Oncogenic transformation confers a selective susceptibility to the combined suppression of the proteasome and autophagy

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Abstract

The proteasome and the autophagy systems are two evolutionarily conserved mechanisms for degrading intracellular materials. They are functionally coupled and suppression of the proteasome promotes autophagy. Although suppression of the proteasome leads to cell death, suppression of autophagy can be either prodeath or prosurvival. To understand the underlying mechanism of this dichotomy and its potential clinical implications, we treated various transformed and nontransformed human cells with proteasome inhibitors. We found that whether autophagy served a prosurvival role in this scenario was contingent on the cellular oncogenic status. Thus, autophagy suppression enhanced apoptosis induced by proteasome inhibitors in transformed cells, but not in nontransformed cells. Oncogenic transformation enhanced the ability of cells to initiate autophagy in response to stress, reflecting a stronger dependence of transformed cells on autophagy for survival. Indeed, a combined use of bortezomib, the only Food and Drug Administration–approved proteasome inhibitor for clinical use, and chloroquine, which inhibits autophagy by disturbing lysosomal functions, suppressed tumor growth more significantly than either agent alone in a xenograft model. These findings indicate that suppression of both intracellular degradation systems could constitute a novel strategy for enhanced cancer control in a tumor-specific way. [Mol Cancer Ther 2009;8(7):2036–45]

Introduction

The ubiquitin-proteasome system is a major degradation system for short-lived proteins (1). Proteins to be degraded are labeled with ubiquitin. The ubiquitinated proteins are degraded by the 26S proteasome complex. The degradation is thus specifically targeted to a fraction of proteins. Prompt removal of these proteins is critical to the precise and timely regulation of intracellular signaling involved in multiple cellular processes, including cell proliferation and cell death. Macroautophagy (referred to as autophagy hereafter) is another major intracellular degradation system. Unlike the ubiquitin-proteasome system, autophagy is mainly responsible for the degradation of long-lived proteins and subcellular organelles (2). Autophagy plays important roles in development, cellular homeostasis, and cell survival (3, 4).

We had previously reported that the proteasome system and the autophagy system could be functionally coupled so that suppression of the former can activate autophagy via endoplasmic reticulum (ER) stress–mediated IRE-1 pathway (5). Consistently, ER stress could directly induce autophagy (6–8). Autophagy in this setting plays a compensatory role for the removal of misfolded proteins, thus mitigating ER stress. As a result, suppression of proteasome inhibitor–induced autophagy (5) or ER stress–induced autophagy resulted in enhanced cell death (8). Interestingly, this prosurvival activity of autophagy was notable in cancer cells but not in non-cancer cells (8). In the latter, suppression of ER stress–induced autophagy did not promote cell death. It is not clear why and how autophagy could play different roles in regulating cell death. Indeed, autophagy could be prodeath as well in a number of cases (9).

The differential role of autophagy in regulating cell death in cancer cells versus normal cells could be explored for tumor-specific therapy. Although direct ER stress inducers, such as thapsigargin or tunicamycin, have not been used in clinics, proteasome inhibitors have been examined as a novel class of anticancer drug (10–12). Currently, one proteasome inhibitor, bortezomib (Velcade), has been approved for treating refractory or relapsed multiple myeloma. There are clearly benefits in exploring the role of autophagy in proteasome inhibition for clinical application. On one hand, proteasome inhibitors have not been found to be effective in treating solid tumor in patients (13–15). On the other hand, resistance to proteasome inhibitors can develop in patients with myeloma (12). Novel therapeutic strategies combining proteasome inhibitors with other chemotherapeutic agents or radiation to overcome the resistance to or to broaden the therapeutic spectrum of proteasome inhibitors are needed (11, 14, 16).

In the present study, we investigated the effect of autophagy on cell death induced by proteasome inhibitors in different types of cells, including paired human cell lines that
differ in the transformation status. We showed that inhibition of autophagy only enhanced cell death in the transformed but not in the nontransformed cells. Furthermore, combined suppression of proteasome and autophagy was more effective than the suppression of either system alone in inhibiting tumor growth in a xenograft tumor model. These findings point out the importance of oncogenic status in how autophagy affects cell death, and indicate the benefits of suppressing both cellular degradation systems as a novel tumor-specific therapeutic strategy.

Materials and Methods

Reagents

The following antibodies were used: anti-Beclin 1 (BD Biosciences), anti-β-actin (Sigma), anti-green fluorescent protein (GFP; Santa Cruz Biotechnology), anti-caspase-3 (Cell Signaling), and horseradish peroxidase–labeled secondary antibodies (Jackson ImmunoResearch Lab). The rabbit polyclonal anti-Atg5 antibody was provided by Noboru Mizushima (17). The rabbit polyclonal anti-LC3B antibody was made using a peptide representing the NH2-terminal 14 amino acids of human LC3B and an additional cysteine (PSEKTFKQRRTFEQC). All chemicals were from Sigma, Invitrogen, or Calbiochem.

Construct, Small Interfering RNA, and Transfection

Adenovirus expressing GFP-LC3B (human; Ad-GFP-LC3) was used as previously described (8). RNAi-mediated inhibition of gene expression was conducted by transfecting respective small interfering RNA (siRNA; 0.24 μmol/L) into 1 × 10^6 cells using OligofectAMINE (Invitrogen) for 48 h before analysis. The following siRNAs (Invitrogen) against human genes were used: Beclin-1 (5′-GGUCUAAGACGUCAACCAA-3′) and LC3B (5′-GAAGGCGCUUACAGCUCAA-3′). A scrambled siRNA (5′-UUCUCAGGUCGACGCU-3′; Qiangen) was used as a negative control.

Cell Culture

The colon cancer cell line, HCT116, was maintained in McCoy’s 5A with routine supplements (18). HCT116 cell line stably expressing GFP-LC3 had been described previously (5). The immortalized or transformed ovarian surface epithelial cell lines were generated as described previously (5). McCoy’s5A with routine supplements (18). HCT116 cell line (PSEKTFKQRRTFEQC). All chemicals were from Sigma, Invitrogen, or Calbiochem.

Analysis of Cell Death

General cell death was determined using propidium iodide staining (1 μg/mL). Apoptotic cells with condensed or fragmented nuclei were determined with Hoechst 33342 staining (5 μg/mL). Analysis of the effector caspase activity was done as previously described (20) using Ac-DEVD-afc as the substrate. Terminal nucleotidyl transferase–mediated nick end labeling staining was done as described previously (21).

Immunoblot Assay

Cells were washed in PBS and lysed in radioimmunoprecipitation assay buffer. Tumor tissues were suspended in radioimmunoprecipitation assay buffer followed by sonication and centrifugation. Forty micrograms of protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were stained with the indicated primary and secondary antibodies and developed with SuperSignal West Pico chemiluminescent substrate (Pierce). Images were obtained using a Kodak Image Station 4000 MM and analyzed by Kodak Image Software (Carestream Health, Inc.).

Animal Work

HCT116 cells were harvested and washed twice in PBS. Approximately 4 × 10^6 cells were resuspended in 200 μL of PBS and inoculated on the right flank of each nude mice (6- to 8-week-old female BALB/c strain; Charles River). By day 14, tumors were well established in the mice with an average size of ~300 mm^3. Mice were then randomly assigned into four groups with five to six mice in each group. Mice were intraperitoneally given saline, bortezomib (0.33 mg/kg body weight, dissolved in saline with 0.3% DMSO), chloroquine (45 mg/kg body weight), or bortezomib together with chloroquine. These agents were administrated every 3 days for a total of six times. Tumor growth was measured every 2 days after the first treatment and the volume of the tumor (mm^3) was determined using the formula, \( V = \pi/6 \times A \times B^2 \) (A is the larger diameter and B is the diameter perpendicular to A). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Statistical Analysis

Experimental data were subjected to z test, Student’s t test, or one-way ANOVA with Scheffe’s post hoc test where appropriate.
Results

Induction of Autophagy by Bortezomib

Previously, we had determined that a commonly used proteasome inhibitor, MG-132, could induce autophagy in HCT116, a colon cancer cell line and DU145, a prostate cancer cell line, which could be inhibited by knocking down essential autophagy genes or by a pharmacologic agent, 3-methyladenine (3-MA; ref. 5). We verified and extended these findings with several other proteasome inhibitors, including bortezomib, the only proteasome inhibitor currently approved by the Food and Drug Administration for clinical use. Treatment of HCT116 with bortezomib induced a dose-dependent increase in the endogenous LC3-II form (Fig. 1A) and in the exogenously introduced GFP-LC3II (Fig. 1B). In addition, GFP-LC3 became punctated, indicating a translocation to the autophagic membranes (Fig. 1C and D). The change in GFP-LC3 localization induction was not dependent on the expression of Bax, a key molecule known to contribute to the sensitivity of HCT116 cells to many types of cell death stimulations, including proteasome inhibitors (18, 22).

To confirm that the accumulation of LC3-II and GFP-LC3 puncta was due to an increased induction of autophagy (23), but not to the blockage of the degradation of the exiting autophagosomes, we determined whether the autophagic flux was increased. We did this analysis based on the degradation of GFP-LC3. The autophagosomal GFP-LC3-II is degraded in the lysosome, but the GFP moiety is relatively resistant to hydrolysis. The appearance of the GFP moiety in cells could be used to indicate the breakdown of the autophagosomes (23). Basal autophagic activity in HCT116 cells stably expressing GFP-LC3 resulted in a low level of GFP-LC3 breakdown, which was significantly elevated following bortezomib treatment (Fig. 1B). Furthermore, the accumulation of GFP moiety could be suppressed by the lysosomal protease inhibitors, E64D and pepstatin A, in parallel with a further accumulation of GFP-LC3-II as the result of the inhibition of the breakdown. The blockage of GFP-LC3 degradation led to a backup accumulation of GFP-LC3-II (Fig. 1B) and the GFP-LC3 punctation (Fig. 1C and D). These observations thus indicated that inhibition of proteasome by bortezomib indeed led to an elevated autophagic flux.

Combined Inhibition of Proteasome and Autophagy Resulted in Enhanced Tumor Cell Death and Suppression of Tumor Expansion

To determine whether the antitumor effects of proteasome inhibitors could be enhanced by the modulation of autophagy, we cotreated the HCT116 cells with bortezomib and an autophagy inhibitor, 3-MA or chloroquine. 3-MA can suppress the class III phosphoinositide-3-kinase, which is required for the initiation of autophagy by many agents,
including proteasome inhibitors (5, 24). Chloroquine can interfere with the lysosome pH level and thus can suppress lysosome-mediated degradation, which would lead to the inhibition of autophagy (23).

Indeed, such a combination led to an enhanced caspase-mediated apoptotic cell death, as indicated by the increased nuclear fragmentation, condensation, and membrane permeability, which could be suppressed by z-VAD, a pan-caspase inhibitor (Fig. 2A and B). HCT116 cells with deficiency in Bax had comparable autophagy response to proteasome inhibitors (Fig. 1), but were relatively resistant to proteasome inhibitors (refs. 5, 22; Fig. 2B) and other chemotherapeutic

**Figure 2.** Bortezomib-induced autophagy is cytoprotective. A, Bax-positive HCT116 cells were either untreated (a) or treated with bortezomib (20 nmol/L) alone (b) or in the presence of 3-MA (10 mmol/L; c), chloroquine (10 μmol/L; d), or z-VAD (50 μmol/L; e) for 24 h. Cell death was determined by nuclear staining with Hoechst 33342 for apoptotic cells with fragmented or condensed nuclei (arrows) and by propidium iodide staining for cytoplasmic membrane permeability change. B, Bax-positive and Bax-negative HCT116 cells were treated as in A, and the percentages of cells with defined changes were quantified. Top, apoptotic cells with fragmented or condensed nuclei; bottom, propidium iodide staining-positive cells. C, Bax-deficient HCT116 cells were transfected with a negative siRNA (Neg) or Beclin-1 (Bec) or LC3B-specific siRNA (120 nmol/L) for 48 h and analyzed by immunoblot with indicated antibodies. D, siRNA-transfected cells were then treated with bortezomib (20 nmol/L) for another 24 h. Cell death was determined as in A, a, negative siRNA only; b, negative siRNA plus bortezomib; c, siRNA against Beclin-1 plus bortezomib; and d, siRNA against LC3B plus bortezomib. Arrows, fragmented or condensed nuclei. E, siRNA-transfected cells were treated with lactacystin (5 μmol/L), ALLN (10 μmol/L), or bortezomib (20 nmol/L) for another 24 h. Cell death was determined as in A. Representative of at least three independent experiments (columns, mean; bars, SD); *, P < 0.01 (z test).
agents (18). However, these cells were not completely apoptosis-deficient, as they still express Bak, which could be activated under stronger apoptotic signals (25). Notably, suppression of autophagy in Bax-deficient HCT116 cells also significantly enhanced apoptotic death caused by bortezomib (Fig. 2B). These findings were consistent with the notion

Figure 3. Combined suppression of the proteasome and autophagy enhances the inhibition of tumor growth in vivo. A, BALB/c nude mice were implanted with \(4 \times 10^6\) Bax-positive HCT116 cells on the right flank. Fourteen days after inoculations, mice were grouped and intraperitoneally given saline, bortezomib (Bort, 0.33 mg/kg), bortezomib plus chloroquine (Bort + CQ, 45 mg/kg), or chloroquine alone (CQ) every 3 days for six times (arrows). The first treatment day was designated as day 0. Tumor volume (mm\(^3\)) was determined every 2 days after the first treatment (day 0) until day 16, and the mean tumor volumes were calculated (n = 5–6/group); *, \(P < 0.01\) (bortezomib/chloroquine group versus the saline group, one-way ANOVA with Scheffe’s post hoc test). B, total lysates were prepared from each tumor sample and pooled in equal amounts of protein within each group. The lysates were then analyzed for effector caspase activities using Ac-DEVD-AFC as the substrate. Independent triplicate measurements were expressed as fold of increase over the saline control (columns, mean; bars, SD). C, tumor samples from each group were subjected to terminal deoxynucleotidyl transferase–mediated nick end labeling staining and the percentage of positive cells were determined (columns, mean; bars, SD); *, \(P < 0.01\) (z test). D, electron microscopic examination of tumor samples recovered from nude mice treated for 16 days with saline (a), bortezomib (b), bortezomib + chloroquine (c), or chloroquine only (d). Arrows, autophagic vesicles; N, nucleus; bar, 1 \(\mu\)m. E, the number of autophagic vesicles per 100 \(\mu\)m\(^2\) area was determined (columns, mean; bars, SD); *, \(P < 0.01\); #, \(P < 0.02\) (one-way ANOVA with Scheffe’s post hoc test). F, an equal amount of protein from each tumor sample within the same group (n = 5–6) was combined and analyzed by immunoblot assay with anti-LC3 and anti-\(\beta\)-actin antibodies. Digital data (mean \pm SD) were from densitometry analysis of the LC3-II band from at least three independent experiments.
that autophagy could play a compensatory mechanism to remove misfolded proteins and thus mitigate ER stress in the case of proteasome inhibition (5, 26). By suppression of autophagy, the ER stress level and therefore the magnitude of death stimulation would be elevated so that Bak could be readily activated (25).

To confirm the specificity of 3-MA and chloroquine in promoting apoptosis by suppressing autophagy, we transfected HCT116 cells with specific siRNA against the mammalian Atg6 homologue, *Beclin-1* or one of the mammalian Atg8 homologues, *LC3B*, two important molecules for autophagy. Immunoblot analysis indicated that both molecules could be effectively knocked down by the specific siRNAs but not a nonspecific scrambled siRNA (Fig. 2C). Consistently, such treatment enhanced bortezomib-induced apoptotic cell death (Fig. 2D and E). Moreover, we found that apoptosis induced by other types of proteasome inhibitors, such as lactacystin and ALLN, could be similarly enhanced (Fig. 2E), indicating the generality of the prosurvival function of autophagy in this setting.

The above finding also suggested that a simultaneous inhibition of autophagy might enhance the therapeutic efficacy of proteasome inhibitors. This could be potentially significant as proteasome inhibitors are currently only approved for the treatment of refractory or relapsed multiple myeloma and their therapeutic effects on other tumors have not been proven in vivo (13–15). We thus investigated whether suppression of autophagy could enhance the killing of solid tumors by bortezomib in a xenograft model. HCT116 were inoculated to the lateral side of the right flank in nude mice. The average tumor volume in each mouse reached ~300 mm³ in 2 weeks. The mice were then treated with saline (as the control), bortezomib, chloroquine, or bortezomib plus chloroquine every 3 days for six times. Tumor progression was followed up by determining the tumor volumes every other day. Chloroquine was chosen over 3-MA because of the comparable efficacy (Fig. 2A and B) and its Food and Drug Administration–approved status for clinic use.

Bortezomib alone retarded tumor growth, compared with the saline treatment (Fig. 3A). Interestingly, chloroquine alone also had inhibitory effects, suggesting that the basal autophagy activity was also beneficial for tumor growth. However, it was the combination of the bortezomib and chloroquine that led to the most significant inhibition of tumor expansion, indicating that suppression of the proteasome together with the compensatory autophagy would cause the maximal stress and demise to cancer cells. Consistently, the combined treatment induced a higher level of apoptosis, as indicated by the increased caspase activities and terminal nucleotidyl transferase–mediated nick end labeling staining in the tumor samples (Fig. 3B and C). The average body weight of mice in each group was ~21 g, which was not significantly changed during the treatment.

Electron microscopic examination of the tumor samples indicated that there was indeed an increased number of autophagic vesicles following bortezomib treatment (Fig. 3D and E). Consistently, the LC3-II form was also elevated (Fig. 3F). On the other hand, chloroquine caused the blockage of the degradation of autophagosomes, and therefore

**Figure 4.** Suppression of autophagy in normal human PBMCs does not enhance the toxicity of proteasome inhibitors. A, human PBMC were incubated with MG132 (0.5 μmol/L) or bortezomib (20 nmol/L) in the presence or absence of 3-MA (10 mmol/L) for 24 h. Immunoblot was then done using the anti-LC3 antibody. B to D, human PBMC were treated with MG132 (0.5 μmol/L), ALLN (10 μmol/L), or bortezomib (20 nmol/L) in the presence or absence of 3-MA for 24 h. Cell death was determined by propidium iodide staining (B) or by nuclear Hoechst 33342 staining for fragmented or condensed nuclei (C). Effector caspase activities were determined using Ac-DEVD-AFC as the substrate (D).
LC3-II, in the lysosome, resulting in an arrest of the autophagic flux and the accumulation of autophagic vesicles (Fig. 3D and E) and LC3-II (Fig. 3F). Combined use of bortezomib and chloroquine led to a more significant manifestation of these changes, reflecting the effects on both the input and the output of the autophagy flux. Overall, the status of autophagy in the tumor samples (Fig. 3D and F) recapitulated that of cultured cells (Fig. 1) following the same type of treatment, indicating that the same signaling pathway was followed and targeted.

Inhibition of Autophagy in Normal or Nontransformed Cells Did Not Enhance Proteasome Inhibitor–Induced Apoptosis

To determine the potential toxicity of the combined suppression of the proteasome and autophagy in normal cells, we first examined the effect of such treatment on freshly isolated normal human PBMC, commonly used for assessing the toxic side effects of chemotherapy. We found that bortezomib and other proteasome inhibitors could induce autophagy in PBMC, based on LC3-II formation, which could be suppressed by 3-MA (Fig. 4A; data not shown). Toxicity was low for these proteasome inhibitors, particularly for bortezomib (refs. 11, 12; Fig. 4B–D). Notably, in contrast to what was observed in the cancer cells, suppression of autophagy with 3-MA did not enhance the toxicity of the proteasome inhibitors in normal PBMC.

We further confirmed the low toxicity of this combined treatment in a normal human colon cell line, CCD-18Co. Suppression of autophagy induced by proteasome inhibitors in this cell line by either 3-MA or siRNA-mediated knockdown of Beclin-1 did not increase apoptosis caused by the same proteasome inhibitors, based on propidium iodide staining, apoptotic nuclear morphology, or caspase activity (Fig. S14; data not shown). Finally, we used nontransformed but immortalized MEFs, in which autophagy is suppressed by the genetic deletion of Atg5 (Fig. S2A).4 Consistently, apoptosis induced by proteasome inhibitors was no higher in Atg5-deficient cells than in the wild-type cells (Fig. S2B–D).4 Similarly, when the wild-type MEFs were cotreated with 3-MA and a proteasome inhibitor, there was also no increase in cell death compared with the proteasome inhibitor alone (data not shown). In fact, the toxicity of proteasome inhibitors in the MEFs could be reduced by the suppression of autophagy in these cells. These data thus indicate that inhibition of autophagy in the normal and/or nontransformed cells did not enhance the toxicity of proteasome inhibitors, which was contrary to the effect in the cancer cells.

4 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
Oncogenic Transformation Was Correlated with a Protective Role of Autophagy Induced by Proteasome Inhibitors

To understand the potential mechanisms that might account for this differential role of autophagy in regulating cell death, we hypothesized that oncogenic transformation might be a key event that leads to such a difference. To explore this possibility, we adopted a recently developed cellular system that used matched isogenic human ovarian epithelial cell lines (19). SV40 T/+/transfected ovarian epithelial cell lines (IOSE-29 and IOSE-80) were further infected with retrovirus expressing a full-length hTERT cDNA. Immortalized sublines (T-29 and T-80) were then transformed with the constitutively activated K-RasV12 to generate the transformed T-29-K-RasV12 and T-80-K-RasV12. Previous studies had shown that T-29-K-Ras and T-80-K-Ras cells exhibited neoplastic behaviors, capable of forming anchorage-independent foci in soft agar and tumors in nude mice, whereas their respective nontransformed counterparts, T-29 and T-80, did not exhibit any of these behaviors (19). Thus, these cell lines were appropriate for the investigation of the role of oncogenic transformation in the autophagic regulation of cell death.

Bortezomib could readily induce autophagy in both the T-29 (Fig. 5) and the T-80 (Fig. S3) cells. Accumulation of GFP-LC3 puncta could be further increased by a concurrent treatment of chloroquine, indicating that there was a net increase of autophagy flux following proteasome inhibition. Interestingly, we observed that the transformed T-29-K-Ras and T-80-K-Ras cells seemed to have a significantly stronger autophagy response than their nontransformed counterparts, T-29 and T-80, did not exhibit any of these behaviors (19). Thus, these cell lines were appropriate for the investigation of the role of oncogenic transformation in the autophagic regulation of cell death.

Discussion

Autophagy Might Play Different Roles in Regulating Apoptosis in Cancer Cells and in Nontransformed Cells Under Stress

Inhibition of autophagy promotes proteasome inhibitor-induced cell death in cancer cells, but not in primary cells or in nontransformed cell lines. This difference might not be due to the disparity in cell type because it could also be observed in matched isogenic ovarian epithelial cell lines differing only in transformation status. It is not quite understood how autophagy may regulate cell death in different ways. Autophagy has been shown in earlier studies to be either cytoprotective (28–30) or cytotoxic (9, 31) under various stress conditions. Although this difference might be related to the type of stress and the types of cell death being affected, a clear pattern has not emerged that could confer a clear mechanistic insight.

Our earlier studies and the present study together suggest that the oncogenic status of the cell is correlated with the effects of autophagy on cell death during the response to proteasome inhibitors or ER stress (5, 8). It had been previously noted that normal cells were much less sensitive to the toxicity of proteasome inhibitors compared with the transformed cancer cells (11, 12). The contribution of the
Selective Protection of Cancer Cells by Autophagy

Oncogenic status rather than the cell type to this difference in death susceptibility could be further shown with the use of matched isogenic transformed and nontransformed ovarian epithelial cell lines (Fig 6; Fig S5; refs. 27, 32). Transformed cells may in general be more sensitive to stress and cell death, including that caused by misfolded proteins (16). Transformed cells may thus be more ready to mount any protective mechanisms, such as autophagy, and become more dependent on these mechanisms for survival. Indeed, a higher level of autophagy could be induced by proteasome inhibitors or starvation in the transformed cells than in the nontransformed cells (Fig 5; Figs S3 and S4). In addition, autophagy is clearly protective in the setting of prodeath stimulation, which may reduce the development of resistance. This is best illustrated by the enhanced killing of Bax-deficient HCT116 cells (Fig. 2), which is otherwise quite resistant to bortezomib despite the presence of Bak. Third, the combination could simultaneously target the cell survival and cell death pathways. Finally, such a combination maintains the relative selectivity of proteasome inhibitors toward cancer cells with a low toxicity for normal cells.

The last point, as shown in the present study, is particularly relevant and significant in cancer therapy, considering that several other regimes have also been examined that suppress autophagy to enhance cancer cell death induced by hypoxia (29), alkylating agents (35), or histone deacetylase inhibitors (36). Although the effect of these combinations on the survival of nontransformed normal cells has not been examined, it would be reasonable to speculate that the rationale defined in the present work may also be applicable in these scenarios, that is, the normal cells might be less affected by these combinations as well.

In the setting of animal studies and clinical trials, the use of chloroquine may be preferred due to its well-defined pharmacologic dynamics, being well tolerated, and the plentiful clinical experience in using this drug in other disease conditions. There could be foreseeable concerns with chloroquine because it is not a specific suppressor for autophagy, but seems to disturb the lysosomal function in general. Whether the long-term use of this agent in the context of cancer therapy might elicit additional side effects is not known. However, in the current absence of any autophagy-specific inhibitors, chloroquine would be a reasonable candidate for use in combination with proteasome inhibitors in clinical trials to promote better cancer control.

It has to be pointed out that suppressing autophagy is not a generic strategy to enhance therapeutic efficacy for all types of cancers. Depending on the types of cancer and the primary therapeutic agents, promoting autophagy might instead be necessary to enhance cancer cell death. In these cases, cytotoxic effects rather than cytoprotective effects, were shown to be caused by autophagy (37).

Finally, the physiologic role of autophagy in normal cells seems to be related to the control of metabolic stress and maintenance of genome and chromosomal stability (38). Autophagy may help to control intracellular ROS level and DNA adducts by removing damaged mitochondria (39). These effects might contribute to the control of tumorigenesis, as supported by the demonstration of the tumor suppressor role of Beclin-1 (40). Although these observations do not necessarily contradict with those made in the context of cancer therapy regarding the function of autophagy, the effect of long-term suppression of autophagy via pharmacologic agents has yet to be defined.

In conclusion, we have provided evidence that oncogenic status may decide how autophagy affects cell death and that the combined use of proteasome inhibitors and autophagy inhibitors could selectively enhance cell death in transformed cancer cells. This novel strategy may offer unique advantages in cancer control.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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