for analysis. (B) Knockdown of PARP-1 and treatment with MNNG were performed as described in Fig. 3D and cell lysates were subjected to Western blot for analysis.

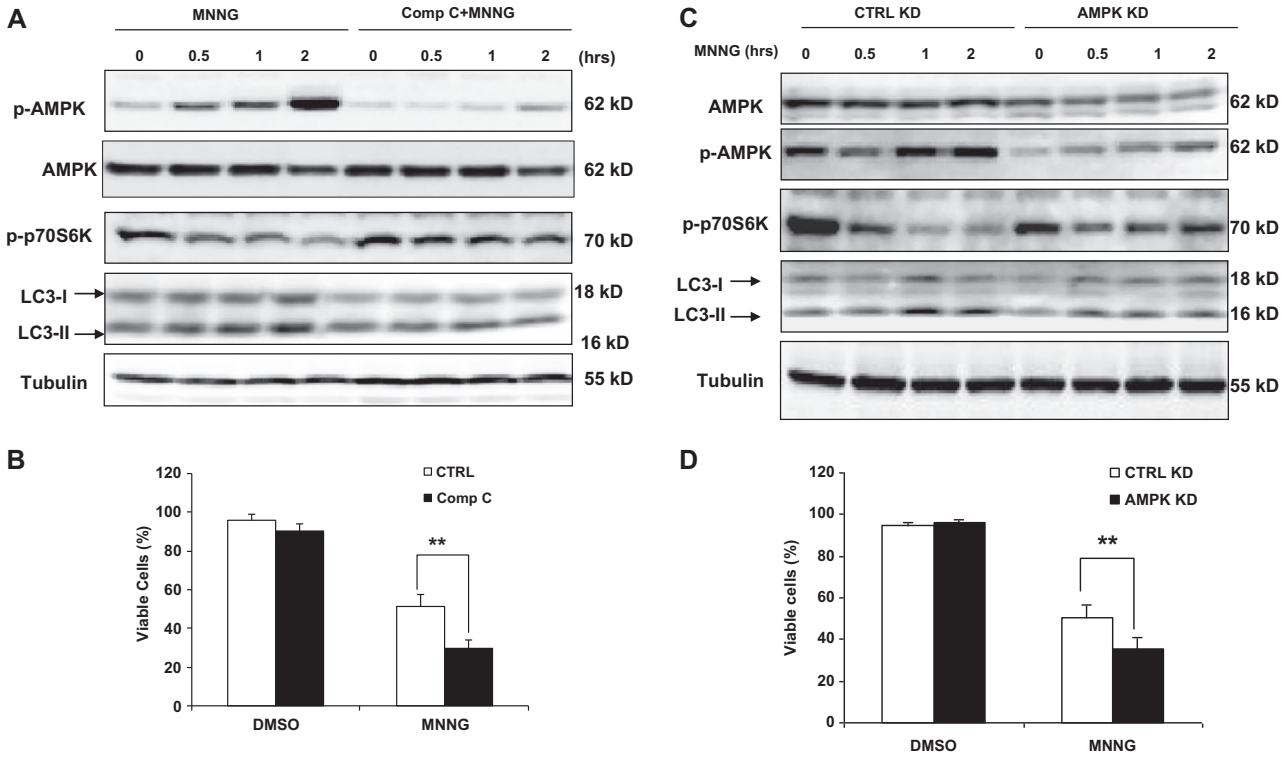

Fig. 5. Suppression of AMPK inhibits MNNG-mediated autophagy and sensitizes MNNG-induced cell death. (A) DKO MEFs were exposed to MNNG (100 μM) with or without 1 h pretreatment of Compound C (10 μM). Cells were collected and subjected to Western blot for analysis. (B) DKO MEF cells were treated with MNNG and Compound C as in panel A for 24 h and cell viability was determined using the PI exclusion test coupled with flow cytometry. (C) DKO MEFs with AMPK KD were treated as in panel A and cell lysates were collected for Western blot. (D) Cells were subjected to AMPK KD and MNNG treatment (for 24 h) and cell death was then quantified as in panel (B). (E) DKO MEFs were treated with MNNG (100 μM), with or without rapamycin (10 nM) for the indicated time. Cell lysates were collected and subsequently analyzed with Western blotting. (F) DKO MEF cells were treated with MNNG (100 μM), with or without rapamycin (20 nM) for 24 h and cell viability was quantified as described in Fig. 1B. Data in panel B, D and F were shown as means ± S.D. of three independent experiments ($^{**}P < 0.01$, *t*-test). (E) Illustration of the mechanisms for MNNG-induced autophagy and cell death, involving PARP-1 activation, ATP depletion, AMPK activation, and mTOR suppression.

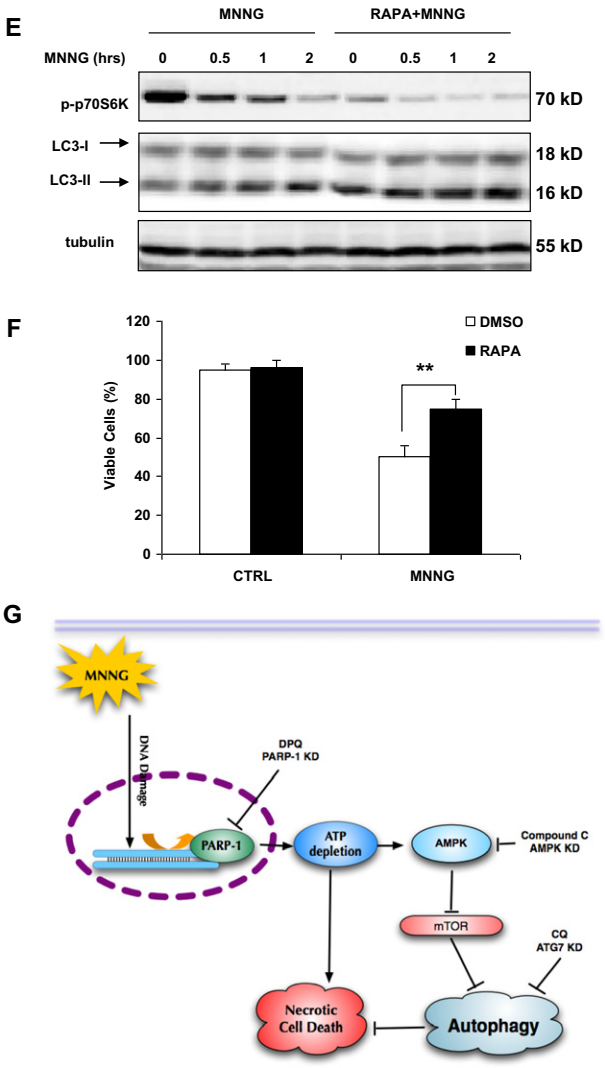

Fig. 5. (*continued*)

activation in cell death induced by MNNG, we used a specific chemical PARP inhibitor, DPQ [23]. DPQ markedly reduced the PAR formation (Fig. 3A), enhanced the intracellular ATP level (Fig. 3B) and effectively suppressed MNNG-induced cell death (Fig. 3C). Next, we used a genetic approach to knockdown PARP-1 using siRNA. PARP-1 knockdown almost completely abolished MNNG-induced PARP-1 activation (Fig. 3D), restored ATP level (Fig. 3E) and prevented MNNG-induced cell death (Fig. 3F). These data collectively suggest that PARP-1 activation and subsequent ATP depletion plays a critical role in MNNG-induced necrotic cell death.

3.4. AMPK mediates autophagy downstream of PARP activation in MNNG-treated cells

After establishing the role of PARP activation MNNG-induced cell death, here we aimed to study the signaling events downstream of PARP activation in MNNG-treated cells. Based on the well-established role of AMPK in autophagy via inhibition of mTOR [24,25], we hypothesize that, in response to MNNG-induced PARP-1 activation and ATP depletion, AMPK activation and subsequent mTOR inhibition might contribute to the induction of autophagy. To verify this, we first examined the changes of AMPK and mTOR activity in cells treated with MNNG. As shown in Fig. 4A and B, treatment with MNNG markedly enhanced PAR formation, with concurrent increase of p-AMPK level and reduction of p-p70S6K, suggesting the close association between PARP-AMPK activation and mTOR suppression. In order to establish the causative role of PARP-1 in MNNG-induced AMPK activation, mTOR suppression and autophagy induction, we used two different approaches to block PARP function, First, a chemical inhibitor DPQ markedly blocked MNNG-induced AMPK activation, restored the phosphorylation of p70S6K, and subsequently reduced LC3-II level (Fig. 4A). Second, similar effect was also found in cells with PARP-1 siRNA knockdown (Fig. 4B). Taken together, it is clearly indicated that MNNG-induced AMPK activation, mTOR suppression and autophagy induction is mediated by PARP-1 activation and ATP depletion.

3.5. Inhibition of AMPK sensitizes MNNG-induced cell death via suppression of autophagy

In this part of our study, we first examined the effect of a specific chemical inhibitor of AMPK, Compound C [26]. As shown in Fig. 5A, Compound C significantly suppressed MNNG-induced activation of AMPK and restored the phosphorylation of p70S6K, leading to reduction of LC3-I to LC3-II conversion. Notably, Compound C also enhanced MNNG-induced cell death (Fig. 5B). Compound C was recently reported to stimulate autophagy via a mechanism independent of AMPK [27]. In order to substantiate the findings from the chemical inhibitor, we used a genetic approach by AMPK knockdown. As shown in Fig. 5C, AMPK knockdown remarkably inhibited AMPK activation (shown by reduction of p-AMPK level) and reversed the inhibitory effect of MNNG on p70S6K phosphorylation, and subsequently suppressed LC3-I to LC3-II conversion (Fig. 5C). Similarly, AMPK knockdown also sensitized MNNG-induced cell death (Fig. 5D). Moreover, in order to further test the pro-survival function of autophagy in MNNG-mediated cell death, cells were treated with rapamycin, a specific inhibitor of mTOR, together with MNNG in Bax-Bak DKO MEFs. Rapamycin markedly reduced p-p70S6K level and increased LC3-II (Fig. 5E) and was able to offer significant protection against MNNG-induced cell death (Fig. 5F). It is thus believed that AMPK activation is an important pro-survival mechanism in MNNG-induced cell death via suppression of mTOR and activation of autophagy.

4. Discussion

Although autophagy is known to be implicated in the therapeutic effect of DNA alkylating agents such as MNNG [28–30], the molecular mechanisms underlying autophagy induced by MNNG remain largely elusive. In this study, we present evidence showing that PARP-1 activation following MNNG-induced DNA damage leads to pro-survival autophagy via the AMPK-mTOR signaling pathway. Therefore, PARP-1 appears to have dual roles in determining the cell fate in response to MNNG (summarized in Fig. 5G): (i) PARP-1 activation is the cause of non-apoptotic cell death via ATP depletion depletion [4,31]; and (ii) PARP-1 activation is able to elicit a self-protective mechanism by induction of autophagy. Our findings are indeed generally consistent with a very recent report on MNNG-induced necrotic cell death in human embryonic kidney HEK293 via PARP1 activation, ATP depletion, AMPK activation and mTOR suppression [32], although the role of autophagy was less studied.

One of the key findings of this study is the critical role of AMPK in MNNG-induced autophagy and cell death, downstream of PARP-1 activation and ATP depletion. AMPK is a well-conserved heterotrimeric kinase complex composed of a catalytic (α) subunit and two regulatory (β and γ) subunits. Serving as a cellular fuel gauge, AMPK is activated under stress conditions, particularly those that decrease ATP levels while increasing the AMP:ATP ratio, such as

hypoxia, ischeamia and glucose starvation [33]. At present, AMPK has been increasingly appreciated as one of the key tumor suppressors via suppression of mTOR and activation of autophagy [24,25]. AMPK serves as an important kinase that negatively regulates mTOR activity, through the following two pathways: (i) activated AMPK phosphorylates and stimulates the GAP activity of TSC1/TSC2 complex, causing conversion of GTPase Rheb to its inactive GDP from, and subsequently leads to the downregulation of mTOR activity [33]; and (ii) activated AMPK suppresses mTOR function by direct phosphorylation of raptor, an essential component of the mTOR complex I (mTORC1), on two well-conserved serine residues [34]. On the other hand, there is strong evidence setting AMPK as a key upstream regulator of autophagy. First, AMPK activation may lead to autophagy via suppression of mTORC1 as discussed above. Second, recent studies have set AMPK as the direct upstream activator of autophagy via its direct effect on ULK1, the ATG1 homolog in mammalians that plays a key role in autophagy initiation stage [35,36]. Consistently, data from our study demonstrate that activation of AMPK leads to suppression of mTORC1 and induction of autophagy in cells exposing to MNNG. In fact, the effect of MNNG is found be similar to that of hydrogen peroxide which is known to elicit a pro-survival autophagy via activation of PARP-1-AMPK and suppression of mTOR in both DKO MEFs and human cancer cells [37]. Therefore, our findings may represent a general mode of reaction in cancer cells in response to DNA damage.

After establishing the signaling pathway in MNNG-mediated autophagy, we then studied the function role of autophagy in MNNG-mediated necrotic cell death. Data from this study clearly suggest that autophagy is a cell survival mechanism in MNNG-induced cell death, based on the observations that suppression of autophagy by knockdown of ATG7 or blockage of lysosomal function by CQ sensitizes MNNG-induced cell death. Our data are found to be consistent with the earlier report that alkylating agent induces the pro-survival autophagy [13]. The pro-survival function of autophagy was further supported by the findings that induction of autophagy by rapamycin was able to offer significant protection against MNNG-mediated cell death. At present, there is growing understanding that autophagy is a protective mechanism under stress conditions, especially against necrotic cell death, with evidence from a series of in vivo studies in which autophagy is found to inhibit necrotic cell death of cancer cells under metabolic stress [38–40]. Therefore, targeting autophagy or its upstream signaling pathways (PARP-1-AMPK-mTOR) should be considered in developing more effective cancer therapeutic approaches.

In conclusion, as illustrated in Fig. 5G, in response to MNNG-induced DNA damage, PARP-1 appears to have dual roles in determining the cell fate in response to MNNG: PARP-1 activation is the cause of non-apoptotic cell death via ATP depletion and PARP-1 activation is able to elicit a self-protective mechanism by induction of autophagy via the AMPK-mTOR pathway. Our findings thus provide novel insights into the complex relationship among alkylating agent-induced DNA damage, autophagy and cell death.

Acknowledgements

We would like to thank Drs. Emily Cheng and T. Yoshimori for reagents. S.N. and Y.T.W. were supported by a NUS scholarship and Q.H. was supported by the Toxicology Program, Office of Life Science, NUS. The research work was supported by research grants from Singapore National Medical Research Council (NMRC/1260/2010) to S.Q.Y. and H.M.S.

References

[1] Schabel Jr., F.M. (1976) Nitrosoureas: a review of experimental antitumor activity. Cancer Treat. Rep. 60, 665–698.
[2] Wyatt, M.D. and Pittman, D.L. (2006) Methylating agents and DNA repair responses: Methylated bases and sources of strand breaks. Chem. Res. Toxicol. 19, 1580–1594.
[3] Chatterjee, S., Berger, S.J. and Berger, N.A. (1999) Poly(ADP-ribose) polymerase: a guardian of the genome that facilitates DNA repair by protecting against DNA recombination. Mol. Cell Biochem. 193, 23–30.
[4] Ha, H.C. and Snyder, S.H. (1999) Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. Proc. Natl. Acad. Sci. USA 96, 13978–13982.
[5] Keil, C., Grobe, T. and Oei, S.L. (2006) MNNG-induced cell death is controlled by interactions between PARP-1, poly(ADP-ribose) glycohydrolase, and XRCC1. J. Biol. Chem. 281, 34394–34405.
[6] Affar el, B., Shah, R.G., Dallaire, A.K., Castonguay, V. and Shah, G.M. (2002) Role of poly(ADP-ribose) polymerase in rapid intracellular acidification induced by alkylating DNA damage. Proc. Natl. Acad. Sci. USA 99, 245–250.
[7] Kroemer, G., Marino, G. and Levine, B. (2010) Autophagy and the integrated stress response. Mol. Cell 40, 280–293.
[8] Levine, B. and Kroemer, G. (2008) Autophagy in the pathogenesis of disease. Cell 132, 27–42.
[9] Mizushima, N. and Komatsu, M. (2011) Autophagy: renovation of cells and tissues. Cell 147, 728–741.
[10] Shen, H.M. and Codogno, P. (2011) Autophagic cell death: Loch Ness monster or endangered species? Autophagy 7, 457–465.
[11] Shen, S. et al. (2011) Association and dissociation of autophagy, apoptosis and necrosis by systematic chemical study. Oncogene 30, 4544–4556.
[12] Resau, J.H., Cottrell, J.R., Hudson, E.A., Trump, B.F. and Jones, R.T. (1985) Studies on the mechanisms of altered exocrine acinar cell differentiation and ductal metaplasia following nitrosamine exposure using hamster pancreatic explant organ culture. Carcinogenesis 6, 29–35.
[13] Amaravadi, R.K., Yu, D., Lum, J.J., Bui, T., Christophorou, M.A., Evan, G.I., Thomas-Tikhonenko, A. and Thompson, C.B. (2007) Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J. Clin. Invest. 117, 326–336.
[14] Ng, S., Wu, Y.T., Chen, B., Zhou, J. and Shen, H.M. (2011) Impaired autophagy due to constitutive mTOR activation sensitizes TSC2-null cells to cell death under stress. Autophagy 7, 1173–1186.
[15] Wu, Y.T. et al. (2010) Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. J. Biol. Chem. 285, 10850–10861.
[16] Wei, M.C. et al. (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292, 727–730.
[17] Zong, W.X., Ditsworth, D., Bauer, D.E., Wang, Z.Q. and Thompson, C.B. (2004) Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev. 18, 1272–1282.
[18] Degterev, A. and Yuan, J. (2008) Expansion and evolution of cell death programmes. Nat. Rev. Mol. Cell Biol. 9, 378–390.
[19] Galluzzi, L. et al. (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ. 19, 107–120.
[20] D'Amours, D., Desnoyers, S., D'Silva, I. and Poirier, G.G. (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J. 342 (Pt 2), 249–268.
[21] Shall, S. and de Murcia, G. (2000) Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? Mutat. Res. 460, 1–15.
[22] Haince, J.F., Rouleau, M., Hendzel, M.J., Masson, J.Y. and Poirier, G.G. (2005) Targeting poly(ADP-ribosyl)ation: a promising approach in cancer therapy. Trends Mol. Med. 11, 456–463.
[23] Lee, M.W., Kim, W.J., Beardsley, D.I. and Brown, K.D. (2007) N-methyl-N′-nitro-N-nitrosoguanidine activates multiple cell death mechanisms in human fibroblasts. DNA Cell Biol. 26, 683–694.
[24] Inoki, K., Kim, J. and Guan, K.L. (2012) AMPK and mTOR in cellular energy homeostasis and drug targets. Annu. Rev. Pharmacol. Toxicol. 52, 381–400.
[25] Alers, S., Loffler, A.S., Wesselborg, S. and Stork, B. (2012) Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. Mol. Cell Biol. 32, 2–11.
[26] Zhou, G. et al. (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108, 1167–1174.
[27] Vucicevic, L. et al. (2011) Compound C induces protective autophagy in cancer cells through AMPK inhibition-independent blockade of Akt/mTOR pathway. Autophagy 7, 40–50.
[28] Mathew, R., Karantza-Wadsworth, V. and White, E. (2007) Role of autophagy in cancer. Nat. Rev. Cancer 7, 961–967.
[29] Amaravadi, R.K. and Thompson, C.B. (2007) The roles of therapy-induced autophagy and necrosis in cancer treatment. Clin. Cancer Res. 13, 7271–7279.
[30] Dang, C.V. (2008) Antimalarial therapy prevents Myc-induced lymphoma. J. Clin. Invest. 118, 15–17.
[31] Jagtap, P. and Szabo, C. (2005) Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat. Rev. Drug Discov. 4, 421–440.
[32] Ethier, C., Tardif, M., Arul, L. and Poirier, G.G. (2012) PARP-1 modulation of mTOR signaling in response to a DNA alkylating agent. PLoS One 7, e47978.
[33] Hardie, D.G. (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat. Rev. Mol. Cell Biol. 8, 774–785.
[34] Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E. and Shaw, R.J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30, 214–226.

[35] Kim, J., Kundu, M., Viollet, B. and Guan, K.L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. 13, 132–141.
[36] Egan, D.F. et al. (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331, 456–461.
[37] Huang, Q. and Shen, H.M. (2009) To die or to live: the dual role of poly(ADP-ribose) polymerase-1 in autophagy and necrosis under oxidative stress and DNA damage. Autophagy 5, 273–276.
[38] Karantza-Wadsworth, V., Patel, S., Kravchuk, O., Chen, G., Mathew, R., Jin, S. and White, E. (2007) Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev. 21, 1621–1635.
[39] Shen, S., Kepp, O. and Kroemer, G. (2012) The end of autophagic cell death? Autophagy 8, 1–3.
[40] Shen, H.M. and Codogno, P. (2012) Autophagy is a survival force via suppression of necrotic cell death. Exp. Cell Res. 318, 1304–1308.