Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells

Yujin Lee [a], Young Hye Kwon [a, b, *]

[a] Department of Food and Nutrition, Seoul National University, Seoul, South Korea
[b] Research Institute of Human Ecology, Seoul National University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 6 July 2019
Accepted 19 July 2019
Available online xxx

Keywords:
Autophagy
Apoptosis
ER stress
Hepatocellular carcinoma
Luteolin
p53

ABSTRACT

In several cancer cells, luteolin (3′,4′,5,7-tetrahydroxyflavone) exerts anticancer effects by upregulation of oxidative stress and endoplasmic reticulum (ER) stress, which are shown to activate p53-dependent cell death. Since luteolin-mediated ER stress regulation has not been investigated in hepatocellular carcinoma (HCC) cells, we investigated the role of ER stress in anti-carcinogenic effects using p53-wild type and p53-null HCC cells treated with luteolin. Trypan blue exclusion test was implemented to determine cell viability. Western blot was applied to compare the difference of autophagy, apoptosis, and proliferation event between cell lines. ER stress and p53 activation were determined by RT-PCR. Our results showed that luteolin at 5–10 μmol/L induced higher cytotoxicity in p53-null Hep3B cells than in p53-wild type HepG2 cells. Cytotoxicity was not observed in normal liver cells. Apoptosis activation and proliferation inhibition occurred in only p53-null Hep3B cells in response to luteolin treatment. Also, luteolin induced oxidative stress and ER stress in p53-null Hep3B cells. Although we observed the induction of p21 in Hep3B cells, the concomitant increases in mRNA levels of p53 family members, including TAp63 and TAp73, were not observed. Furthermore, luteolin induced autophagy in Hep3B cells only, which enhanced cell viability. Taken together, this study suggests that luteolin-induced ER stress may exert anticancer effects in a p53-independent manner.

© 2019 Published by Elsevier Inc.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common primary liver malignancy and the major cause of cancer-related mortality worldwide [1]. Furthermore, HCC is diagnosed late for unnoticeable symptoms [2]. In cancer cells, there is an imbalance between cell proliferation and death [3]. As basal apoptotic signal of cancer is low, an induction of apoptosis plays a key role in anti-cancer mechanisms. p53, the tumor suppressor protein, is one of targets in cancer treatment. In response to stress stimuli, such as DNA damage or oxidative stress, p53 is activated to induce the expression of target genes involved in the regulation of apoptosis, cell cycle arrest, and autophagy [4]. As autophagy acts in buffering stress stimuli, its induction could be crucial for cancer cells to survive. However, deficiency in autophagy causes the failure in sustaining homeostasis, such as eliminating damaged proteins and managing oxidative stress, thus regulating cellular conditions that favor to be either pro-tumorigenic or susceptible to cell death [5].

Endoplasmic reticulum (ER) is involved in calcium homeostasis, protein quality control, and lipid synthesis. Under prolonged or severe ER stress, unfolded protein reaction (UPR) system is induced to alleviate stress so that cells can survive. Through UPR system, protein accumulation in ER is decreased by downregulation of protein translocation to ER and folding capacity is enhanced by activating transcription of ER-resident chaperone protein. Upon a prolonged ER stress, X-box-binding protein-1 (Xbp-1) mRNA is activated by splicing, leading to apoptosis via activation of the C/EBP homologous protein (CHOP). In addition, phosphorylation of inositol-requiring enzyme 1 (IRE1) is known to induce apoptosis by activation of c-Jun N-terminal kinase. Thus, regulation of ER stress becomes an important control point because it affects both cell survival and cell death [6]. Furthermore, Xbp-1 is known to upregulate the formation of autophagosome by transcriptional activation of beclin 1 [7].

Luteolin (3′,4′,5,7-tetrahydroxyflavone), a flavonoid that is found in broccoli, pepper, thyme, and celery, has been shown to exert anticancer effects via p53 and autophagy activation in HCC

* Corresponding author. 1 Gwanak-ro, Gwanak-gu, Seoul, 08826, South Korea.
E-mail address: hye0414@snu.ac.kr (Y.H. Kwon).

https://doi.org/10.1016/j.bbrc.2019.07.073
0006-291X/© 2019 Published by Elsevier Inc.

Please cite this article as: Y. Lee, Y.H. Kwon, Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.073

cells [8,9]. However, the anticancer effects of luteolin via regulating ER stress in HCC have not been studied yet. In addition, ER stress is known to activate p53, thus influencing p53-dependent anticancer mechanism, such as apoptosis, cell proliferation, and autophagy. Therefore, we used p53-wild type HepG2 cells and p53-null Hep3B cells to investigate whether ER stress is involved in anticancer effects of luteolin in HCC cells.

2. Materials and methods

2.1. Cell culture and treatment

Human HCC cells, HepG2 (p53 wild type; American Type Culture Collection, USA) and Hep3B (p53 null type; KCLB; Korean Cell Line Bank, Korea) were cultured in Dulbecco's modified Eagle's medium (DMEM; WelGene, Korea) supplemented with 10% heat-inactivated fetal bovine serum (WelGene). Murine normal liver cell line, NCTC clone 1469 (KCLB) were cultured in DMEM (WelGene) supplemented with 10% horse serum (WelGene). All cell lines were incubated in a humidified 95% air, 5% CO_2 atmosphere at 37 °C. When the cultures reached about 40–60% confluence, they were serum starved for 16 h and were treated with various concentrations of luteolin for 48 h. For the inhibition of autophagy, cells were treated with 10 μmol/L chloroquine (CQ; Sigma, USA) or 5 mmol/L 3-methyladenine (3-MA; Sigma) in the absence or presence of luteolin. Cell viability was determined using trypan blue exclusion test. The effect of each treatment on cell viability was assessed by the relative value compared with control value.

2.2. Measurement of protein levels by immunoblotting

After treatment, the cells were scraped from the dishes and lysed in ice-cold lysis buffer. The protein contents of the lysates were measured with Protein Assay Dye Reagent (Bio-Rad, USA), and equal amounts of protein were loaded into the lanes of an SDS-PAGE gel, electrophoresed, and blotted onto a PVDF membrane (Millipore, USA). After the membranes had been blocked with either 5% nonfat milk or bovine serum albumin, they were probed with a specific primary antibody as follows: anti-catalase (Abcam, UK), anti-microtubule-associated protein light chain 3 (LC3; Novus Biology, USA), anti-p53 (Santa Cruz Biotechnology, USA), anti-poly (ADP-ribose) polymerase (PARP; Cell Signaling Technology, USA), anti-proliferation cell nuclear antigen (PCNA; Santa Cruz Biotechnology), or anti-heat shock protein 70 (HSC70; Santa Cruz Biotechnology). Membranes were then incubated with a horseradish peroxidase-linked secondary antibody for chemiluminescent detection. The band intensities were quantified with Quantity One software (Bio-Rad).

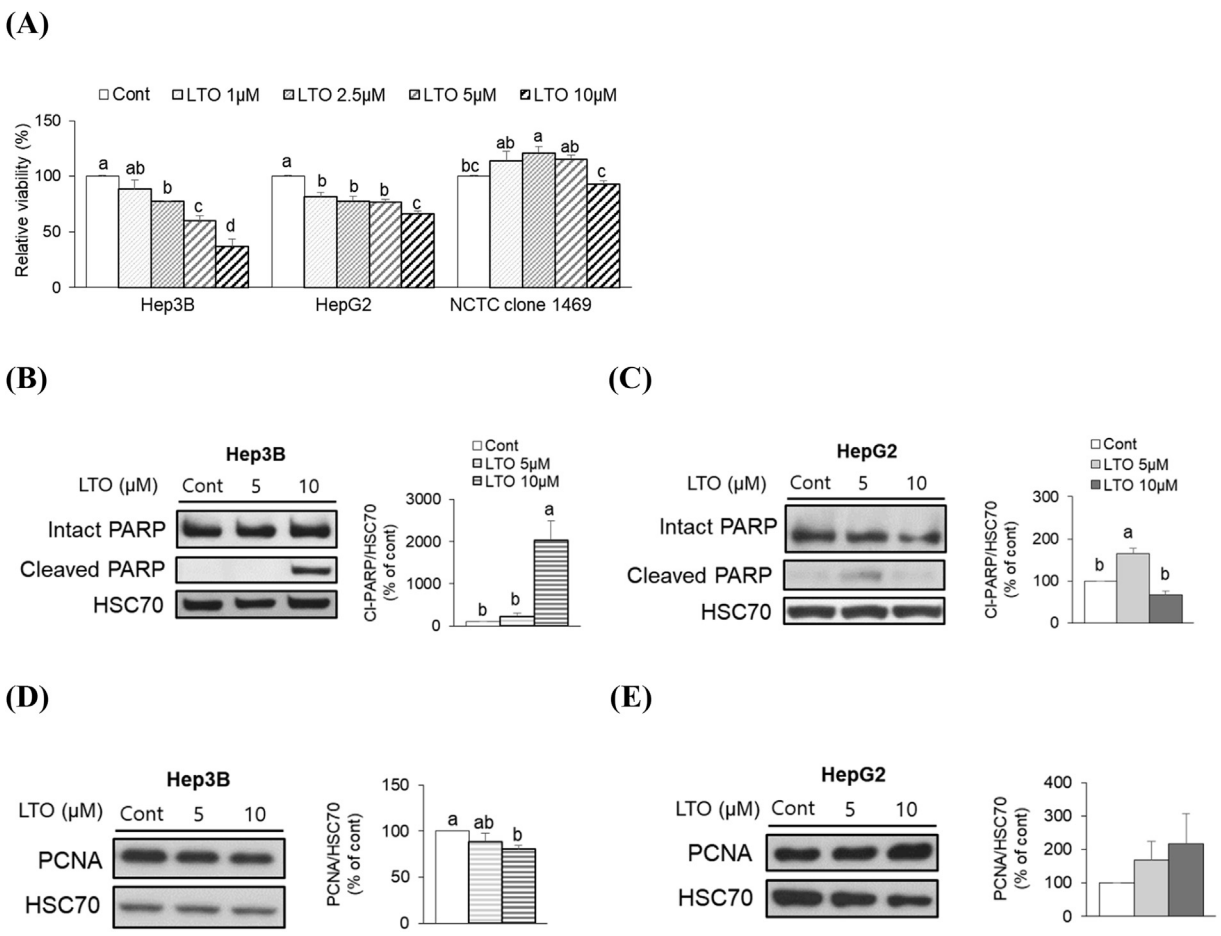

Fig. 1. Luteolin induced apoptosis and inhibited cell proliferation in p53-null Hep3B cells. (A) The effect of luteolin on cell viability of hepatic carcinoma cells, including Hep3B and HepG2 cells, and normal liver cells, NCTC clone 1469. Cell were treated with luteolin (0–10 μmol/L, 0.2% DMSO) for 48 h. The cytotoxicity of luteolin was assessed by trypan blue exclusion assay. (B&C) The cleavage of PARP protein levels and (D&E) PCNA protein levels were determined by immunoblotting in Hep3B cells and HepG2 cells treated with luteolin at 5 and 10 μmol/L. HSC70 was used to normalize the data. Data were presented as mean ± SEM (n = 3–4). Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan multiple range test, $p < 0.05$). CONT: 0.2% DMSO only.

Please cite this article as: Y. Lee, Y.H. Kwon, Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.073

2.3. Measurement of gene expression using semiquantitative and quantitative PCR analyses

After treatment, total RNA was extracted with RNAiso Plus (Takara, Japan). cDNA was synthesized using 2 μg of total RNA with Superscript II Reverse Transcriptase (Invitrogen, USA). To determine mRNA levels of *Xbp-1,* semi-quantitative PCR was used. Amplified products were further digested by *PstI* (Promega, USA) to check whether a *PstI* restriction site was lost after IRE1-mediated splicing of mRNA. Amplified products were separated on an agarose gel and visualized with a Loading STAR nucleic acid staining solution (Dyne Bio, Korea). For quantitative PCR analysis, mRNA expressions were analyzed by StepOne™ Real Time PCR System (Applied Biosystems, USA) using SYBR® Green PCR Master Mix (Applied Biosystems) according to supplier's protocol. *Actb* was used as a reference gene and relative gene expression levels were analyzed using $2^{-\Delta\Delta Ct}$ method. The primer sequences are described in Supplementary Table 1.

2.4. Statistical analyses

All data were analyzed using SPSS software (ver. 23.0, SPSS Inc., USA). For multiple comparison, significant difference was determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Student's t-test was used for two-group comparison. The data are reported as the means ± SEMs, and differences were considered significant at $p < 0.05$.

3. Results

3.1. Luteolin induced apoptosis and inhibited cell proliferation in p53-null Hep3B cells, but not in p53-wild type HepG2 cells

First, we determined the effect of luteolin on viability of HCC cells, including p53-null Hep3B and p53-wild type HepG2 cells, and normal liver cells, cells were treated with luteolin at concentrations of 1–10 μmol/L for 48 h. As a result, treatment of HCC cells with

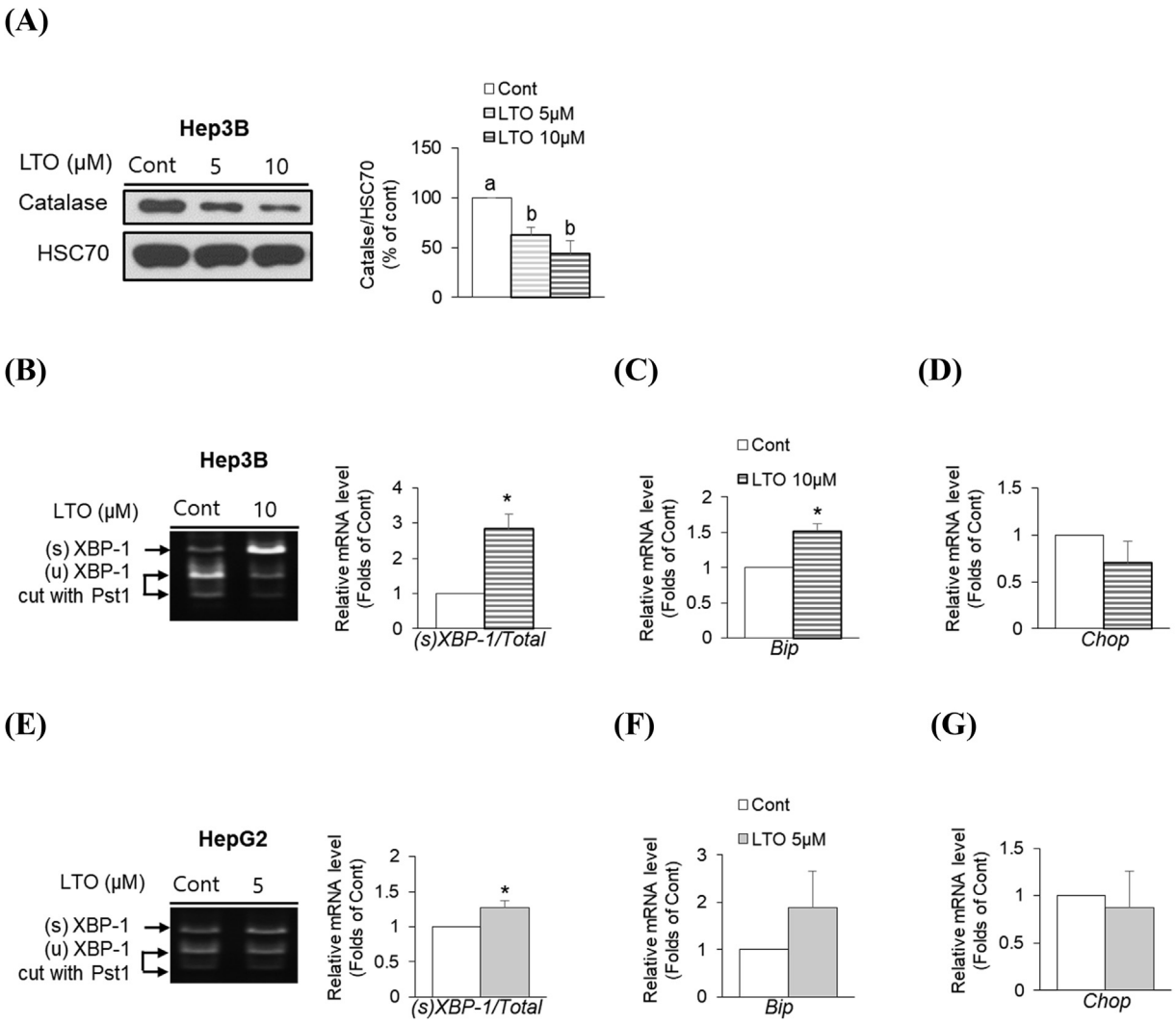

Fig. 2. Luteolin induced ER stress in p53-null Hep3B cells. (A) Antioxidant enzyme catalase protein levels were determined by immunoblotting in Hep3B cells treated with 5 and 10 μmol/L luteolin. HSC70 was used to normalize the data. Gene expression levels of (B) spliced *Xbp-1*, (C) *Bip*, and (D) *Chop* in Hep3B cells treated with 10 μmol/L luteolin, and (E) spliced *Xbp-1*, (F) *Bip*, and (G) *Chop* in HepG2 cells treated with 5 μmol/L luteolin. Spliced *Xbp*-1 mRNA was determined by semiquantitative PCR and total *Xbp-1* was used to normalize spliced *Xbp-1*. *Bip* and *Chop* mRNA levels were determined by quatitative PCR and *Actb* was used to normalize the PCR data. Bars represent the means ± SEM (n = 3–4). Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple comparison test, $p < 0.05$). *$p < 0.05$ compared with CONT (Student's test). CONT: 0.2% DMSO only.

Please cite this article as: Y. Lee, Y.H. Kwon, Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.073

luteolin at 2.5–10 μmol/L significantly decreased cell numbers (Fig. 1A). However, NCTC clone, normal murine liver cells, showed resistance against the cytotoxicity of luteolin at 1–10 μmol/L. To investigate whether apoptosis is involved in a decrease in cell viability, we measured the cleavage of PARP, a well-recognized marker for apoptosis. Protein levels of PARP cleaved form were significantly increased about 20 times in 10 μmol/L luteolin-treated Hep3B cells compared to DMSO-only treated control cells (Fig. 1B). Such a dramatic increase was not observed in luteolin-treated HepG2 cells (Fig. 1C). We also determined whether luteolin decreased Hep3B cell numbers by inhibition of cell proliferation. PCNA, an indicator of cell proliferation, was downregulated in luteolin-treated Hep3B cells in a dose-dependent manner (Fig. 1D). However, no change was detected in HepG2 cells (Fig. 1E).

3.2. Luteolin induced ER stress in p53-null Hep3B cells, but not in p53-wild type HepG2 cells

It has been reported that luteolin increased ER stress via oxidative stress in cancer cells [10]. Consistently, we observed downregulation of catalase protein levels in luteolin-treated Hep3B cells, suggesting the generation of oxidative stress in response to luteolin (Fig. 2A). To investigate whether luteolin induced ER stress in HCC cells, we measured the mRNA levels of ER stress biomarkers, spliced form of *Xbp*-1, *Bip*, and *Chop*. Luteolin treatment at 10 μmol/L significantly upregulated the mRNA levels of both *Bip* (Fig. 2B) and spliced *Xbp-1* (Fig. 2C) in p53 null Hep3B cells. No significant effect was observed in *Chop* mRNA level in Hep3B cells (Fig. 2D). Expression of genes involved in ER stress were also measured in p53 wild type HepG2 cells treated with 5 μmol/L of luteolin, at which a slight increase of apoptosis was observed. There were no significant changes in mRNA levels of *Bip* and *Chop* (Fig. 2E&F). Spliced *Xbp*-1 mRNA levels showed only a slight increase in HepG2 cells (Fig. 2G).

3.3. Induction of TAp63 and TAp73 is not involved in luteolin-induced apoptosis in p53-null Hep3B cells

A previous report showed that luteolin treatment induced anticancer effects via p53 activation in HCC cells [8]. To confirm whether p53 is activated in HepG2 cells in response to luteolin treatment at 5 and 10 μmol/L, we measured protein levels of p53 and mRNA levels of p53 downstream genes, including the pro-apoptotic regulators *Bim, Bax,* and *Puma*, the autophagy modulator *Dram1*, and the proliferation regulator *p21*. Although increases in p53 protein levels were observed (Fig. 3A), there were no change in mRNA levels of p53 target genes, except *Dram1* in luteolin treated-HepG2 cells (Fig. 3B). Interestingly, luteolin treatment upregulated *p21* gene expression in Hep3B cells (Fig. 3C). Thus, we further examined whether p53 family members, including TAp63 and TAp73, are involved in the transactivation of p53-dependent target gene expression. However, we observed downregulation of *TAp63* mRNA levels and no changes in *TAp73* mRNA levels in Hep3B cells in response to luteolin (Fig. 3D).

3.4. Luteolin induced autophagy, which enhanced viability of p53-null Hep3B cells, but not in p53-wild type HepG2 cells

To investigate whether autophagy was regulated by luteolin treatment, we measured the protein levels of LC3-II and p62, which are representative autophagy markers. During the formation of autophagosomal membranes, the level of LC-II is increased by

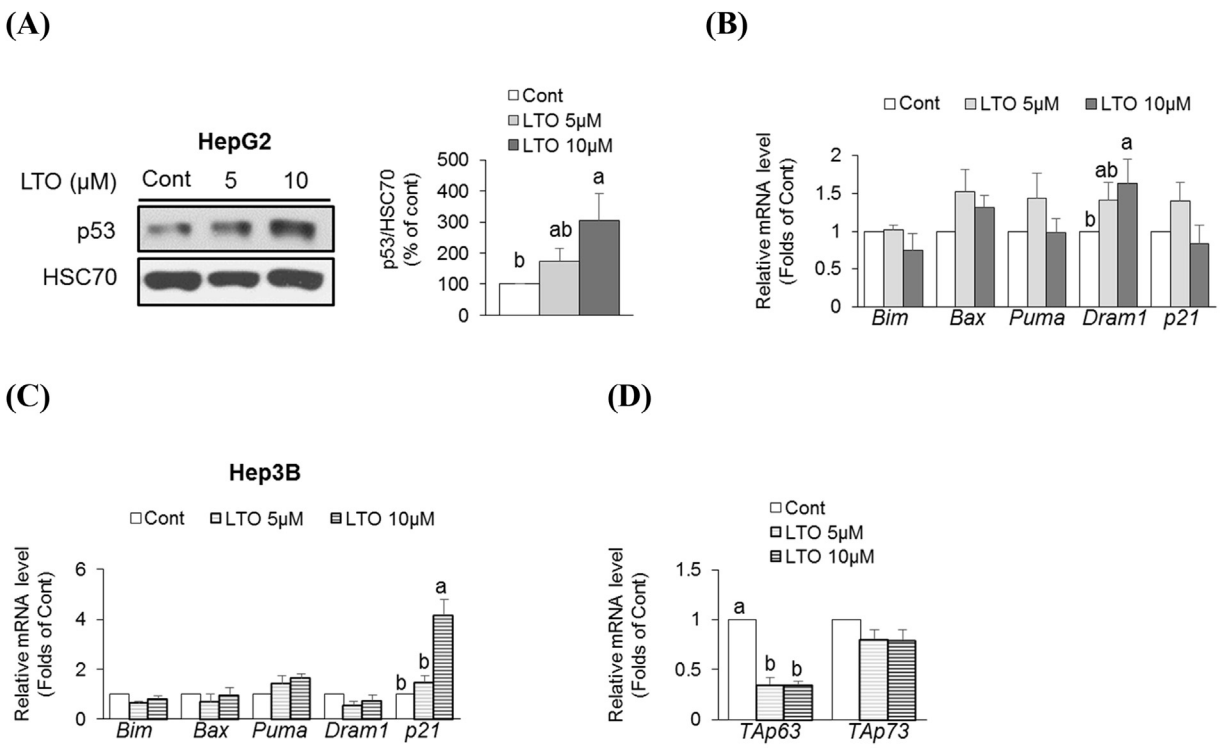

Fig. 3. Induction of TAp63 and TAp73 is not involved in luteolin-induced apoptosis in p53-null Hep3B cells. (A) p53 protein levels were determined by immunoblotting in HepG2 cells treated with 5 and 10 μmol/L luteolin. HSC70 was used to normalize the data. (B) The mRNA levels of p53 downstream genes were determined in p53-wild HepG2 cells treated with 5 and 10 μmol/L luteolin. The mRNA levels of (C) p53 downstream genes and (D) p53 family members were determined in Hep3B cells treated with 5 and 10 μmol/L luteolin. *Actb* was used to normalize the PCR data. Bars represent the means ± SEM (n = 3). Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple comparison test, $p < 0.05$). CONT: 0.2% DMSO only.

Please cite this article as: Y. Lee, Y.H. Kwon, Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.073

conjugation cytosolic LC3 with phosphatidylethanolamine. During the fusion of the autophagosome with lysosomes, LC3-II proteins, located both in- and outside of autophagosome, are also degraded with receptor protein p62 [11,12]. Thus, the level of LC3-II is increased and the level of p62 is decreased simultaneously when autophagy occurs. Indeed, the protein level of LC3-II was upregulated and the protein level of p62 was downregulated via luteolin treatment in p53 null Hep3B cells (Fig. 4A). However, none of changes were observed in p53 wild type HepG2 cells treated with luteolin (Fig. 4B).

To further investigate whether luteolin-induced autophagy increases cell death in Hep3B cells, we measured viable cell numbers in the presence of luteolin with the autophagy inhibitors, 3-MA and CQ. Interestingly, we observed that cell death was increased when luteolin-induced autophagy was inhibited by 3-MA (Fig. 4C) and CQ (Fig. 4D) treatment. Taken together, we identified that luteolin-induced autophagy promotes cancer cell survival.

4. Discussion

The present study investigated anticancer effects of luteolin on p53 activation via ER stress in HCC cells. Unexpectedly, ER stress was only increased in p53-null Hep3B cells. In consistent with ER stress regulation, luteolin-induced anticancer effects were also significantly higher in p53-null Hep3B cells compared to p53-wild type HepG2 cells. Although luteolin at concentrations of

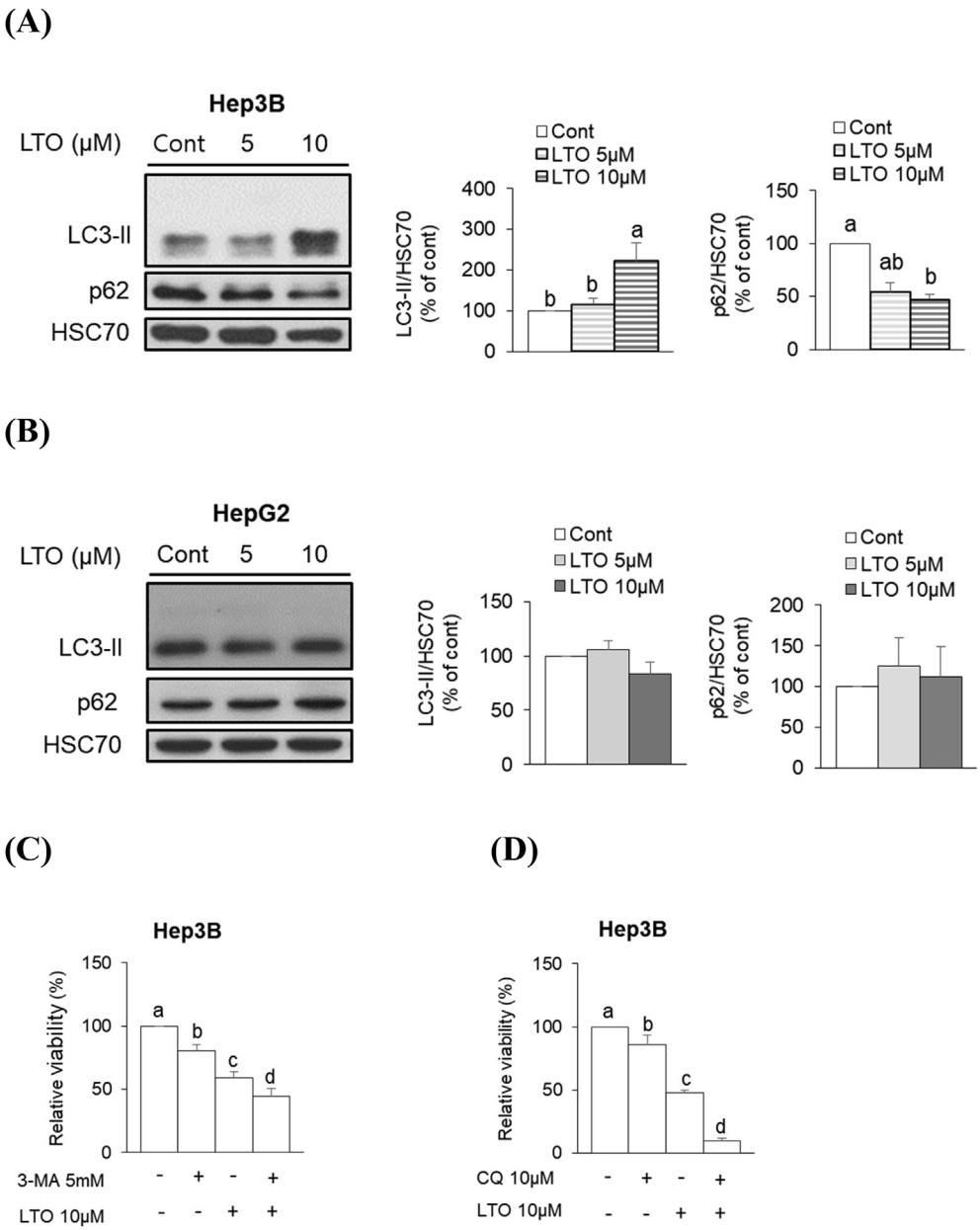

Fig. 4. Luteolin induced autophagy, which enhanced viability of p53-null Hep3B cells. (A) LC3-II and p62 protein levels were determined by immunoblotting in Hep3B cells treated with 5 and 10 μmol/L luteolin. (B) LC3-II and p62 protein levels were determined by immunoblotting in HepG2 cells treated with 5 and 10 μmol/L luteolin. HSC70 was used to normalize the data. (C) Cell viability was measured when luteolin-induced autophagy was blocked in autophagosome formation step by co-incubation 3-MA with luteolin. (D) Cell viability was measured when luteolin-induced autophagy was blocked in autolysosome degradation step by co-incubation CQ with luteolin. Cell viability was assessed by trypan blue exclusion assay. Bars represent the means ± SEMs (n = 3–5). Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple comparison test, $p < 0.05$). CONT: 0.2% DMSO only.

Please cite this article as: Y. Lee, Y.H. Kwon, Regulation of apoptosis and autophagy by luteolin in human hepatocellular cancer Hep3B cells, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.073

1–10 μmol/L induced cytotoxicity of HepG2 cells, the intensity was relatively lower than that in p53-null Hep3B. Also, we identified that there was no upregulation of most of p53 downstream target gene expressions, except *Dram1*, in HepG2 cells. Damage-regulated autophagy modulator *Dram1* is an autophagy-related gene that promotes autophagy via regulating the degradation of autophagosome [13]. However, autophagy was not induced in HepG2 cells in response to luteolin treatment. Taken together, it is possible that luteolin exerts p53-independent anticancer effects in HCC cells. Consistently, a previous study reported that luteolin inhibited proliferation and increased apoptosis in p53-mutant HCC cells [14]. Indeed, sustained ER stress has been shown to induce GCN2/eIF2α phosphorylation and ATF4 expression, which upregulate expression of pro-apoptotic genes, such as Bid and Trb3 [15].

Interestingly, mRNA levels of p21, which is involved in cell cycle arrest, were significantly increased in p53-null HCC cells, but not in p53-wild type HCC cells. Several studies have reported the role of p53 family in cell cycle arrest under stress condition [16]. Indeed, TAp63 and TAp73, the p53 family members, were shown to transactivate p53 downstream genes. However, in the present study, TAp73 was not altered and even TAp 63 was decreased in luteolin-treated p53-null HCC cells. So, the anticancer effects of luteolin seem to be induced via p53-independent. Further investigations are needed to identify the mechanism of p21 regulation.

Here, we observed that luteolin induced autophagy only in p53-null Hep3B cells. Unlike the cytotoxic effects of luteolin via apoptosis, it was identified that luteolin-induced autophagy inhibited a reduction of cell number by co-incubation with autophagy inhibitors. In contrast, luteolin exerted autophagy, which promoted apoptosis in p53-wild SMMC-7721 HCC cells [9]. The concentration of luteolin treatment in the previous study was 10 times higher than that in the present study. In other studies, lower concentration of luteolin treatment (50 μmol/L) induced cytoprotective autophagy in cutaneous squamous cell carcinoma cells [17], while higher concentration (200 μmol/L) of luteolin treatment induced lethal autophagy in lung carcinoma cells [18]. Taken together, the concentration might be an important factor in determining whether luteolin-induced autophagy induces or inhibits cell death.

In conclusion, this study showed that luteolin induces ER stress in p53-null Hep3B cells. Furthermore, apoptosis, proliferation, and autophagy were regulated only in luteolin-treated Hep3B cells. Thus, anticancer effects of luteolin seem to be induced in both p53-dependent and p53-independent manners. The extent of cellular stress may play an important role in switching the involved mechanism of luteolin-induced anticancer effects.

Conflicts of interest

The authors declare that there is no conflict of interests.

Acknowledgments

This research was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT). (No. 2017R1A2B4011432).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.07.073.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424.
[2] N. Patel, A.C. Yopp, A.G. Singal, Diagnostic delays are common among patients with hepatocellular carcinoma, J. Natl. Compr. Cancer Netw. 13 (2015) 543–549.
[3] R.S. Wong, Apoptosis in cancer: from pathogenesis to treatment, J. Exp. Clin. Cancer Res. 30 (2011) 87.
[4] B.J. Aubrey, G.L. Kelly, A. Janic, M.J. Herold, A. Strasser, How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ. 25 (2018) 104–113.
[5] M.T. Rosenfeldt, K.M. Ryan, The multiple roles of autophagy in cancer, Carcinogenesis 32 (2011) 955–963.
[6] Y.P. Vandewynckel, D. Laukens, A. Geerts, et al., The paradox of the unfolded protein response in cancer, Anticancer Res. 33 (2013) 4683–4694.
[7] A. Margariti, H. Li, T. Chen, et al., XBP1 mRNA splicing triggers an autophagic response in endothelial cells through BECLIN-1 transcriptional activation, J. Biol. Chem. 288 (2013) 859–872.
[8] S.B. Yee, H.J. Choi, S.W. Chung, et al., Growth inhibition of luteolin on HepG2 cells is induced via p53 and Fas/Fas-ligand besides the TGF-beta pathway, Int. J. Oncol. 47 (2015) 747–754.
[9] Z. Cao, H. Zhang, X. Cai, et al., Luteolin promotes cell apoptosis by inducing autophagy in hepatocellular carcinoma, Cell. Physiol. Biochem. 43 (2017) 1803–1812.
[10] Q. Wang, H. Wang, Y. Jia, H. Pan, H. Ding, Luteolin induces apoptosis by ROS/ER stress and mitochondrial dysfunction in gliomablastoma, Cancer Chemother. Pharmacol. 79 (2017) 1031–1041.
[11] I. Tanida, T. Ueno, E. Kominami, LC3 and autophagy, Methods Mol. Biol. 445 (2008) 77–88.
[12] M. Cicchini, V. Karantza, B. Xia, Molecular pathways: autophagy in cancer–a matter of timing and context, Clin. Cancer Res. 21 (2015) 498–504.
[13] X.D. Zhang, L. Qi, J.C. Wu, Z.H. Qin, DRAM1 regulates autophagy flux through lysosomes, PLoS One 8 (2013), e63245.
[14] J. Chang, Y. Hsu, P. Kuo, et al., Increase of Bax/Bcl-XL ratio and arrest of cell cycle by luteolin in immortalized human hepatoma cell line, Life Sci. 76 (2005) 1883–1893.
[15] S. Shin, G.R. Buel, L. Wolgamott, et al., ERK2 mediates metabolic stress response to regulate cell fate, Mol. Cell 59 (2015) 382–398.
[16] M. Napoli, E.R. Flores, The p53 family orchestrates the regulation of metabolism: physiological regulation and implications for cancer therapy, Br. J. Canc. 116 (2017) 149–155.
[17] L. Verschooten, K. Barrette, S. Van Kelst, et al., Autophagy inhibitor chloroquine enhanced the cell death inducing effect of the flavonoid luteolin in metastatic squamous cell carcinoma cells, PLoS One 7 (2012), e48264.
[18] S.H. Park, H.S. Park, J.H. Lee, et al., Induction of endoplasmic reticulum stress-mediated apoptosis and non-canonical autophagy by luteolin in NCI-H460 lung carcinoma cells, Food Chem. Toxicol. 56 (2013) 100–109.