Autophagy Controls p38 Activation to Promote Cell Survival under Genotoxic Stress*§

Lei Qiang‡, Chunli Wu‡§, Mei Ming‡, Benoit Viollet‡¶§, and Yu-Ying He‡§

From the ‡Section of Dermatology, Department of Medicine, University of Chicago, Chicago, Illinois 60637, the §Department of Radiation Oncology, 4th Affiliated Hospital, China Medical University, Shenyang, Liaoning 110001, China, and ¶INSERM, U1016, Institut Cochin, CNRS, UMR8104, and **Université Paris Descartes, Sorbonne Paris Cité, 75014 Paris, France

Deregulated cell survival under carcinogen-induced genotoxic stress is vital for cancer development. One of the cellular processes critical for cell survival under metabolic stress and energy starvation is autophagy, a catabolic process involved in capture and delivery of cytoplasmic components to lysosomes for degradation. However, the role of autophagy following carcinogen-induced genotoxic stress remains unclear. Here we show that UVB radiation, a known human skin carcinogen that induces autophagy under relevant carcinogen-induced genotoxic stress, sensitizes cells to UVB-induced apoptosis through p62-dependent p38 activation. Compared with normal human skin, autophagy was activated in human squamous cell carcinomas, in association with decreased phosphorylation of p38, and increased phosphorylation of ATR and formation of γ-H2AX, two markers of DNA damage response. Our results demonstrate that autophagy promotes cell survival through suppressing p62-mediated p38 activation and thus may facilitate tumor development under genotoxic stress. These findings suggest that autophagy plays an oncogenic role in epithelial carcinogenesis by promoting cell survival.

Autophagy is a catabolic process by which cellular proteins, cytoplasm, and organelles are captured and targeted for proteolytic degradation in lysosomes (1). The role of autophagy in tumor development is complex because it can be a tumor-promoting or -suppressing process (2, 3). Both normal and tumor cells use autophagy to survive metabolic stress, and as such, autophagy inhibitors are predicted to have the potential for cancer therapy (4–7). On the other hand, autophagy may act as a tumor suppressor. Probable causes of cancer associated with defects in autophagy and impaired tolerance to stress include enhanced cell death resulting in an inflammatory response and cytokine production, and genome damage (8). Another possibility is that functional autophagy might limit accumulation of mutated and harmful proteins, and chromosomal instability (9).

Autophagy is regulated at multiple levels. Two of the important regulators are mammalian target of rapamycin (mTOR)² and AMP kinase (AMPK). mTOR is a key sensor for cellular nutrient and energy levels (10); inhibition of mTOR induces autophagy (11, 12). AMPK is a ubiquitous serine/threonine kinase that acts as a highly sensitive sensor of cell energy stores (13). In conditions where nutrients are scarce, AMPK acts as a metabolic checkpoint inhibiting cellular growth, which has recently been connected to cellular processes such as autophagy (14). AMPK regulates autophagy through suppression of the mTORC1 pathway (15), involving the direct phosphorylation of the tumor suppressor TSC2 and the mTORC1 subunit raptor (16, 17). Recently AMPK has been shown to induce autophagy by directly phosphorylating several sites in the autophagy factor ULK1 (12, 18), in addition to unbiased mass spectrometry studies showing that endogenous AMPK subunits are ULK1 interactors (19, 20). However, the role of AMPK remains controversial. AMPK activation either promotes or inhibits cell death in a context-dependent and/or cell type-dependent manner (21, 22).

Autophagy is induced in response to nutrient deprivation (23), metabolic stress (9), endoplasmic reticulum stress (24), and anticancer treatments (25, 26). However, the role of autophagy under relevant carcinogen-induced genotoxic stress remains unclear. In this study, we have investigated whether and how UVB radiation, a known human skin carcinogen that

---

* This work was supported, in whole or in part, by National Institutes of Health Grants ES016936 (to Y.-Y. H.) and UL1RR024999 through the Clinical and Translational Science Award (CTSA) Program. This work was also supported by American Cancer Society Grant RSG-13-078-01 (to Y.-Y. H.), University of Chicago Comprehensive Cancer Center Pilot Program Grant P30 CA014299, and the University of Chicago Friends of Dermatology Research.

§ This article contains supplemental Methods and additional references.

* To whom correspondence should be addressed. Tel.: 773-795-4696; Fax: 773-702-8398; E-mail: yyhe@medicine.bsd.uchicago.edu.

---

** The abbreviations used are: mTOR, mammalian target of rapamycin; ACC, acetyl-CoA-carboxylase; AICAR, 5-amino-1-[β-D-ribofuranosyl]-imidazole-4-carboxamide; AMPK, AMP kinase; BfnA1, baflomycin A1; 3-MA, 3-methyladenine; MEF, mouse embryonic fibroblast; mJ, millijoules; NHEK, normal human epidermal keratinocyte; PARP, poly(ADP-ribose) polymerase; SCC, squamous cell carcinoma.
acts by causing DNA damage (27), activated autophagy and what the functional relevance is to skin cancer pathogenesis. We found that UVB induces autophagy through AMPK activation. Accumulation of p62 in autophagy-deficient cells increases UVB-induced activation of the stress response pathway p38 and subsequent apoptosis. Our findings uncover the indispensable function of autophagy in cell survival under carcinogen-induced genotoxic stress, suggesting an oncogenic role of autophagy.

MATERIALS AND METHODS

Human Skin Tumor Samples—All human specimens were studied after approval by the University of Chicago Institutional Review Board.

UVB Irradiation—Cells were washed twice with PBS and then exposed to UVB radiation system (UV Stratalinker 2400 with UVB bulbs; Stratagene) at different doses. Control samples were sham-irradiated under the same conditions. The UVB doses were monitored weekly using a Goldilux UV meter equipped with a UVB detector (Oriel Instruments).

Statistical Analyses—Data were expressed as the mean of three independent experiments and analyzed by Student’s t test. A two-sided value of p < 0.05 was considered significant in all cases.

The details for cell culture, plasmids and transfection, immunoprecipitation, cell apoptosis, Western blotting, and immunofluorescence assays can be found in the supplemental Methods.

RESULTS

UVB Induces Autophagy—A primary hallmark of mammalian autophagy is the conversion of LC3-I, one form of the mammalian homolog of the yeast Atg8 gene product, to another form, LC3-II, through proteolytic cleavage and lipidation (28, 29). Another distinctive hallmark is the translocation of LC3 from the cytosol to the autophagic vacuoles (30). In addition, the protein p62 (also known as SQSTM1) has been reported to interact with the autophagic effector protein LC3 and is degraded through an autophagy-lysosome pathway (31). To determine the effect of UVB on autophagy in skin, we exposed normal human epidermal keratinocytes (NHEK) to UVB irradiation and then analyzed the levels of LC3-I, LC3-II, and p62.

FIGURE 1. UVB induces autophagy and autophagic flux. A, NHEK cells were exposed to UVB (10 and 20 mJ/cm²) and incubated for the indicated times. The levels of LC3-I, LC3-II, and p62 were analyzed by immunoblot assay. B, NHEK cells were exposed to UVB (10 mJ/cm²) and incubated for the indicated times or treated with rapamycin (Rap, 500 nM) for 6 h with or without BfnA1 pretreatment (50 nM, 2 h). The levels of LC3-I, LC3-II, and p62 were analyzed by immunoblot assay. C, WT and Atg5 KO MEF cells were exposed to UVB (20 mJ/cm²) and incubated for 1.5, 3, 6, and 12 h. The punctuate localization of LC3 was analyzed by immunofluorescence assay. Scale bar, 50 μm. Red, LC3; blue, DAPI nuclear counterstain. D, quantification of the LC3 puncta using ImageJ software (mean ± S.D. (error bars), n = 3; *, p < 0.05). The data shown are representative of three independent experiments. E, WT and Atg5 KO MEF cells were exposed to UVB (20 mJ/cm²) and incubated for 6 h or treated with rapamycin (500 nM) for 6 h with or without pretreatment with BfnA1 (50 nM) for 2 h. The levels of LC3-I, LC3-II, and p62 were analyzed by immunoblot assay. F and H, WT (F) and Atg5 KO (H) MEF cells were transfected with mRFP-EGFP-LC3 and exposed to UVB (20 mJ/cm²) and incubated for 6 h or treated with rapamycin (500 nM) for 6 h with or without BfnA1 pretreatment (50 nM, 2 h). The punctuate localization of LC3 ( GFP and RFP) was analyzed by fluorescence microscopy. Scale bar, 20 μm. G and I, images from F (G) and H (I) were subsequently quantified for the LC3 puncta using the ImageJ Green and Red Puncta Colocalization plugin. The results were obtained from three independent experiments with at least 100 cells analyzed (mean ± S.D. (error bars), n = 3; *, p < 0.05; **, p < 0.01, compared with the sham group; #, p < 0.05, compared with the sham/bafilomycin group).
mTOR (32), was used as a positive control. Similar to rapamycin, UVB induced autophagic flux (Fig. 1B). Similarly, UVB also induced autophagy in wild-type (WT) mouse embryonic fibroblasts (MEFs), as indicated by the accumulation of LC3 around and inside the autophagic vacuoles, with strong punctuate dots, whereas deletion of the autophagy gene \textit{Atg5} in \textit{Atg5} knock-out (KO) MEFs abolished the basal and UVB-induced autophagy (Fig. 1C, and D). Using the tandem mRFP-EGFP-LC3 construct, another tool for analyzing autophagic flux, we found that UVB induced the autophagic flux in WT MEFs but not in \textit{Atg5} KO MEFs (Fig. 1E–I). Our results clearly demonstrate that UVB induces autophagy and autophagic flux in both NHEK and MEF cells.

**FIGURE 2. Activation of the AMPK pathway is required for UVB-induced autophagy.** A, WT and \textit{Ampk} KO MEF cells were exposed to UVB (20 mJ/cm$^2$) and then incubated for the indicated times. The levels of AMPK, p-AMPK, ACC, p-ACC, and p-ULK1 were analyzed by immunoblot assay. B, WT and \textit{Ampk} KO MEF cells were exposed to UVB (20 mJ/cm$^2$) and incubated for the indicated times, and the levels of LC3-I, LC3-II, AMPK, and p-AMPK were analyzed by immunoblot assay. C, WT and \textit{Ampk} KO MEF cells were exposed to UVB (20 mJ/cm$^2$) and incubated for 6 h or treated with AICAR (AI, 1 mM) for 6 h with or without BfnA1 pretreatment (50 nm, 2 h). The levels of LC3-I, LC3-II, AMPK, and p-AMPK were analyzed by immunoblot assay. D and F, WT (D) and \textit{Ampk} KO (F) MEF cells were transfected with mRFP-EGFP-LC3, exposed to UVB (20 mJ/cm$^2$), and incubated for 6 h or treated with AICAR (1 mM) for 6 h with or without BfnA1 pretreatment (50 nm, 2 h). The puncta of LC3 (GFP and RFP) were analyzed by fluorescence microscopy. Scale bar, 20 mm. E and G, images from D (E) and F (G) were quantified for the LC3 puncta using the ImageJ Green and Red Puncta Colocalization plugin. The results were obtained from three independent experiments with at least 100 cells analyzed (mean ± S.D. (error bars), n = 3; *, p < 0.05; **, p < 0.01, compared with the sham group; #, p < 0.05, compared with the sham/bafilomycin group).

UVB-induced Autophagy Requires a Functional AMPK Pathway—To determine the mechanism by which UVB induces autophagy, we first analyzed the role of AMPK, an activator of autophagy, by phosphorylating the autophagy component ULK1/2 (12, 18). In WT MEF cells, UVB increased phosphorylation of AMPK, and acetyl-CoA-carboxylase (ACC) and ULK1, two downstream targets of the AMPK pathway, whereas no phosphorylation of AMPK, ACC, or ULK1 was detected in \textit{Ampk} KO MEF cells (Fig. 2A, and B). At early time points UVB induced AMPK activation and increased the levels of LC3-II, whereas \textit{Ampk} deletion markedly reduced the levels of LC3-II (Fig. 2B). Autophagic flux assay showed that, similar to AICAR, a widely used AMPK activator, UVB activated the AMPK pathway and induced autophagic flux (Fig. 2C). Similar results were observed in tan-
These data indicate that AMPK is required for UVB-induced autophagy and autophagic flux.

**Autophagy Promotes Cell Survival under UVB-induced Genotoxic Stress**—To determine the role of AMPK-mediated autophagy in cell survival following UVB damage, we analyzed apoptosis in WT, Ampk KO, and Atg5 KO MEF cells. UVB-induced apoptosis in Ampk KO cells at the 24-h time point but not in WT cells (Fig. 3, A–C). In addition, autophagy deficiency sensitized MEF cells to UVB-induced apoptosis, as indicated by increased levels of PARP cleavage and cleaved caspase-3 and by increased number of cells in the sub-G1 phase in Atg5 KO cells compared with WT cells (Fig. 3, D and E). Treatment with 3-MA, a widely used autophagy inhibitor (33), significantly sensitized cells to UVB-induced apoptosis in Atg5 WT cells but not in Atg5 KO cells (Fig. 3, D and E). A similar effect of 3-MA was detected in WT and Ampk KO cells (Fig. 3, F and G). These data indicate that AMPK-mediated autophagy is essential for cell survival under UVB-induced genotoxic stress.

**Autophagy Deficiency Results in the Activation of p38**—It has been reported that an increase in p38 activation sensitizes cells to UVB-induced apoptosis in several cell types (34–37). In addition, ATF3 has been shown to be the downstream effector of p38, mediating its proapoptotic function (38, 39). To determine the downstream effectors mediating the role of autophagy in cell survival, we assessed the effect of autophagy deficiency on p38 activation and ATF3 expression. Compared with WT MEF cells, p38 activation and ATF3 protein levels were aug-
Autophagy in Cell Survival under Genotoxic Stress

In Autophagy-deficient Cells—To further investigate the role of p38 activation in the effect of autophagy on UVB-induced apoptosis, WT, Atg5 KO, and Ampk KO MEF cells were pre-pretreated with 3-MA (5 mM) alone or with the p38 pathway inhibitors SB202190 (5 μM) or SB203580 (5 μM) for 3 h and then exposed to UVB. Inhibition of p38 activation reduced the cleavage of PARP and caspase-3 and the percentage of sub-G1 cells induced by UVB in 3-MA-treated WT cells, and in vehicle- or 3-MA-treated Atg5 KO (Fig. 5, A and B) or Ampk KO cells (Fig. 5, C and D). The same effect was also detected in HaCaT cells (Fig. 5E). Inhibition of autophagy by 3-MA significantly increased UVB-induced apoptosis, which was largely reversed by the p38 pathway inhibitors SB202190 (5 μM) or SB203580 (5 μM) (Fig. 5E). Taken together, these data indicate that inhibition of p38 activation reduces the UVB-induced apoptosis in autophagy-deficient cells.

Accumulation of p62 Is Required for p38 Hyperactivation and Apoptosis—To determine the mechanism by which autophagy regulates p38 activation following UVB damage, we investigated the role of p62, which has been reported to accumulate upon autophagy inhibition (31). Immunoprecipitation analysis showed that p62 bound to p38 and the binding was increased in Ampk KO and Atg5 KO cells compared with that in WT cells (Fig. 6, A and B). As p38 was activated following UVB irradiation and p62 levels were increased in Ampk KO and Atg5 KO cells, the binding of p-p38 to p62 was increased by UVB irradiation and further increased in Ampk KO and Atg5 KO cells (Fig. 6, A and B). These data indicate that autophagy deficiency increases the binding of p62 to p38. To determine the role of p62 in p38 activation and the proapoptotic effect of autophagy inhibition, we used siRNA targeting p62. Knockdown of p62 in Atg5 KO cells decreased basal p38 phosphorylation in the absence of UVB irradiation (Fig. 6C). In particular, knockdown of p62 inhibited UVB-induced p38 phosphorylation and ATF3 up-regulation in WT cells, Ampk KO, and Atg5 KO cells (Fig. 6, D and E). Knockdown of p62 significantly reduced UVB-induced apoptosis in Ampk KO and Atg5 KO cells, whereas little effect was detected in WT MEF cells (Fig. 6, F and G). Taken together, our findings indicate that accumulation of 62 is required for UVB-induced hyperactivation of p38 and increased apoptosis in autophagy-deficient cells.

Autophagy Induction Is Associated with Increased DNA Damage Response and Decreased p38 Phosphorylation in Human Squamous Cell Carcinoma (SCC)—To further investigate the specific function of autophagy in cancer development, we evaluated the levels of LC3-II, the autophagy-related protein Beclin 1, p38 phosphorylation, and DNA damage response status in human skin SCC derived from sun-exposed areas and unpaired sun-protected normal human skin. Compared with normal skin samples, all three SCC samples showed increased autophagy and Beclin 1 levels and were positive for phosphorylation of ATR (ataxia telangiectasia and Rad3-related protein) and formation of γ-H2AX, two known markers for DNA damage response, whereas p38 phosphorylation was reduced (Fig. 7). These data suggest that autophagy induction is associated with reduced p38 activation and DNA damage response in UV-associated human SCC, which likely promotes cancer cell survival following UVB stress or secondary genotoxic stress due to genomic instability.

DISCUSSION

As a tightly regulated cellular self-eating process, autophagy has been linked to a wide range of pathological conditions including neurodegenerative diseases, infection, and cancer (40). In established tumors, autophagy is activated in response to multiple stresses including nutrient starvation, endoplasmic reticulum stress, and hypoxia to maintain mitochondrial function (2, 3). However, the role of autophagy in response to human carcinogen-induced or endogenous genotoxic stress remains unclear. In this study, we showed that the human skin carcinogen UVB induces autophagy in keratinocytes and MEF cells through AMPK activation. AMPK-mediated autophagy inhibits UVB-induced apoptosis, which may block the eradication of potentially mutagenic cells. Autophagy inhibition also augmented p38 activation through p62 up-regulation, which sensitized cells to UVB-induced apoptosis. In human SCC, autophagy was activated in association with the activation of the DNA damage response pathway and reduced p38 phosphorylation, suggesting that during cancer development, autophagy may provide a survival advantage by inhibiting the p38 pathway following UVB-induced DNA damage and later endogenous genotoxic stress due to genomic instability.
UVB, the major skin carcinogen for humans, induced autophagy in an AMPK-dependent manner. UVB-induced AMPK activation seems time-dependent (41, 42): AMPK is activated at earlier time points whereas it is inhibited at later time points. In contrast to the proapoptotic role of AMPK activation determined using siRNA and pharmacological activators (42), our findings using genetic deletion clearly support an antiapoptotic role of AMPK activation through inducing autophagy following UVB damage. This is consistent with the antiapoptotic action of AMPK in tumor cells treated with the chemotherapy drug cisplatin (43).

Our findings demonstrate that autophagy deficiency sensitizes cells to UVB-induced apoptosis through increasing the activation of p38. p38 activation has been shown to play an important role in UVB-induced apoptosis, although its precise function is controversial (27, 36, 44–46). A previous study showed that UVB-mediated activation of the p38 pathway enhances the resistance of normal human keratinocytes to apoptosis by stabilizing cytoplasmic p53 (44). Down-regulation of NF-κB activity and Fas expression may also be involved in this process (45). p38-mediated COX-2 up-regulation following UVB radiation may also promote cell survival (27). In contrast,
several studies have demonstrated that p38 activation promotes UVB-induced apoptosis (34–37) through multiple downstream effectors including Bax (36) and ATF3 (46). In addition, the p38 pathway has also been demonstrated to play an important role in the regulation of autophagy (47, 48). However, whether autophagy also regulates p38 and its underlying mechanism remains unknown. We found that following UVB damage, autophagy deficiency facilitates the activation of p38 and subsequent apoptosis. Although autophagy deficiency may trigger an inflammatory response and chronic tissue damage, leading to genetic instability and promoting tumorigenesis (8), we found that compared with normal human skin, autophagy is increased in human SCC in parallel with decreased activation of p38 and activation of DNA damage response pathways, suggesting that UVB stress or secondary endogenous genotoxic stress in skin tumor cells induces autophagy to promote cell survival.

During autophagy, p62 had been demonstrated to be an important adaptor between ubiquitinated protein aggregates and the autophagic machinery (49) and to play an important role in autophagic degradation of some soluble cytosolic proteins (31, 50). We found that p62 availability is critical for the capacity of the cell for UVB-induced p38 activation. Indeed, earlier studies have reported that p62 binds directly to p38 and facilitates the activation of p38 in response to cytokines (51, 52). Accumulation of p62 in autophagy-deficient cells inhibits the clearance of ubiquitinated proteins destined for proteasomal degradation by delaying their delivery to the proteasome proteases (53). Thus, binding of p62 could block the degradation of p38 through the autophagic degradation system. However, these mechanisms seem unlikely because no difference in total p38 was detected between autophagy-functional and -deficient cells. There are two possibilities for the function of autophagy upon UVB-induced p38 activation: (i) p62 binds to p38 and ...
Autophagy in Cell Survival under Genotoxic Stress

FIGURE 7. Autophagy induction is correlated with reduced p38 activation and presence of DNA damage response in SCC derived from sun-exposed areas as compared with unpaired sun-protected normal human skin. Immunoblot analysis of ATR, p-ATR, Beclin 1, γ-H2AX, p38, p-p38, LC3-I, and LC3-II in normal human skin and SCC.

facilitates the activation; or (ii) binding of p62 prevents p38 from dephosphorylating and thus prevents its inactivation. Further investigations are needed to elucidate the mechanism by which p62 regulates p38 activation.

In conclusion, our findings demonstrate that UVB induces AMPK-dependent autophagy. Autophagy deficiency augments UVB-induced apoptosis through increasing p62-mediated p38 activation. Such an antiapoptotic function of autophagy may play an important role in promoting cell survival under genotoxic stress during tumorigenesis and in established tumors. Our findings may provide unique insights into the indispensable role of autophagy in cell survival and carcinogenesis under genotoxic stress and facilitate the development of better chemopreventive and therapeutic strategies by targeting autophagy to reduce the cancer burden in the skin and many other organs in which DNA-damaging carcinogens are involved.

Acknowledgments—We thank Dr. Noboru Mizushima (Tokyo Medical and Dental University) for kindly providing WT and Atg5 KO MEFs and Dr. Ann Motten for a critical reading of the manuscript.

REFERENCES

1. Yokimitsu, T., and Klionsky, D. J. (2005) Autophagy: molecular machinery for self-eating. Cell Death Differ. 12, 1542–1552
2. Roy, S., and Debnath, J. (2010) Autophagy and tumorigenesis. Semin. Immunopathol. 32, 383–396
3. White, E. (2012) Deconvoluting the context-dependent role for autophagy in cancer. Nat. Rev. Cancer 12, 401–410
4. 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
5. Dang, C. C. (2008) Antimalarial therapy prevents Myc-induced lymphoma. J. Clin. Invest. 118, 15–17
6. Jin, and White, E. (2007) Role of autophagy in cancer: management of metabolic stress. Autophagy 3, 28–31
7. Yue, Z., Jin, S., Yang, C., Levine, A. J., and Heintz, N. (2003) Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc. Natl. Acad. Sci. U.S.A. 100, 15077–15082
8. Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., Mukherjee, C., Shi, Y., Gélinas, C., Fan, Y., Nelson, D. A., Jin, S., and White, E. (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10, 51–64
9. Mathew, R., Kongara, S., Beaudoin, B., Karp, C. M., Bray, K., Degenhardt, K., Chen, G., Jin, S., and White, E. (2007) Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev. 21, 1367–1381
10. Wallischler, S., Loewith, R., and Hall, M. N. (2006) TOR signaling in growth and metabolism. Cell 124, 471–484
11. Ravikumar, B., Vacher, C., Berger, Z., Davies, J. E., Luo, S., Oroz, L. G., Scaravilli, F., Easton, D. F., Duden, R., O’Kane, C. J., and Rubinstein, D. C. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat. Genet. 36, 585–595
12. Kim, J., Kundu, M., Violet, B., and Guan, K. L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. 13, 1016–1023
13. Steinberg, G. R., and Kemp, B. E. (2009) AMPK in health and disease. Physiol. Rev. 89, 1025–1078
14. Mihaylova, M. M., and Shaw, R. J. (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat. Cell Biol. 13, 132–141
15. Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., and Ohsumi, Y. (2010) Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol. Cell. Biol. 30, 1049–1058
16. Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Merry, 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
17. Hay, N., and Sonenberg, N. (2004) Upstream and downstream of mTOR. Genes Dev. 18, 1926–1945
18. Egan, D. F., Shackelford, D. B., Mihaylova, M. M., Gelino, S., Kohzin, R. A., Mair, W., Vasquez, D. S., Joshi, A., Gwinn, D. M., Taylor, R., Asara, J. M., Fitzpatrick, J., Dillain, A., Violet, B., Kundu, M., Hansen, M., and Shaw, R. J. (2011) Phosphorylation ofULK1(hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331, 466–461
19. Behrends, C., Sowa, M. E., Gygi, S. P., and Harper, J. W. (2010) Network organization of the human autophagy system. Nature 466, 68–76
20. Lee, J. W., Park, S., Takahashi, Y., and Wang, H. G. (2010) The association of AMPK withULK1 regulates autophagy. PLoS One 5, e15394
21. Shaw, M. M., Gurr, W. K., McCormin, R. J., Schorderet, D. F., and Sherrwin, R. S. (2007) S’AMP-activated protein kinase α deficiency enhances stress-induced apoptosis in BHK and PC12 cells. J. Cell. Mol. Med. 11, 286–298
22. Mukherjee, P., Mulrophy, T. J., Marsh, J., Blair, D., Chiles, T. C., and Seyfried, T. N. (2008) Differential effects of energy stress on AMPK phosphorylation and apoptosis in experimental brain tumor and normal brain. Mol. Cancer 7, 37
23. Komatsu, M., Waguri, S., Ueno, T., Iwata, J., Murata, S., Tanida, I., Ezaki, J., Mizushima, N., Ohsumi, Y., Uchiyama, Y., Kominami, E., Tanaka, K., and Chiba, T. (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J. Cell Biol. 169, 425–434
24. Sakaki, K., Wu, J., and Kaufman, R. J. (2008) Protein kinase Cβ is required for autophagy in response to stress in the endoplasmic reticulum. J. Biol. Chem. 283, 15370–15380
25. Ito, H., Daido, S., Kanzawa, T., Kondo, S., and Kondo, Y. (2005) Radiation-induced autophagy is associated with LC3 and its inhibition sensitizes malignant glioma cells. Int. J. Oncol. 28, 1401–1410
26. Gills, J. J., Lopiccolo, J., and Dennis, P. A. (2008) Nelfinavir, a new anti-HIV drug with pleiotropic effects and many paths to autophagy. Autophagy 4, 107–109
27. Bowden, G. T. (2004) Prevention of non-melanoma skin cancer by targeting ultraviolet-B light. Nat. Rev. Cancer 4, 23–35
28. Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T., and Mizushima, N. (2004) The role of autophagy during the early neonatal starvation period. Nature 432, 1032–1036
Autophagy in Cell Survival under Genotoxic Stress

29. Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhisa, T., Ohsumi, Y., and Yoshimori, T. (2001) Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. J. Cell Biol. 152, 657–668

30. Kabeya, Y., Mizushima, N., Yamamoto, A., Oshitani-Okamoto, S., Ohsumi, Y., and Yoshimori, T. (2004) LC3, GABARAP, and GATE16 localize to autophagosomal membrane depending on form-II formation. J. Cell Sci. 117, 2805–2812

31. Pankiv, S., Clausen, T. H., Lamark, T., Brech, A., Ruehle, S., Lamark, T., Janssens, S., Vandenheede, J. R., Garmyn, M., and Agostinis, P. (2004) Juhnn, Y. S. (2007) G protein-coupled receptors mediate apoptosis via the epidermal platelet-activating factor receptor. J. Biol. Chem. 279, 1472–1480

32. Sarkar, S., Ravikumar, B., Floto, R. A., and Rubinsztein, D. C. (2009) Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Cell Death Differ. 16, 46–56

33. Seglen, P. O., and Gordon, P. B. (1982) 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 79, 1889–1892

34. Shimizu, H., Banno, Y., Sumi, N., Naganawa, T., Kitajima, Y., and Nozawa, Y. (1999) Activation of p38 mitogen-activated protein kinase and caspases in UVB-induced apoptosis of human keratinocyte HaCaT cells. J. Invest. Dermatol. 112, 769–774

35. Landis, M., Yi, Q., Hyatt, A. M., Travers, A. R., Lewis, D. A., and Travers, J. B. (2007) Involvement of p38 MAP kinase in the augmentation of UVB-induced apoptosis in HaCaT cells. Am. J. Physiol. Cell Physiol. 298, C542–C549

36. Van Laethem, A., Van Kelst, S., Lippens, S., Declercq, W., Vandenabeele, P., Janssens, S., Vandenheede, J. R., Garmyn, M., and Agostinis, P. (2004) Activation of p38 MAPK is required for Bax translocation to mitochondria, cytochrome c release and apoptosis induced by UVB irradiation in human keratinocytes. FASEB J. 18, 1946–1948

37. Seo, M., Lee, M. I., Heo, J. H., Lee, Y. I., Kim, Y., Kim, S. Y., Lee, E. S., and Juhnn, Y. S. (2007) G protein β subunits augment UVB-induced apoptosis by stimulating the release of soluble heparin-binding epidural growth factor from human keratinocytes. J. Biol. Chem. 282, 24720–24730

38. Lu, D., Chen, J., and Hai, T. (2007) The regulation of ATF3 gene expression by mitogen-activated protein kinases. Biochem. J. 401, 559–567

39. Lu, D., Wolfgang, C. D., and Hai, T. (2006) Activating transcription factor 3, a stress-inducible gene, suppresses Ras-stimulated tumorigenesis. J. Biol. Chem. 281, 10473–10481

40. Levine, B., and Kroemer, G. (2008) Autophagy in the pathogenesis of disease. Cell 132, 27–42

41. Zhang, J., and Bowden, G. T. (2008) UVB irradiation regulates Cox-2 mRNA stability through AMPK and HuR in human keratinocytes. Mol. Carcinog. 47, 974–983

42. Cao, C., Lu, S., Kivlin, R., Wallin, B., Card, E., Bagdasarian, A., Tamakloe, T., Chu, W. M., Guan, K. L., and Wan, Y. (2008) AMP-activated protein kinase contributes to UV- and H2O2-induced apoptosis in human skin keratinocytes. J. Biol. Chem. 283, 28897–28908

43. Harhaji-Trajkovic, L., Vilimanovich, U., Kravic-Stevovic, T., Bumbasirevic, V., and Trajkovic, V. (2009) AMPK-mediated autophagy inhibits apoptosis in cisplatin-treated tumor cells. J. Cell. Mol. Med. 13, 3644–3654

44. Chouinard, N., Valerie, K., Rouablia, M., and Huot, J. (2002) UVB-mediated activation of p38 mitogen-activated protein kinase enhances resistance of normal human keratinocytes to apoptosis by stabilizing cytoplasmic p53. Biochem. J. 365, 133–145

45. Ivanov, V. N., and Ronai, Z. (2000) p38 protects human melanoma cells from UV-induced apoptosis through down-regulation of NF-κB activity and Fas expression. Oncogene 19, 3003–3012

46. Turchi, L., Aberdam, E., Mazure, N., Pouysségur, J., Deckert, M., Kitajima, S., Aberdam, D., and Virolle, T. (2008) Hif-2α mediates UV-induced apoptosis through a novel ATF3-dependent death pathway. Cell Death Differ. 15, 1472–1480

47. Webber, J. L., and Tooze, S. A. (2010) Coordinated regulation of autophagy by p38α MAPK through mAtg9 and p38IP. EMBO J. 29, 27–40

48. McClung, J. M., Judge, A. R., Powers, S. K., and Yan, Z. (2010) p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. Am. J. Physiol. Cell Physiol. 298, C542–C549

49. Kraft, C., Peter, M., and Hofmann, K. (2010) Selective autophagy: ubiquitin-mediated recognition and beyond. Nat. Cell Biol. 12, 836–841

50. Kim, P. K., Hailey, D. W., Mullen, R. T., and Lippincott-Schwartz, J. (2008) Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes. Proc. Natl. Acad. Sci. U.S.A. 105, 20567–20574

51. Sudo, T., Maruyama, M., and Osada, H. (2000) p62 functions as a p38 MAPK mediator of recognition and beyond. Nat. Cell Biol. 12, 836–841

52. Kim, P. K., Hailey, D. W., Mullen, R. T., and Lippincott-Schwartz, J. (2008) Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes. Proc. Natl. Acad. Sci. U.S.A. 105, 20567–20574

53. Sudo, T., Maruyama, M., and Osada, H. (2000) p62 functions as a p38 MAPK mediator of recognition and beyond. Nat. Cell Biol. 12, 836–841

54. Korolchuk, V. I., Mansilla, A., Menzies, F. M., and Rubinsztein, D. C. (2009) Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol. Cell 33, 517–527