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Modulation of endoplasmic reticulum (ER) stress-induced autophagy by C/EBP homologous protein (CHOP) and inositol-requiring enzyme 1α (IRE1α) in human colon cancer cells

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ABSTRACT

To explore the relationship between UPR and autophagy in intestinal epithelial cells, we investigated whether autophagy was induced by endoplasmic reticulum (ER) stress in colon cancer cell lines. We demonstrated that autophagy was induced by ER stress in HT29, SW480, and Caco-2 cells. In these cells, inositol-requiring enzyme1α (IRE1α) and C/EBP homologous protein (CHOP) were involved in the ER stress–autophagy pathway, and CHOP was a regulator of IRE1α protein expression. Our findings suggest that CHOP promotes IRE1α and autophagy especially in ER stress conditions. This study will provide important insights into the disclosure of the ER stress–autophagy pathway.

1. Introduction

Autophagy is a highly conserved cellular degradation system that controls intracellular components by sequestering cytoplasm and cytoplasmic organelles in double membrane-bound autophagosomes. Autophagy plays important roles in maintaining amino acid levels and adapting to starvation [1,2]. Microtubule-associated protein 1 light chain 3B (LC3B) conjugated with phosphatidylethanolamine, LC3-II, is associated with the autophagosome membranes. Recently, genome-wide association studies have shown that *autophagy related 16-like 1* (*ATG16L1*) [3] and *Immunity-related GTPase family M protein* (*IRGM*) [4], both of which are associated with autophagy, are genes that influence susceptibility to Crohn's disease (CD). In addition, Cadwell et al. revealed that mice hypomorphic for *ATG16L1* and CD patients homozygous for the risk allele displayed a granule abnormality in Paneth cells [5].

When misfolded proteins accumulate in the ER, a condition referred to as ER stress, unfolded protein response (UPR) occurs in the cells to maintain homeostasis [6]. In mammalian cells, the ER transmembrane proteins, RNA-dependent protein kinase-related endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) are known as canonical sensors of ER stress, and they activate UPR. In 2008, it was revealed that *X-box binding protein 1* (*XBP1*) hypomorphic variants influenced susceptibility to inflammatory bowel disease including CD and ulcerative colitis [7]. XBP1 is an ER stress-responsive transcription factor, which is spliced 26-bp nucleotides by activated IRE1α to be an active form [8,9]. It was also revealed that *Xbp1* deletion in intestinal epithelial cells resulted in spontaneous enteritis and a decrease in the number of Paneth and goblet cells [7].

UPR and autophagy play important roles in cell homeostasis and are implicated in inflammatory bowel disease. We hypothesized that a functional pathway between UPR and autophagy exists in intestinal epithelial cells and its failure contributes to the development or maintenance of intestinal inflammation. In the present study, to explore the relationship between UPR and autophagy in intestinal epithelial cells, we investigated whether ER stress induced autophagy in HT29, SW480, Caco-2 cells. We demonstrated that autophagy was induced by ER stress in human colon cancer cell lines and that IRE1α and CHOP modulated the regulation of autophagy.

2. Materials and methods

2.1. Reagents and antibodies

Tunicamycin (Tm) and thapsigargin (Tg), obtained from Sigma–Aldrich (St. Louis, MO), were dissolved in DMSO (Wako, Japan). Bafilomycin A1 was obtained from Sigma–Aldrich, and Hoechst 33342 was from Invitrogen (Carlsbad, CA). Anti-phosphorylated

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0006-291X/

Table 1
Sequences of primers used in this study.

	Sense	Antisense
PERK	CTTATGCCAGACACACAGGACAA	TCCATCTGAGTGCTGAATGGAATAC
IRE1α	GCCGTGGTGAAGATGGACTG	ACAGAACCACCTTTATAGGTCCTGA
ATF6	AAGCCCTGATGGTGCTAACTGAA	CATGTCTATGAACCCATCCTCGAA
XBP1	GTTGAGAACCAGGAGTTAAGACAG	CAGAGGGTATCTCAAGACTAGG
CHOP	TGCTTTCAGGTGTGGTGATGTATG	AATCAGAGCTGGAACCTGAGGA
LC3B	ACTGAATTCCCATGCCGTCGGAGAAG	TTTGAATTCTTACACTGACAATTTCA

The XBP1 gene was simultaneously amplified in both the unspliced and spliced forms (activated form).

Table 2
Sequences of siRNA used in this study.

	Sense	Antisense
siPERK	CGAGAGCCGGAUUUAUUGAtt	UCAAUAAAUCCGGCUCUCGtt
	GGAUGAAAUUUGGCUGAAAtt	UUUCAGCCAAAUUUCAUCCtt
	CAGACACACAGGACAAGUAtt	UACUUGUCCUGUGUGUCUGtt
siIRE1α	CAACCUCUCUUCUGUAUCUtt	AGAUACAGAAGAGAGGUUGtt
	GGAAGGUGAUGCACAUCAAtt	UUGAUGUGCAUCACCUUCCtt
	CUGGAGGAGACGAAUGAUAtt	UAUCAUUCGUCUCCUCCAGtt
	CUGUACUCUUGGAGUAACAtt	UGUUACUCCAAGAGUACAGtt
siATF6	GACUAAACCUGUCCUACAAtt	UUGUAGGACAGGUUUAGUCtt
	GGAGUGAGCUACAAGUGUAtt	UACACUUGUAGCUCACUCCtt
	GCACUGGAAUUCAGAUGCAtt	UGCAUCUGAAUUCCAGUGCtt
siCHOP	GAAGGCUUGGAGUAGACAAtt	UUGUCUACUCCAAGCCUUCtt
	GGAAAGGUCUCAGCUUGUAtt	UACAAGCUGAGACCUUUCCtt
	GUCUCAGCUUGUAUAUAGAtt	UCUAUAUACAAGCUGAGACtt

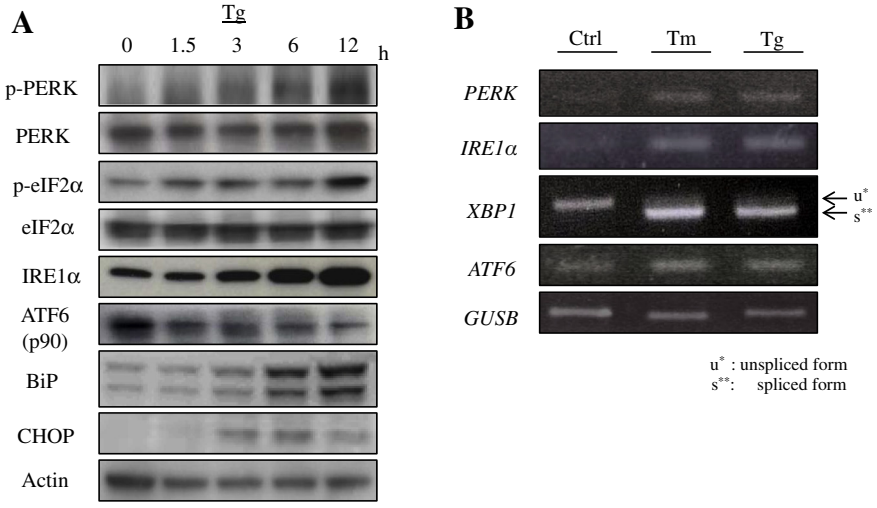


Fig. 1. UPR occurs in HT29 cells. (A) Western blot analysis of p-PERK, PERK, p-eIF2α, eIF2α, IRE1α, ATF6 (p90), BiP, and CHOP is shown in HT29 cells after treatment with 0.5 μM Tg at the indicated time points. (B) mRNA levels are examined by semiquantitative PCR. HT29 cells were incubated in the presence or absence of 3 μg/ml Tm or 0.5 μM Tg for 4 h. The bands represent amplified *PERK*, *IRE1α*, *XBP1*, and *ATF6*. The *XBP1* bands consist of the unspliced form (u^*) and spliced form (s^{**}).

PERK antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA); PERK, phosphorylated-eukaryotic translation initiation factor 2α (p-eIF2α), eIF2α, CHOP, IRE1α, BiP, actin, and LC3B from Cell Signaling (Beverly, MA); and ATF6 from Abcam (Cambridge, UK). These were all used as primary antibodies. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse antibodies, from Cell Signaling, were used as secondary antibodies.

2.2. Cell culture

Human colon cancer cell lines HT29 and SW480 were obtained from the American Type Culture Collection (Manassas, VA), Caco-2 cells were from the European Collection of Cell Culture (Porton Down, UK). These cells were maintained in DMEM (Wako, Japan) supplemented with 10% (v/v) FBS (Life Technologies, Carlsbad, CA), at 37 °C in a humidified atmosphere with 5% CO_2.

2.3. Construction of a green fluorescent protein-labeled LC3B (GFP-LC3B) plasmid vector

LC3B sequences were amplified from human small intestine cDNA (Clontech, Palo Alto, CA) using the primers, listed in Table 1, containing EcoRI sites. The PCR products were inserted, in-frame, into the multicloning site of the pAcGFP1-C3 vector (Clontech)

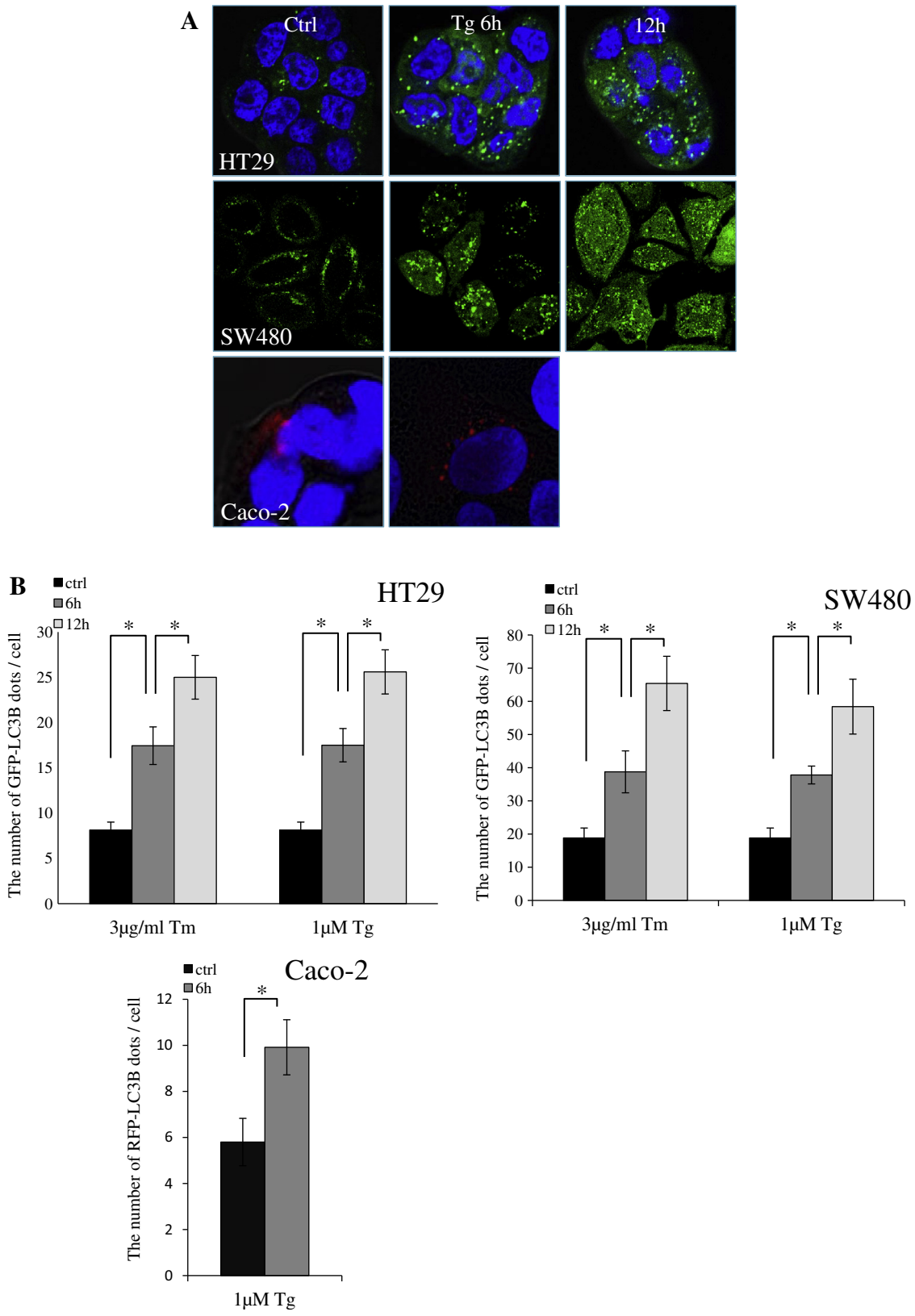
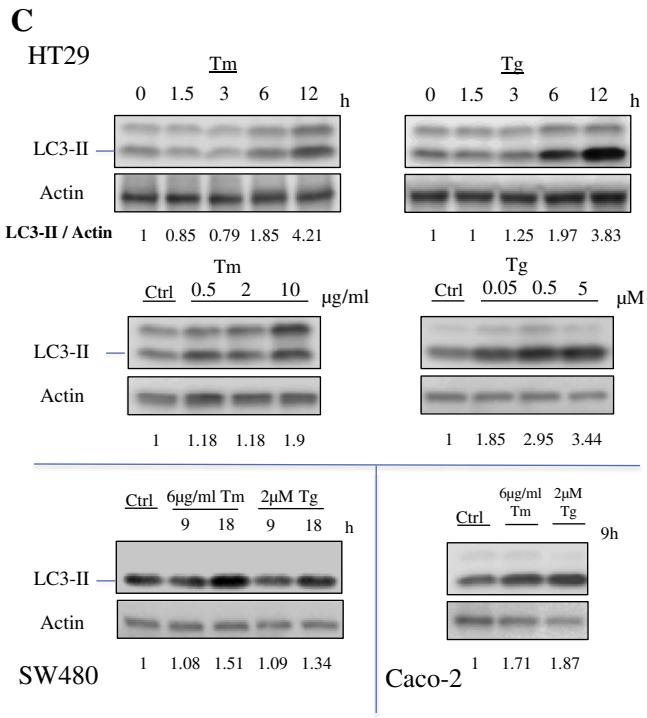


Fig. 2. Autophagy is upregulated by ER stress in human colon cancer cells. (A) GFP-LC3B-transfected HT29 (upper panels) and SW480 (middle panels) cells as well as RFP-LC3B-transfected Caco-2 (lower panels) cells treated with 1 μM Tg at the indicated time points are shown. HT29 and Caco-2 cells were incubated with Hoechst 33342 nucleic acid stain. (B) The number of dots is counted in at least 20 cells. The means ± SEM of the dots/cell are shown (*$P < 0.05$; Student's t test). (C) The protein levels of LC3-II were examined by Western blotting in three types of colon cancer cells with Tm or Tg. The equal amount of DMSO was treated for control cells. The band intensities of LC3-II normalized by actin are compared with that in the control. The intensity ratios are displayed below the actin bands. (D) Electron microscopic analysis of HT29 cells exposed to 3 μg/ml Tm for 6 h. Double-membrane structures of autophagosomes (arrow head) are shown. Scale bar, 200 μm.



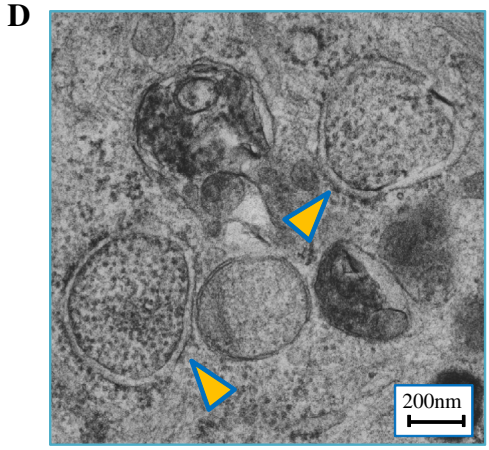


Fig. 2 (*continued*)

cut with EcoRI. The insertion fragment of the targeting DNA was confirmed by sequencing.

2.4. Transfection and establishment of stable transformant cell lines

Cells were transfected with, small interfering RNA (siRNA) and the GFP-LC3B vector using lipofectamin 2000 (Invitrogen, Carlsbad, CA), and Premo autophagy sensor LC3B-RFP (Invitrogen), according to the manufacturer's instructions. We used siRNAs (Santa Cruz, CA) targeting the human genes *PERK, IRE1α, ATF6* and *CHOP*. The siControl RNA (siCtrl; Santa Cruz), which comprises a scrambled sequence not leading to the specific degradation of any cellular message, was transfected as a control. The sequences of these siRNAs are listed in Table 2. The GFP-LC3B transformant was cloned by selection with G418 (Life Technologies, Carlsbad, CA) and placing a cloning ring (IWAKI, Haibara, Japan) around the evident single colony. GFP/red fluorescent protein (RFP) was visualized using a Nikon C2si confocal laser scanning microscope (Tokyo, Japan) with a FITC/tetramethylrhodamine isothiocyanate (TRITC) filter. Knockdown efficiency was monitored by determining the level of mRNA and protein in each experiment.

2.5. Transmission electron microscopy

Cells were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer and postfixed in 2% osmium tetroxide. Then the cells were dehydrated with ethanol and infiltrated with propylene oxide and epoxy resin. Ultrathin sections (60–80 nm) were obtained using a Leica-UCT ultramicrotome (Leica Microsystems, Wetzlar, Germany). Sections were stained with 2% uranyl acetate and examined using a JEM-1200EX transmission electron microscope (JEOL, Akishima, Japan).

2.6. Western blotting

Whole cell lysates were prepared using a Mammalian cell extraction kit (BioVision, Mountain View, CA) and PhosSTOP (Roche, Basel, Switzerland). Protein concentrations in the lysates were measured using the BCA Protein Assay kit (Pierce Chemical Co., Rockford, IL). Equal amounts of proteins were loaded onto SDS–PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Bucks, UK). After incubation with tris-buffered saline and Tween 20 (TBS-T; BIORAD, Hercules, CA) containing ECL blocking agent (GE Healthcare), membranes were incubated overnight at 4 °C with the primary antibodies. All antibodies were diluted to 1:1000 in TBS-T. After washing with TBS-T, membranes were incubated with secondary antibodies (1:10,000 in TBS-T) at 25 °C. After washing with TBS-T, the bound antibodies were visualized by chemiluminescence using the ECL Prime Western Blotting Detection Reagent (GE Healthcare) and the bands were analyzed using a digital imaging system (VersaDoc; Bio-Rad, Hercules, CA). Actin was used as a loading control.

2.7. RNA isolation and PCR analysis

Total RNA was extracted from cells using the illustra RNAspin Mini kit (GE Healthcare). cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA was amplified by PCR with the specific primers listed in Table 1. Reaction products were analyzed by electrophoresis in 1% agarose gels, stained with ethidium bromide, and visualized using a BioDoc-it Imaging System (UVP, Upland, CA). β-Glucuronidase (GUSB) was used as an internal control.

2.8. Real-time PCR and gene expression array

cDNA was amplified with SYBR Premix Ex Taq II (TaKaRa Bio, Otsu, Japan) and specific primers using a StepOnePlus system (Applied Biosystems, Carlsbad, CA) for real-time PCR. The expression levels were expressed as fold changes compared with the indicated control. Gene expression arrays were performed with PCR arrays (SABiosciences, Hilden, Germany) targeting the UPR- and autophagy-associated genes using a StepOnePlus system. Gene expression levels were analyzed using the manufacturer's server on the Qiagen website (http://www.qiagen.com). The array data have been deposited with Gene Expression Omnibus (GEO).

2.9. Statistical analysis

Data from at least three independent experiments were analyzed and expressed as mean ± standard error of mean. The data were analyzed using the unpaired Student's *t* test. A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. UPR occurred in HT29 cells

In HT29 cells treated with 0.5 μM Tg, increase of phosphorylation of PERK and eIF2α, IRE1α, BiP, and CHOP protein levels, and

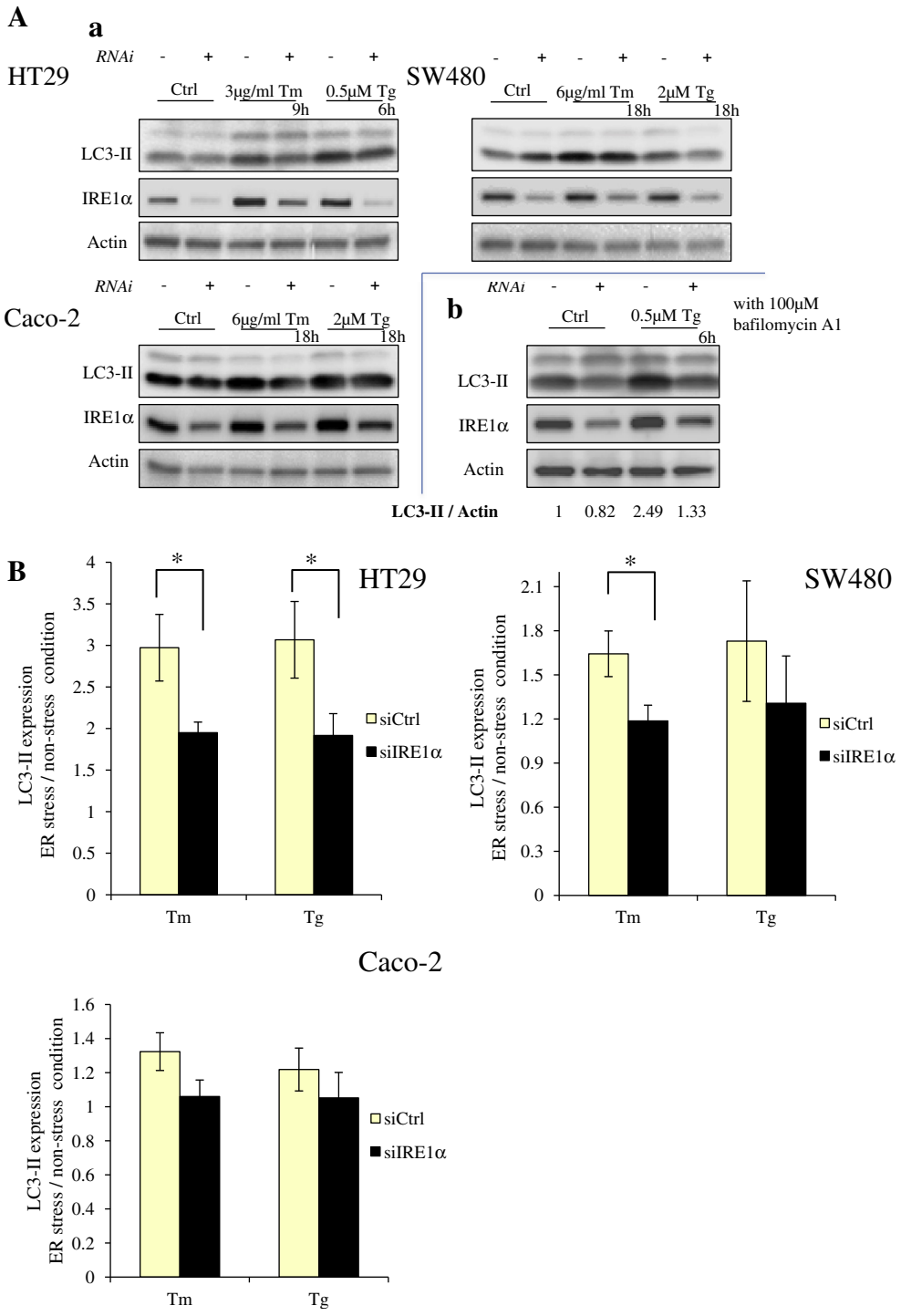


Fig. 3. The gene knockdown of canonical UPR sensors. (A) (a) Cells were incubated in the presence or absence of Tm or Tg, after *IRE1α* gene knockdown in HT29 cells siRNA (left upper), SW480 cells siRNA (right upper), and Caco-2 cells siRNA (left lower). Western blotting of the LC3-II and IRE1α bands from cells transfected with siCtrl or siIRE1α are shown, respectively. (b) HT29 cells incubated in the presence or absence of Tg (0.5 μM for 6 h) and treated with bafilomycin A1 (100 μM) were examined after siCtrl or siIRE1α transfection. LC3-II and IRE1α protein levels are shown. Analyzed intensity ratios are displayed in the same way as shown in Fig. 2C. (B) The intensity ratio of the LC3-II bands analyzed in Tm- or Tg-treated cells transfected with siCtrl or siIRE1α in comparison with that in nontreated cells transfected with siCtrl or siIRE1α. The values are expressed as means ± SEM of three independent experiments (*$P < 0.05$; Student's t test). (C) (a) In GFP-LC3B-transfected HT29 cells, compared with siCtrl treatment, siIRE1α-treated cells show fewer GFP-LC3B dots after treatment with 0.5 μM Tg for 6 h. (b) The number of dots is counted. The means ± SEM of the dots/cell are shown (**$P < 0.005$). (D) *PERK* or *ATF6* gene knockdown. In a manner similar to the IRE1α knockdown as shown in Fig. 3A, after *PERK* or *ATF6* gene knockdown, HT29 cells are chemically treated. The intensity ratio of the LC3-II bands are shown. The values are expressed as means ± SEM of three independent experiments (*$P < 0.05$).

decrease of ATF6 (p90) protein levels were observed along the indicated time points (Fig. 1A; [10–13]). In addition, semiquantitative PCR revealed elevation of *PERK*, *ATF6*, and *IRE1α* as well as splicing of *XBP1* in HT29 cells treated with Tm or Tg (Fig. 1B). These results indicated that UPR occurred in the human colon cancer cells and activated PERK, IRE1α, and ATF6.

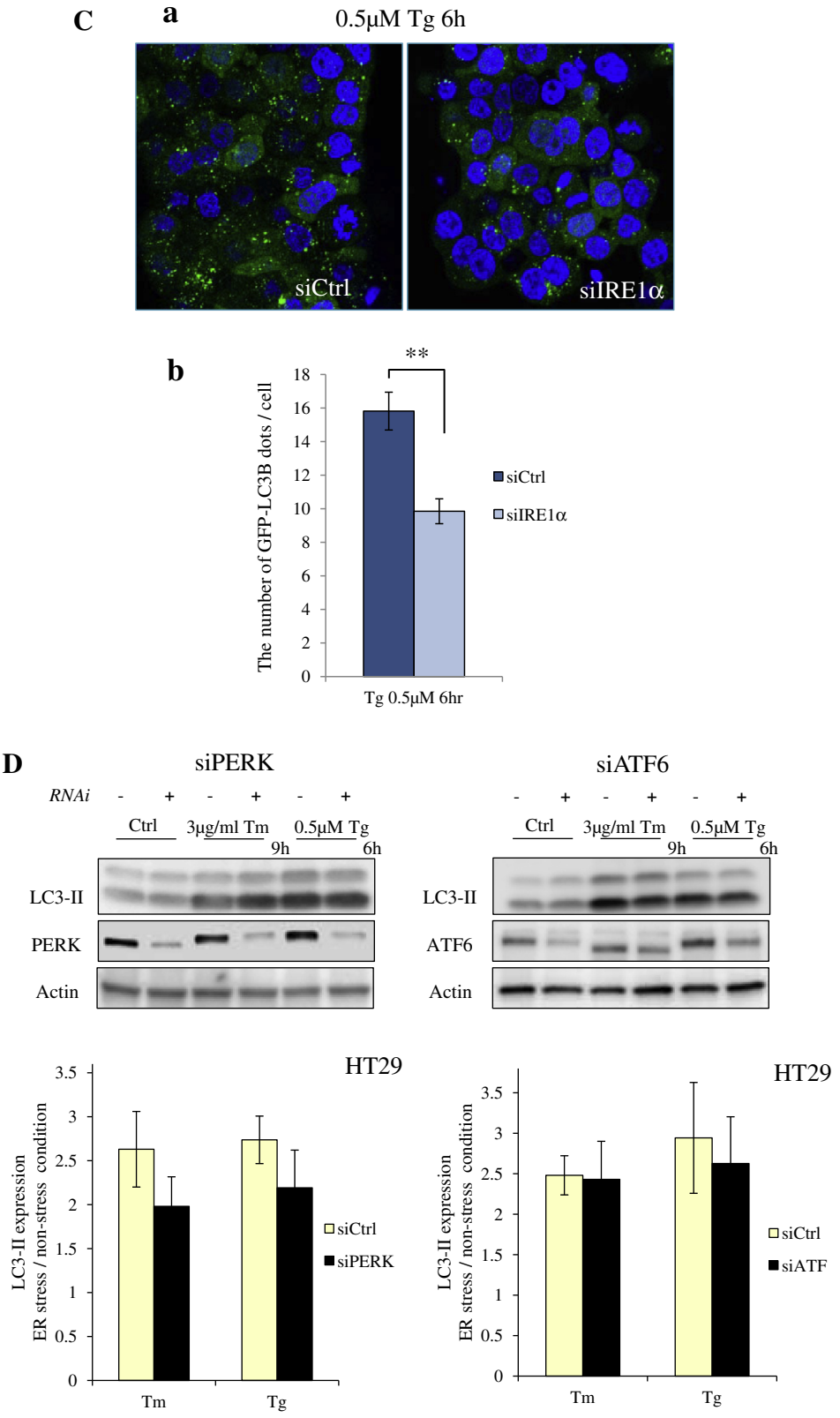


Fig. 3 (*continued*)

3.2. Autophagy was induced in response to ER stress

To investigate whether UPR induced autophagy, HT29, SW480, and Caco-2 cells were treated with Tm or Tg. We engineered a GFP-LC3B plasmid vector and transfected it into HT29 and SW480 cells to obtain stable transformants. These transformants allowed visualization of autophagosomes to evaluate autophagy [14]. We also transiently transfected RFP-LC3B into Caco-2 cells

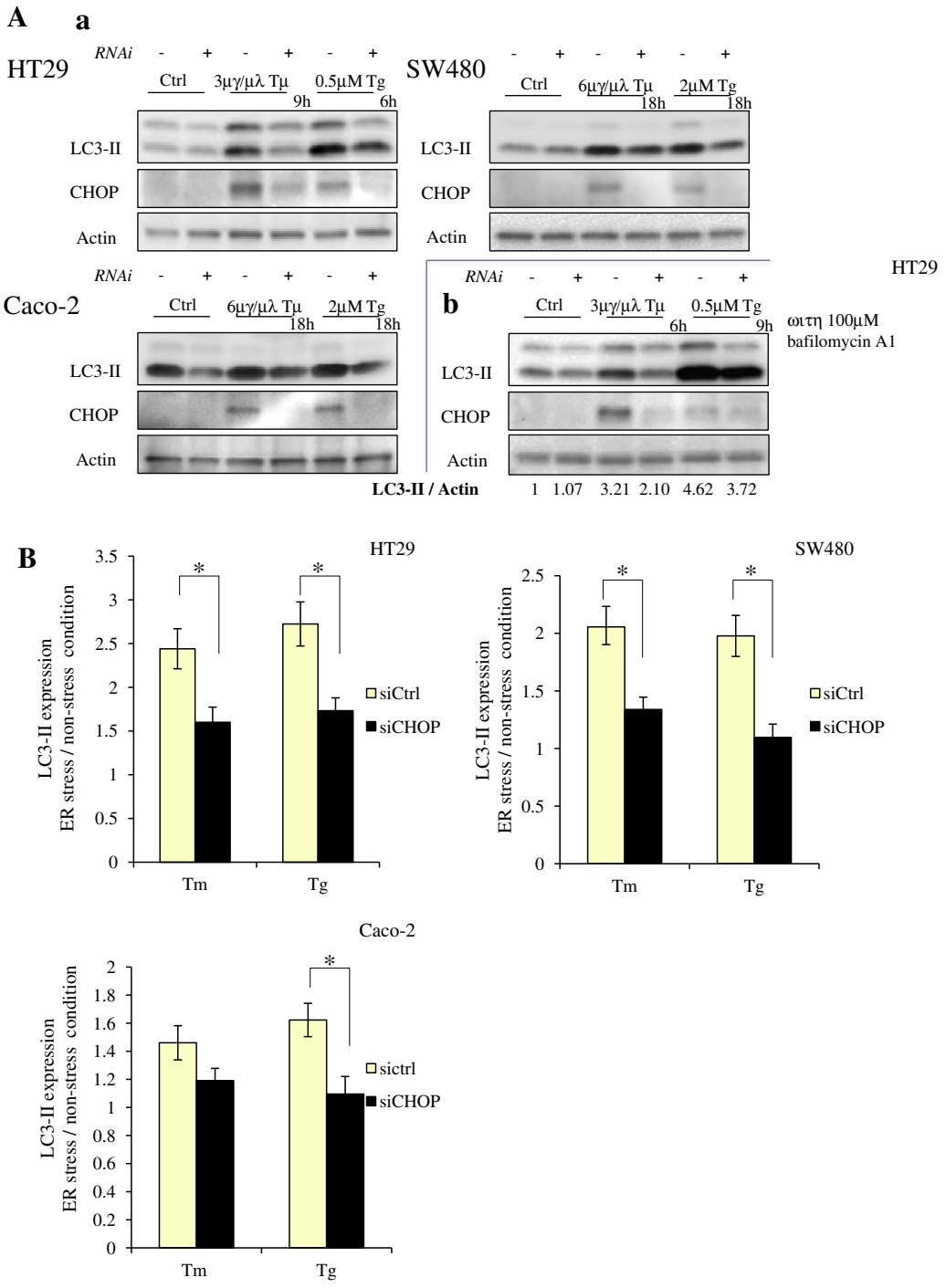


Fig. 4. CHOP is significantly involved in the ER stress–autophagy pathway. (A) (a) LC3-II and CHOP protein levels are shown in three types of colon cancer cells with or without Tm or Tg treatment. From left to right lanes, cells with siRNA targeting for Ctrl and *CHOP* are alternately displayed. Cells were exposed to Tm or Tg at the indicated concentrations and the time points. (b) LC3-II and CHOP bands are examined in a similar manner as mentioned in Fig. 3A (b), with 100 μM bafilomycin A1. Analyzed intensities are also displayed. (B) The intensity ratio of the LC3-II bands normalized by actin from cells transfected with siCtrl or siCHOP are analyzed in Tm- or Tg-treated conditions in comparison with that in nontreated conditions. The values are expressed as means ± SEM of three independent experiments ($^{*}P < 0.05$; Student's *t* test). (C) (a) In GFP-LC3B-transfected HT29 cells, fewer GFP-LC3B dots are observed in siCHOP-treated cells than in siCtrl-treated cells after treatment with 0.5 μM Tg for 6 h. (b) The number of dots is counted. The means ± SEM of the dots/cell are shown ($^{**}P < 0.005$). (D) The levels of CHOP and IRE1α protein in three types of cells are shown upon treatment with siIRE1α or siCHOP and Tm or Tg. (E) Analyzed data from Fig. 4D. The relative *CHOP* or *IRE1α* expression, normalized by actin, of siIRE1α- or siCHOP-treated cells treated with Tm or Tg are compared with that of siCtrl-treated cells treated with Tm or Tg. The left upper chart represents the CHOP expression ratio of siIRE1α-treated cells to siCtrl-treated cells treated with Tm or Tg. The right upper chart represents the IRE1α expression ratio of siCHOP-treated cells to siCtrl-treated cells treated with Tm or Tg. The left lower chart represents the CHOP expression rario of siPERK- or siATF6-treated cells to siCtrl-treated cells treated with Tm or Tg in HT29 cells. Results are expressed as means ± SEM of three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.005$).

to monitor autophagy. After treating these cells with Tm or Tg at the indicated concentrations and time points, the number of GFP- or RFP-LC3B signals (dots) observed using the confocal laser microscope was remarkably increased compared with the baseline of controls (Fig. 2A and B). Subsequently, we examined LC3-II protein levels, used as a marker for autophagy, by Western blotting

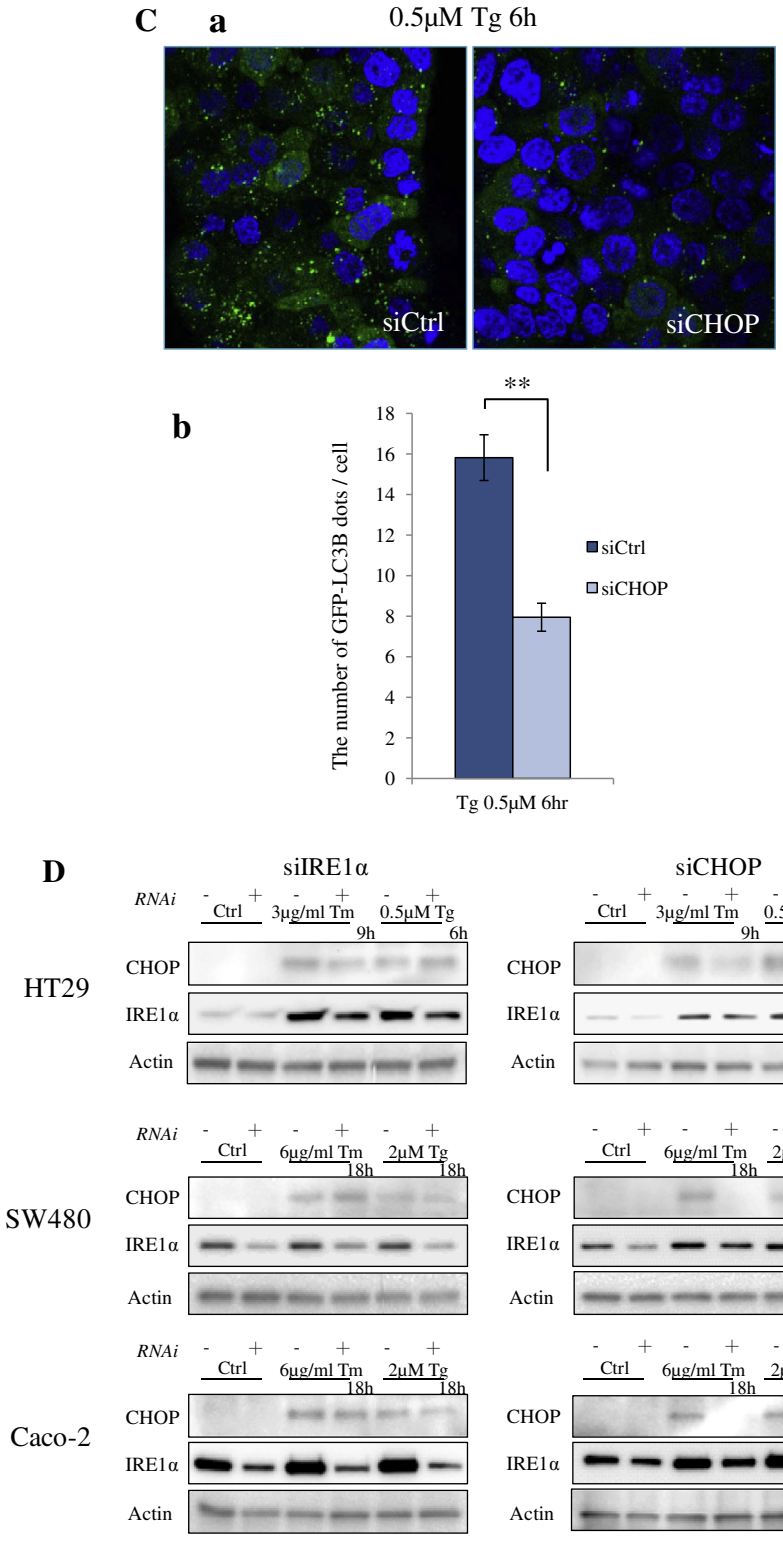


Fig. 4 (*continued*)

[15]. In three types of cells treated with Tm or Tg, the levels of LC3-II protein were elevated at the indicated concentrations of the chemicals and time points (Fig. 2C). Double-membrane structures, autophagosomes, were observed using electron microscopy in chemically treated HT29 cells (Fig. 2D). These data indicated that autophagy was induced by ER stress in all of three types of colon cancer cell lines.

3.3. IRE1α was involved in the induction of autophagy in ER stress-stimulated conditions

To verify whether the UPR was associated with autophagy and to determine which sensor protein was the main contributor to the UPR–autophagy system, we conducted gene knockdown analyses for the three canonical sensors, PERK, IRE1α, and ATF6. Knockdown

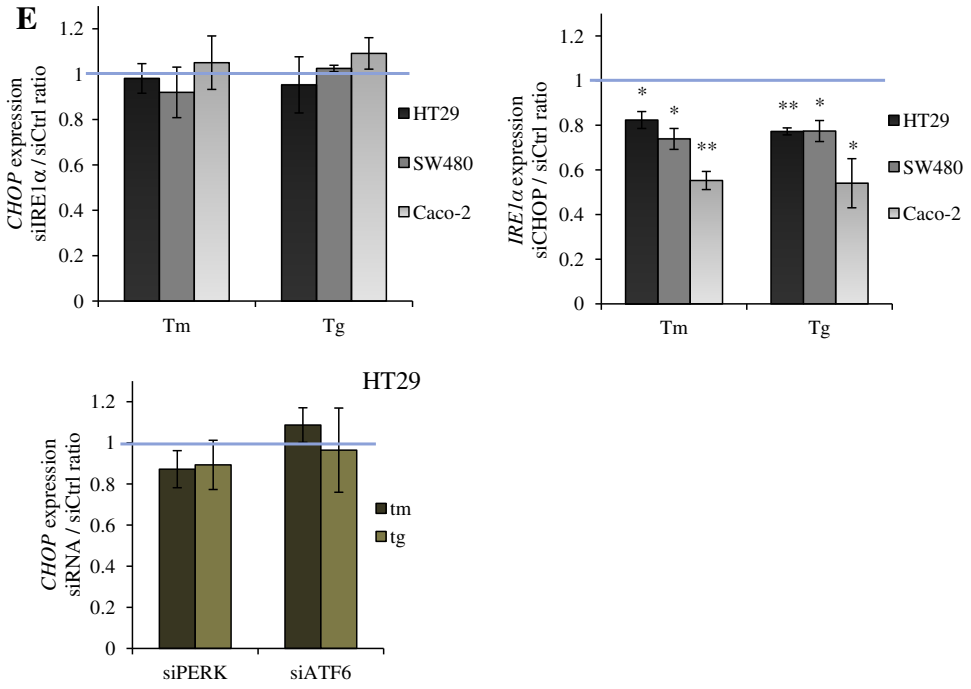


Fig. 4 (*continued*)

of *IRE1α* by siRNA significantly inhibited the upregulation of LC3-II in chemically treated HT29 cells (Fig. 3A(a) and B). When the degradation of autophagosome was inhibited with bafilomycin A1 [16,17], the upregulation of LC3-II was also inhibited (Fig. 3A(b)). These observations indicated that IRE1α was involved in the induction of autophagosomes rather than LC3-II degradation. Similarly, the knockdown of *IRE1α* in SW480 and Caco-2 cells by siRNA suppressed the upregulation of LC3-II induced by the chemical treatment (Fig. 3A(a) and B). In HT29 cells stably transfected with GFP-LC3B, knockdown of *IRE1α* with Tg resulted decrease of the number of GFP-LC3B dots compared with that of siCtrl transfected cells (Fig. 3C(a and b)). Knockdown of *PERK* tended to suppress upregulation of LC3-II in HT29 cells but *ATF6α* resulted in little change of LC3-II levels (Fig. 3D). These results indicated that IRE1α was involved in the induction of autophagy by ER stress in colon cancer cells.

3.4. Gene expression array showed that CHOP was strikingly increased by Tg treatment

To shed light on the mechanisms involved in ER stress-induced autophagy, we examined gene expression arrays of the UPR- and autophagy-related genes. Comparing Tg-treated HT29 cells with nontreated cells, the expression of most genes varied (GEO Database ID: GSE51141). Particularly *CHOP* expression was increased in the Tg-treated cells. To confirm the *CHOP* elevation, we examined mRNA expression in HT 29 cells by real-time PCR. In Tg-treated cells (0.5 μM, 2.5 h), *CHOP* expression was approximately 90 times higher than that in nontreated cells (data not shown). As represented in Figs. 1A and 2C (right upper panel), in HT29 cells treated with 0.5 μM Tg, protein levels of CHOP elevated robustly at 3 h and those of LC3-II increased notably at 6 h while slightly increased at 3 h. *CHOP* elevation was remarkable at the condition (0.5 μM Tg for 2.5 h) just before the elevation of LC3-II protein. Therefore, we next conducted a knockdown examination to uncover the relationship between the UPR–autophagy pathway and CHOP.

3.5. CHOP regulated IRE1α and autophagy

We studied knockdown of *CHOP* in three types of colon cancer cell lines. Importantly, the upregulation of LC3-II was significantly inhibited when *CHOP* was knocked down in Tm- or Tg-treated HT29 and SW480 cells (Fig. 4A(a) and B). Similarly, the upregulation of LC3-II was adequately inhibited in Caco-2 cells. When *CHOP* was knocked down in GFP-LC3B-transfected HT29 cells, the number of GFP-LC3B dots treated with Tg was decreased compared with that of siCtrl-transfected cells (Fig. 4C(a and b)). Moreover, the inhibition of LC3-II upregulation was also observed on co-treatment with bafilomycin A1 (Fig. 4A(b)). These data indicated that CHOP was involved in the induction of autophagosomes. Of note, the three types of colon cancer cells treated with siCHOP exhibited a significant suppression of IRE1α, while cells treated with siIRE1α did not exert any influence on CHOP expression (Fig. 4D and E). Knockdown of *PERK* exhibited decrease of CHOP expression, while knockdown of *ATF6* resulted in little change on CHOP expression (Fig. 4E). These results demonstrated that CHOP significantly regulated the induction of autophagy and IRE1α protein expression.

4. Discussion

In the present study, we showed that ER stress induced autophagy in human colon cancer cells. Our findings were ascertained by the following methods: using different two chemical ER stress inducers to activate UPR; performing analysis in three types of cell lines; immunoblotting for UPR and LC3-II along a specified time course; detecting autophagy for LC3-II protein, LC3B dots, and observing double-membrane structures using electron microscopy.

We observed the activation of UPR in HT29 cells treated with the chemicals along the specified time course and showed that ER stress upregulated autophagy in three types of colon cancer cells. The autophagy induction was likely dependent on the intensity and duration of ER stress, and relied on cell types. Autophagy seemed to be highly activated in SW480 cells under nontreatment conditions, while autophagy was weakly activated in response to

ER stress in Caco-2 cells. Previous reports showed ER stress induced autophagy in human embryonic kidney cells [18] and mouse embryonic fibroblast cells [19]. These data demonstrate that ER stress-induced autophagy is a broad functional pathway in various cell types. Our findings also reveal that UPR and autophagy, both of their related gene variants have been identified as being susceptible to CD, are linked in a functional way in colon cancer cells. This implicates that disorders of the ER stress–autophagy pathway may be involved in the pathology of CD. Further investigation, particularly in vivo on CD patients, is needed to reveal the association between ER stress–autophagy pathway and CD.

We demonstrated that IRE1α and CHOP modulated the autophagy induction by ER stress in this study. PERK-eIF2α was revealed to be important for induction of autophagy in mouse embryonic carcinoma cells [20]. It was demonstrated that IRE1α-mediated activation of JNK resulted in the activation of autophagy [19,21,22], and XBP1 splicing triggered activation of Beclin-1 and autophagy [23]. Among the three canonical ER stress sensors, the difference of participation in ER stress–autophagy pathway may depend on cell types. Our results showed that IRE1α might be involved in the ER stress–autophagy pathway that was different to some extent depending on colon cancer cell type.

We examined comprehensive gene expression arrays to investigate the genes related to the ER stress–autophagy pathway. As expected, the expression of most genes changed in the UPR- and autophagy-related genes. Especially, *CHOP* was strongly elevated under Tg-treated conditions. This result and CHOP knockdown analyses, in three types of colon cancer cell lines with two different ER stress inducers, led to the conclusion that CHOP regulated autophagy and IRE1α. Prior to this report, this association between CHOP and autophagy, and IRE1α, was unknown. Further investigation is needed to explore the mechanism of CHOP and autophagy. Other gene expressions also varied under Tg-treated conditions on gene expression arrays, and these genes could be involved in the ER stress–autophagy pathway as well. For example, *growth arrest and DNA-damage-inducible gene 34* (*Gadd34*) was reported to be a molecule associated with the induction of autophagy [24]. But other genes were unknown to affect in the ER stress–autophagy pathway. The genes can be candidate mediators in this pathway.

In conclusion, ER stress induces autophagy, and IRE1α and CHOP modulate the induction of autophagy in human colon cancer cells. Our findings could be an insight into the disclosure of the ER stress–autophagy pathway. Further investigation to clarify the role of IRE1α and CHOP in this pathway and the association between this pathway and inflammatory bowel disease is needed.

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