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Mechanism of Dose-Dependent Regulation of UBE1L by Polyphenols in Human Bronchial Epithelial Cells

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ABSTRACT

Ubiquitin activating enzyme E1-like (UBE1L) is the activating enzyme for ISG15ylation (ISG15, interferon stimulated gene 15). UBE1L is thought to be a candidate tumor suppressor gene and has positive activity against stress responses such as viral infections. Both type I interferon and retinoic acid are known to induce UBE1L expression. However, the molecular mechanism of UBE1L regulation is unclear. Here, the effect of several chemopreventive polyphenols on UBE1L expression in human bronchial epithelial cells (Beas-2B) was investigated. Lower concentrations of curcumin, (−)-epigallocatechin-3-gallate (EGCG) and resveratrol upregulated UBE1L, while high concentrations of curcumin, EGCG and resveratrol downregulated UBE1L levels. Interestingly, curcumin, EGCG and resveratrol diminished intracellular reactive oxygen species (ROS) at lower concentrations but generated ROS at higher concentrations. The antioxidant N-acetylcysteine (NAC) increased UBE1L protein levels, while pro-oxidants such as hydrogen peroxide and tert-butyl hydroperoxide (tBHP) decreased UBE1L protein levels, indicating that the intracellular redox status is associated with UBE1L expression. Kinase inhibitors were used to examine the contribution of mitogen-activated protein kinase (MAPK) activity to the polyphenol-regulated UBE1L. Only the inhibition of c-Jun N-terminal kinase (JNK) significantly reduced UBE1L expression. Knockdown of nuclear factor erythroid-2 related factor-2 (Nrf2) caused a concomitant decrease in UBE1L protein levels. It is concluded from the above mentioned results that JNK/Nrf2 signal pathway is involved in the regulation of UBE1L via intracellular ROS status when cells came in contact with polyphenols. J. Cell. Biochem. 116: 1553–1562, 2015.

KEY WORDS: CHEMOPREVENTIVE; POLYPHENOLS; UBE1L; ROS; JNK

Ubiquitin activating enzyme E1-like (UBE1L) is the E1-activating enzyme of ubiquitin-like protein ISG15 (interferon-stimulated gene, 15-kDa protein) [Yuan and Krug, 2001]. Human ISG15 is known for antiviral immunity activities. Zhang reported that human intracellular ISG15 is also a key negative regulator for IFN-α/β immunity, preventing interferon-α/β over-amplification and auto-inflammation [Zhang et al., 2015]. UBE1L plays an important role in anti-viral activity [Jeon et al., 2010]. Mice lacking UBE1L demonstrate increased susceptibility to influenza B virus infection [Lai et al., 2009]. UBE1L−/− mice display increased susceptibility to lethality following Sindbis virus infection [Giannakopoulos et al., 2009]. In addition, the UBE1L gene locus on chromosome 3p21 is deleted in many lung cancer cell lines [McLaughlin et al., 2000]. UBE1L overexpression inhibits the growth of human bronchial epithelial cells and lung cancer cells [Feng et al., 2008]. Activation of UBE1L expression in several cancer cell lines promotes cyclin D1 ISGylation and decreases cell growth [Feng et al., 2008]. The promyelocytic leukemia/retinoic acid receptor α (PML/RARα) fusion protein is an oncogene that causes acute promyelocytic leukemia (APL) [Xiao et al., 1993]. Retinoic acid induces UBE1L expression and consequently directs PML/RARα protein ISGylation for degradation [Kitareewan et al., 2002]. Previously, we demonstrated that overexpression of UBE1L reduces both epidermal growth factor receptor (EGFR) expression at the post-translational level and also EGFR downstream of the AKT/NF-κB signaling pathway in human bronchial epithelial cells [Jiang et al., 2014].

Conflicts of interest: There are no conflicts of interest.

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These findings indicate that UBE1L could be a candidate tumor suppressor gene.

Dietary polyphenols are important potential chemopreventive and therapeutic natural agents. Curcumin, (−)-epigallocatechin-3-gallate (EGCG) and resveratrol are dietary polyphenols, which are thought to be natural antioxidants [Zhao et al., 2010]. Reactive oxygen species (ROS) levels reduction may contribute to the chemopreventive properties of polyphenols. EGCG has been reported to inhibit bleomycin-induced pulmonary fibrosis by enhancing antioxidant activities and phase II enzymes such as glutathione-S-transferase and NAD(P)H:quinone oxidoreductase 1; these activities are mediated by the signaling of nuclear factor erythroid 2 related factor 2 (Nrf2), which interacts with the Kelch-like erythroid-cell-derived protein with CNC homology [ECH]-associated protein (Keap1) [Sriram et al., 2009]. However, dietary polyphenols can also act as pro-oxidants [Ramos, 2008]. The pro-oxidant activity of plant polyphenols may be an important mechanism of their anticancer properties. The cytotoxic activity of curcumin in many cancer cells is attributed to its pro-oxidant activity through the generation of ROS [Gandhy et al., 2012]. At cytotoxic concentrations, polyphenols act as chemotherapeutic agents that induce cell apoptosis.

When polyphenols act as antioxidants, one of the major cellular responses is the induction of antioxidative enzymes and carcinogen-detoxification enzymes through the Nrf2–ARE (antioxidant responsive element) system. The activation of several upstream kinases, such as mitogen-activated protein kinases (MAPKs) [Xu et al., 2006] and phosphoinositide 3-kinase (PI3K) [Nakaso et al., 2003], are thought to enhance the nuclear translocation and transcriptional activation of Nrf2. There are three main components of the MAPK family, including the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 MAPK [Coskun et al., 2011]. The ERK and JNK pathways positively activate Nrf2 transactivation domain activity [Shen et al., 2004], while the p38 MAPK pathway can either stimulate or inhibit Nrf2 transactivation activity [Balogun et al., 2003; Shen et al., 2004]. The overexpression of dominant-negative JNK1 suppresses Nrf2-induced ARE reporter gene expression [Keum et al., 2003].

Because UBE1L is a potential tumor suppressor and has anti-viral activity, its regulation is important in cancer prevention, cancer therapy and response against viral infection; however, the mechanism of its regulation remains unclear. Here, we demonstrate a novel mechanism of UBE1L regulation by several chemopreventive polyphenols.

MATERIALS AND METHODS

REAGENTS

Curcumin, EGCG, resveratrol and tert-butyl hydroperoxide (tBHP) were purchased from Sigma–Aldrich Ltd. (Poole, Dorset, UK). SB203580 (p38 inhibitor), PD98059 (ERK1/2 inhibitor), SP600125 (JNK inhibitor), 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) and N-acetyl cysteine (NAC) were purchased from Beyotime (Haimen, China). Hydrogen peroxide (30%, H_2O_2) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

CELL CULTURE

Human bronchial epithelial cells (Beas-2B) were cultured in LHC-9 media (Biofluids, Rockville, MD). The human non-small cell lung cancer cell line LTEP-α-2 was cultures in Dulbecco′s modified Eagle′s medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37°C in a humidified 5% CO_2 atmosphere.

IMMUNOBLOT ANALYSIS

Cell lysates were obtained using a nucleoprotein and cytoplasm protein extraction kit (KeyGEN, Nanjing, China). The protein concentration was measured with bicinchoninic acid (BCA) protein assay kit (Beyotime, Haimen, China). Lysates (60 μg) were loaded on a 7.5% sodium dodecyl sulfate–polyacrylamide gel before transfer to polyvinylidene difluoride membranes (Millipore, MA). Membranes were blocked with 5% non-fat milk solution and sequentially incubated with primary antibody and enzyme-conjugated secondary antibody. β-Actin was used as a loading control. The primary antibodies were β-actin (BOSTER, Wuhan, China), p-JNK1/2, JNK1/2 (Beyotime, Haimen, China) and Nrf2 (sc-365949, Santa Cruz Biotechnology, CA). UBE1L primary antibody was kindly provided by Dr. Ethan Dmitrovsky (Dartmouth Medical School, NH). The protein bands were detected using the Chemiluminesence Detection System (Cell Signaling Technology, Danvers, MA).

REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

Total RNA was isolated from cells using Trizol (Invitrogen, Carlsbad), and cDNA synthesis was performed using the PrimeScript™ RT Master Mix (Takara, Dalian, China). Real time PCR assays were performed with SYBR Premix Ex Taq™ II (Takara, Dalian, China) and the 7300 Real Time PCR System (Applied Biosystems, Foster City, CA) according to the manufacturer′s instructions. Primers were synthesized by Invitrogen (Shanghai, China). The UBE1L forward primer was 5′-TGGGCACCTTGTGTCATAAGC-3′, and the reverse primer was 5′-CTCAGAGTGAGAATGCCAGGG-3′. The GAPDH forward primer was 5′-ACGTGTCAGTCAGTGGTGGACCT-3′, and the reverse primer was 5′-GTCCACCACCCTGTTGCTG-3′.

MTT ASSAY

Cell viability was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Beas-2B cells in 96-well plates were treated with curcumin, EGCG or resveratrol for 24 h. MTT solution (AMRESCO, Solon, OH) (0.5 mg/ml) was added to the culture medium, and the plates were incubated for additional 4 h at 37°C. Dimethyl sulfoxide (DMSO) was added to each well to dissolve the MTT formazan crystal, and the absorbance was measured at 490 nm using a microplate reader (TECAN, Männedorf, Switzerland).

DETERMINATION OF INTRACELLUAR ROS GENERATION

To detect intracellular ROS, Beas-2B cells were incubated with 20 μM 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) for 20 min at 37°C after the indicated treatments. After incubation, the increase in fluorescence resulting from oxidation of DCFH-DA to 2′,7′-dichlorofluorescein (DCF) was measured by flow cytometry (BD, San Jose, CA). The mean fluorescence intensity at 530 nm was calculated. Data were collected from at least 10,000 cells at a flow rate of 250–300 cells/s.

KNOCKDOWN OF NRF2

Cells were cultured in 35-mm culture plates with 2×10^5 cells per well and were transfected with three specific siRNAs targeting Nrf2 or with control siRNA (Ribobio, Guangzhou, China) using Lipofectamine 2000 (Invitrogen, Carlsbad) according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Data were derived from three independent experiments. The parameters are expressed as the mean ± SD. Statistical analysis was performed using analysis of variance with the Newman–Keuls test. $^{*}P < 0.05$ and $^{**}P < 0.01$ values were considered significant. Immunoblots were quantified using ImageJ software [Schneider et al., 2012] (National Institutes of Health).

RESULTS

EFFECT OF POLYPHENOLS ON THE VIABILITY OF BEAS-2B CELLS

We first assessed the effects of polyphenols on the viability of Beas-2B cells with the MTT assay. Figure 1A–C demonstrate that low

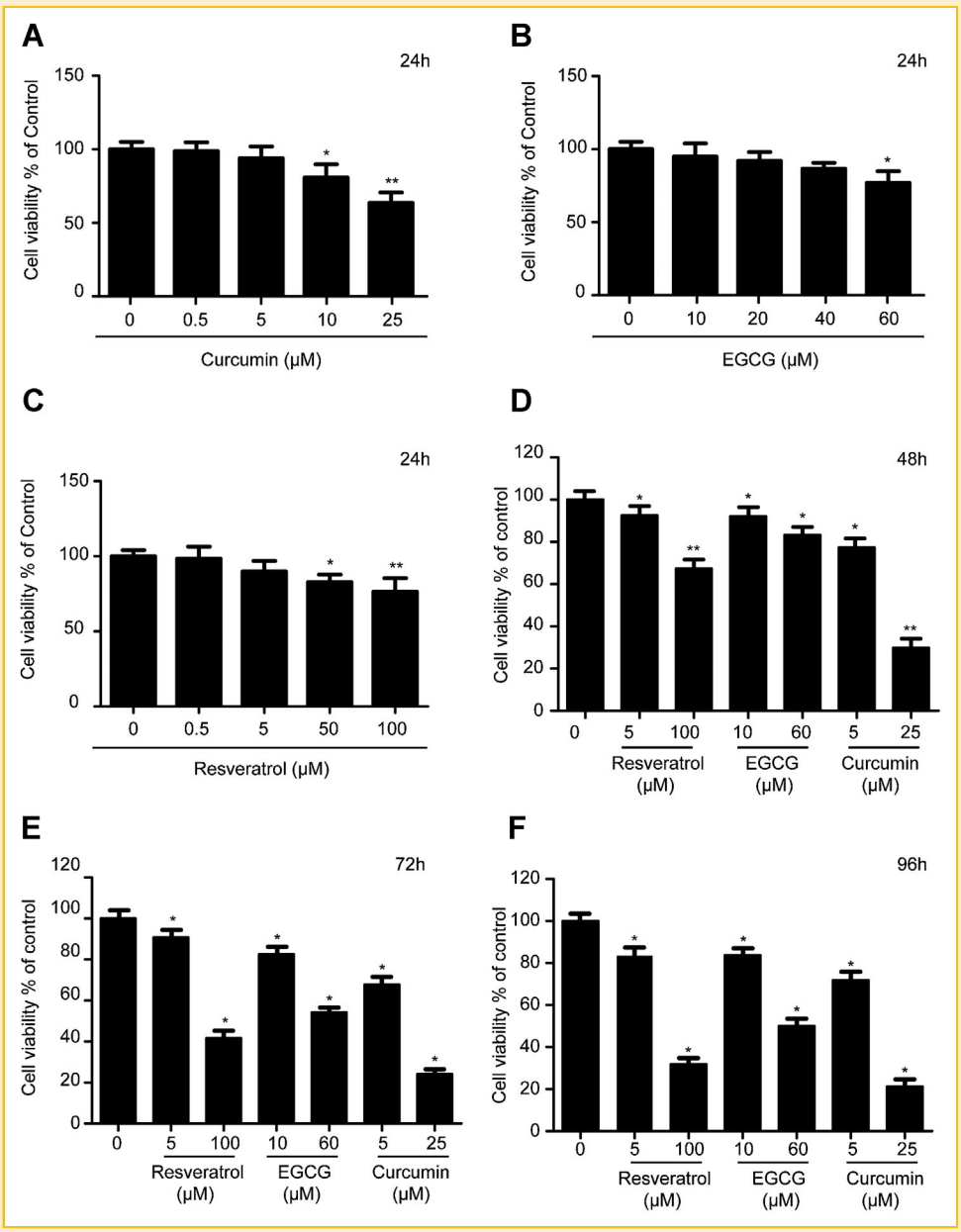

Fig. 1. Effect of polyphenols on cell viability. Beas-2B cells were seeded on 96 well plates. Then the cells were treated with various concentrations of curcumin, EGCG and resveratrol for 24 h (A–C), 48 h (D), 72 h (E), and 96 h (F). The cell viability was then determined by MTT assay. Experiments were done in triplicates. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

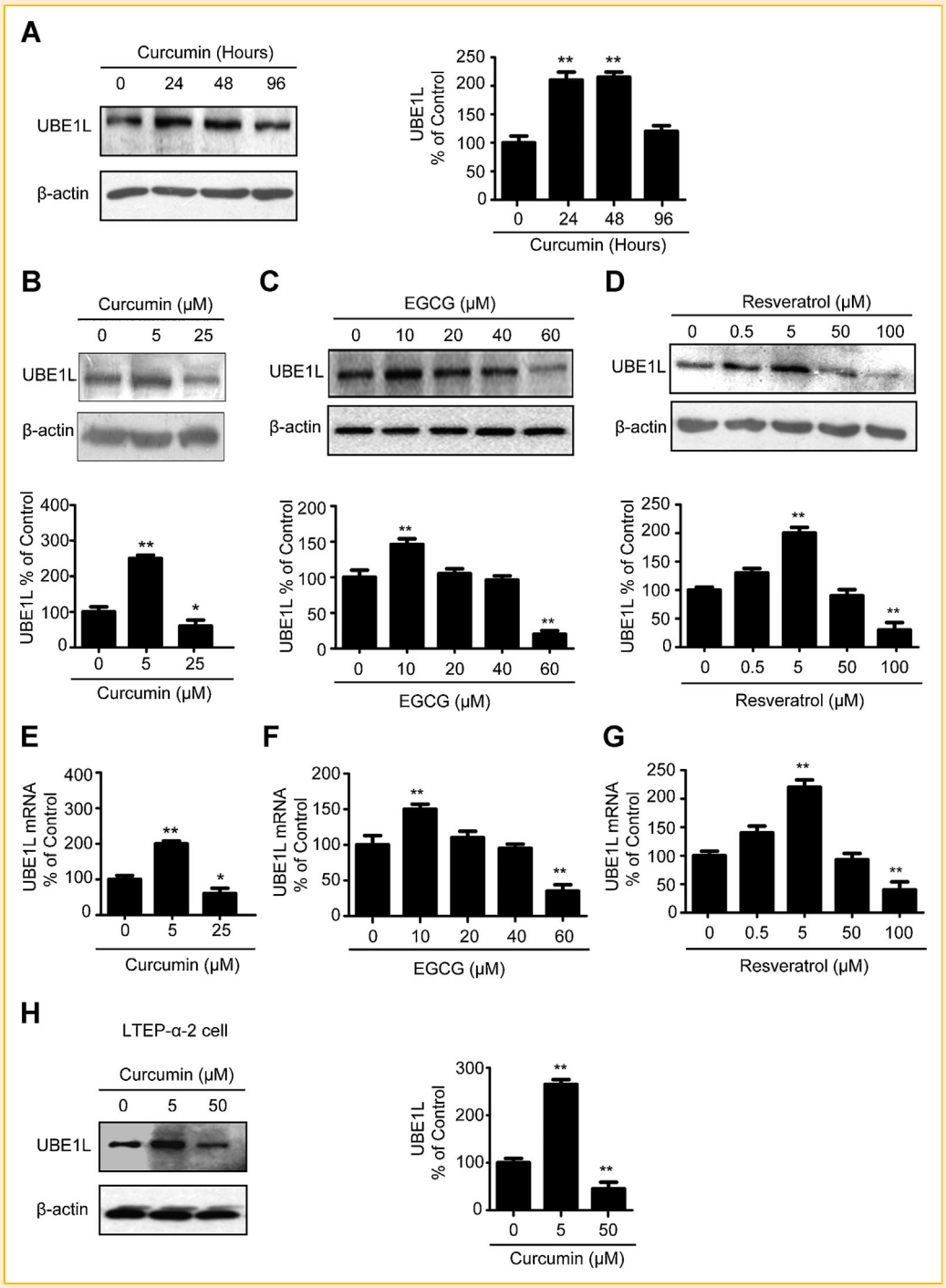

Fig. 2. Concentration-dependent regulation of UBE1L expression by polyphenols. Beas-2B cells were treated with 5 μM of curcumin for 24, 48, and 96 h. A: Cell lysates were subjected to Western blot with anti-UBE1L antibody. Beas-2B cells were treated with 5 and 25 μM of curcumin for 24 h. B: Cell lysates were subjected to Western blot with anti-UBE1L antibody. C: Expressions of UBE1L proteins in Beas-2B cells treated with 10–60 μM of EGCG for 24 h. (D) Expressions of UBE1L proteins in Beas-2B cells treated with 0.5–100 μM of resveratrol for 24 h. E–G: Expressions of UBE1L mRNA levels treated with curcumin, EGCG and resveratrol were analyzed with real time–polymerase chain reaction as described in MATERIALS AND METHODS. H: Expressions of UBE1L proteins in LTEP-α-2 cells treated with 5 and 50 μM of curcumin for 24 h. β-Actin was used as loading control for Western blot. GAPDH was used as internal control for real time PCR. Each blot represents three separate experiments. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

concentrations of curcumin (0.5–5 μM), EGCG (10–40 μM) and resveratrol (0.5–5 μM) did not inhibit Beas-2B cell growth. Additionally, when the cells were incubated with higher concentrations of curcumin (10–25 μM), EGCG (60 μM) and resveratrol (50–100 μM), the cell viability decreased. However, both lower and higher dose of resveratrol (5 and 100 μM), EGCG (10 and 60μM) and curcumin (5 and 25 μM) inhibited the growth of Beas-2B cells after treatment for 48, 72, and 96 h, as shown in Figure 1D–F.

DUAL REGULATION OF UBE1L EXPRESSION BY POLYPHENOLS

Beas-2B cells were treated with 5 μM curcumin for 24, 48, and 96 h. Western blot analysis showed that curcumin significantly induced UBE1L protein expression at 24 and 48 h (Fig. 2A). Therefore, a 24 h incubation time was chosen for the rest of the experiments. Regulation of UBE1L by curcumin in Beas-2B cells was analyzed at both the mRNA and protein levels. Western blot analysis showed that 5 μM but not 25 μM curcumin significantly induced UBE1L protein levels (Fig. 2B). Total RNA was isolated from cells incubated with a range of concentrations of curcumin for 24 h and subjected to real time-PCR. UBE1L mRNA was induced with of 5 μM curcumin, but was reduced by 25 μM curcumin treatment (Fig. 2E). This dose-dependent dual regulation of UBE1L was also observed with EGCG and resveratrol treatment. EGCG induced maximum UBE1L expression at the 10 μM dose. At EGCG dose higher than 40 μM, UBE1L protein and mRNA expression were decreased (Fig. 2C, F). Resveratrol induced UBE1L maximum expression at the 5 μM dose. At 100 μM resveratrol, UBE1L protein and mRNA expression decreased (Fig. 2D, G). To test whether the polyphenols show the same dual regulation of UBE1L in other cell lines, lung cancer LTEP-α-2 cells were treated with curcumin. Figure 2H shows that 5 μM curcumin induced UBE1L protein expression, while 50 μM curcumin reduced UBE1L levels.

ROS REGULATION BY POLYPHENOLS IS RESPONSIBLE FOR UBE1L EXPRESSION

To verify the dual character of plant polyphenols as antioxidants and pro-oxidants, intracellular ROS was measured by flow cytometry. The intracellular ROS levels decreased with 5 μM curcumin, 10 μM EGCG and 5 μM resveratrol. The intracellular ROS levels increased with 25 μM curcumin, 60 μM EGCG and 100 μM resveratrol (Fig. 3A–C). Interestingly, the concentrations of polyphenols that induced or reduced cellular ROS were consistent with the concentrations of polyphenols that regulated UBE1L expression (Fig. 2). This result indicates that intracellular ROS may be responsible for UBE1L regulation.

EFFECT OF ANTIOXIDANTS AND OXIDANTS ON UBE1L EXPRESSION

To investigate whether intracellular ROS was involved in the expression of UBE1L, antioxidants and pro-oxidants were applied to the cells. As Figure 4A showed, when Beas-2B cells were treated with the antioxidant NAC (10–1,000 μM), UBE1L levels increased. Treatment with the oxidant H_2O_2 decreased UBE1L protein levels in a dose dependent manner (Fig. 4B). tBHP, another pro-oxidant, also reduced UBE1L protein levels (Fig. 4C). Co-treatment of Beas-2B cells with 0.5 μM curcumin and 10 μM NAC enhanced UBE1L induction compared to treatment with only curcumin or NAC (Fig. 4D). Pretreatment of cells with 5 μM curcumin partially prevented the 100 μM tBHP-mediated reduction of UBE1L, as shown in Figure 4E. Moreover, Beas-2B cells were pretreated with 10 μM NAC for 4 h, and then incubated with curcumin (25 μM), EGCG (60 μM), or resveratrol (100 μM). Western blot analysis showed that the decreased UBE1L level was reversed by NAC (Fig. 4F–H). These results demonstrate that the regulation of UBE1L may be associated with intracellular redox status.

JNK CONTRIBUTE TO THE REGULATION OF UBE1L BY POLYPHENOLS

It is known that MAPKs play an important role in intracellular redox status [Yu et al., 2000]. To examine the contribution of MAPK activity in the regulation of UBE1L by redox status, Beas-2B cells were treated with MAPK inhibitors including ERK1/2 inhibitor PD98059, p38 inhibitor SB203580, and JNK inhibitor SP600125. Inhibition of JNK significantly reduced UBE1L protein levels, while inhibition of ERK and p38 had no effect on UBE1L expression (Fig. 5A). The effect of the inhibitors on cell viability has been investigated by MTT assay and the results were shown in supplement data (Fig. S1). The JNK inhibitor

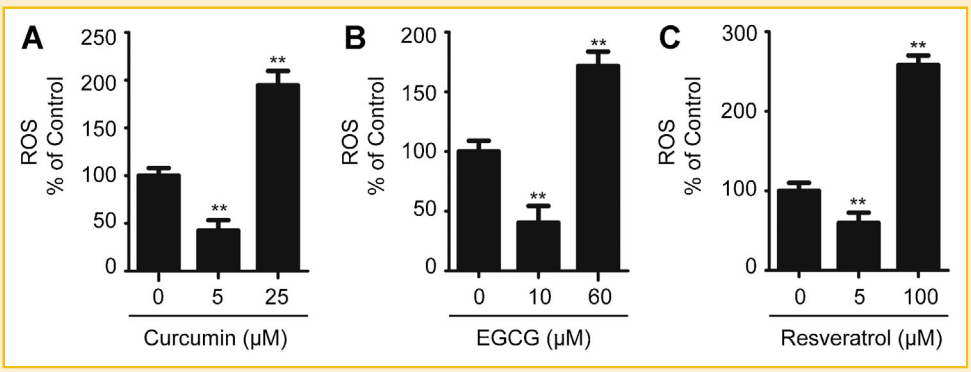

Fig. 3. Dual effect of polyphenol on ROS level. Beas-2B cells were seeded onto 6-well culture plates and treated with various concentrations of curcumin (A), EGCG (B) and resveratrol (C) for 24 h. The DCF fluorescence intensity was measured by a flow cytometry. Data are representative results from three independent experiments. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

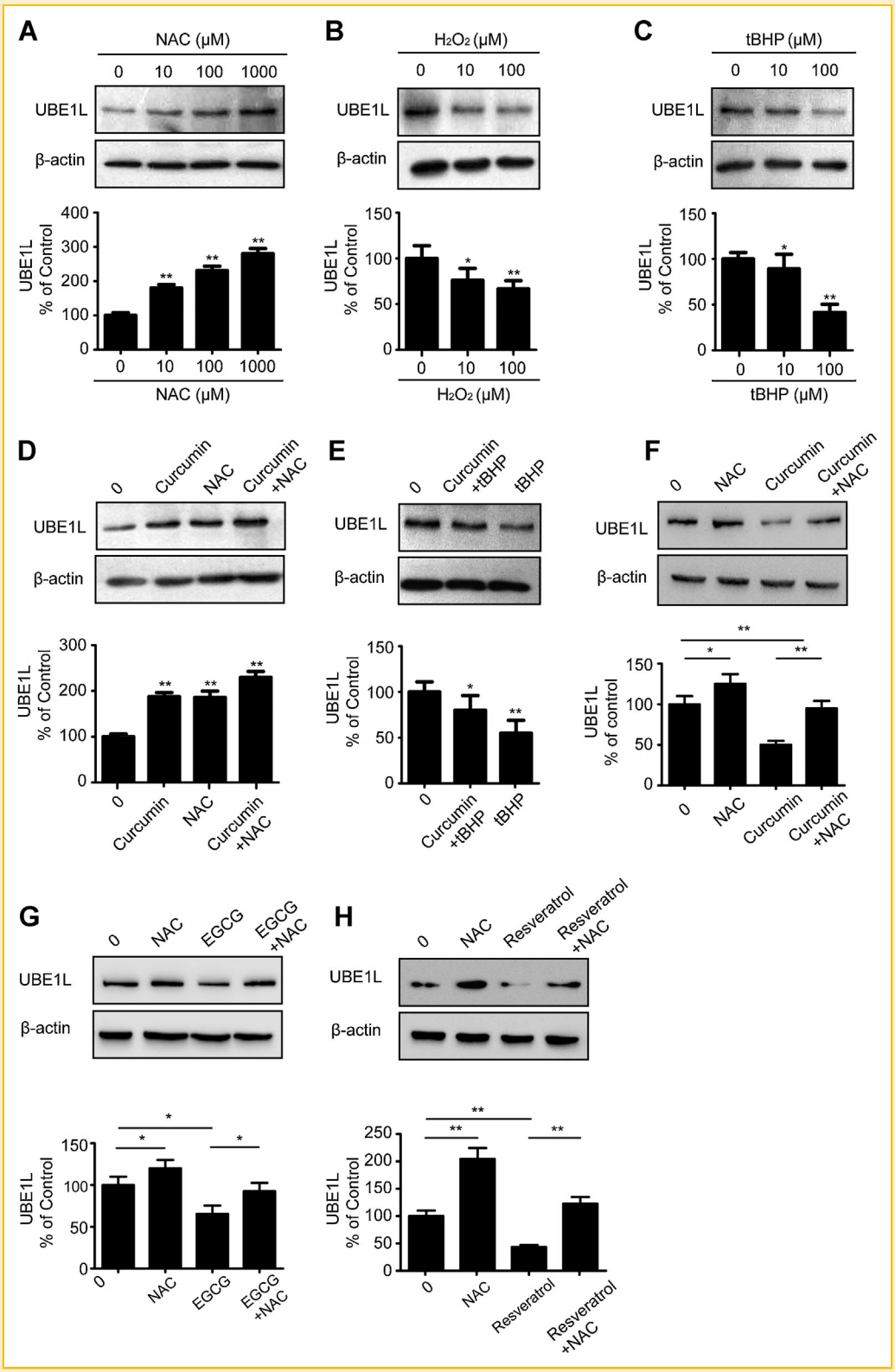

Fig. 4. Antioxidants induce UBE1L, while pro-oxidants inhibit UBE1L expression. Beas-2B cells were treated with various concentrations of NAC (A), H_2O_2 (B) and tBHP (C) for 24 h. Co-treatment of cells with curcumin (0.5 μM) and NAC (10 μM) for 24 h (D). Pretreatment of cells with curcumin for 4 h and then cells were treated with tBHP for 24 h (E). Pretreatment of cells with NAC (10 μM) for 4 h and then the cells were treated with curcumin (25 μM), EGCG (60 μM) or resveratrol (100 μM) for 24 h (F–H). Cell lysates were prepared and subjected to Western blot with anti-UBE1L antibody. β-Actin was used as loading control. Each blot represents three separate experiments. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

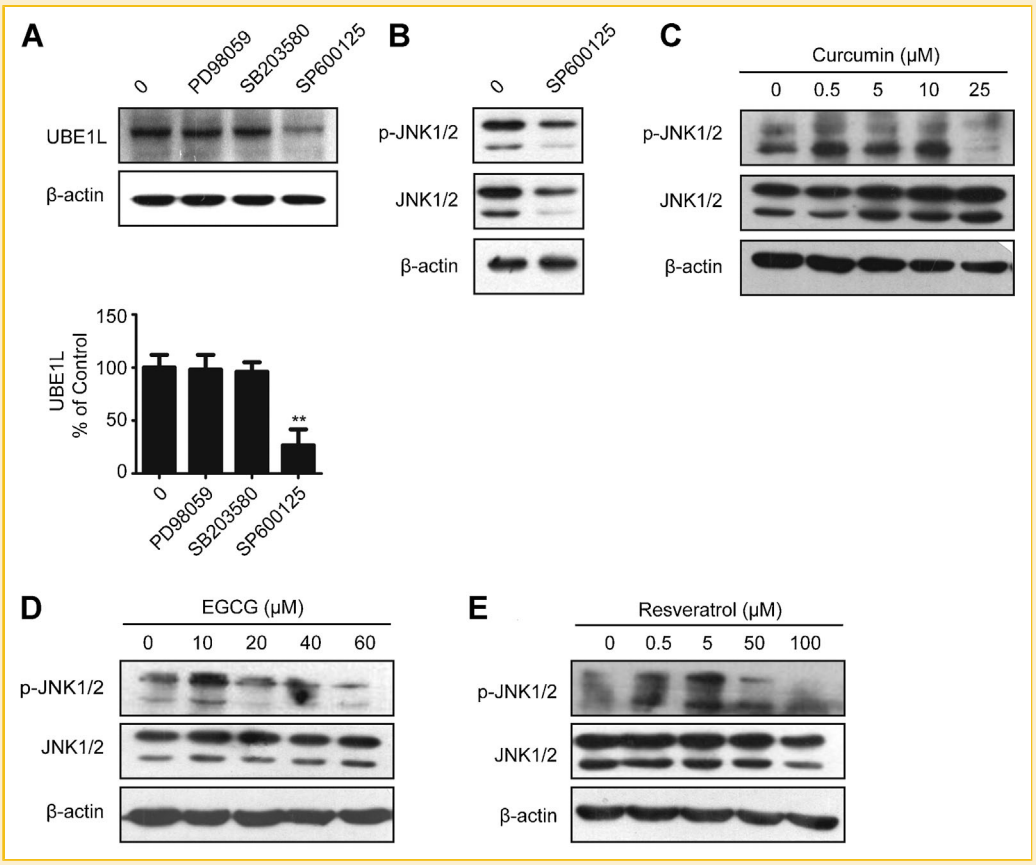

Fig. 5. Effect of JNK on regulation of UBE1L. A: Beas-2B cells were treated with 30 μM ERK1/2 inhibitor PD98059, p38 inhibitor SB203580 and JNK inhibitor SP600125 for 24 h. The UBE1L level was evaluated by Western blot analysis. B: Beas-2B cells were treated with 30 μM JNK inhibitor SP600125 for 24 h. The p-JNK1/2 and JNK1/2 levels were evaluated by Western blot analysis. C–E: Beas-2B cells were treated with various concentrations of curcumin, EGCG and resveratrol for 24 h. The p-JNK1/2 and JNK1/2 levels were evaluated by western blot analysis. β-Actin was used as loading control. Each blot represents three separate experiments. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

SP600125 inhibited p-JNK and JNK protein levels (Fig. 5B). Lower concentrations of curcumin (0.5–10 μM) increased p-JNK protein levels, while at 25 μM, curcumin decreased p-JNK levels (Fig. 5C). The induction of p-JNK by EGCG was observed at 10 μM. However, at 60 μM EGCG, there was a decrease in p-JNK levels (Fig. 5D). Lower concentrations of resveratrol (0.5 and 5 μM) increased p-JNK protein levels. High concentrations of resveratrol (100 μM) suppressed p-JNK protein levels (Fig. 5E). The total JNK expression was not influenced by curcumin, EGCG and resveratrol treatment. These results indicate that activated JNK contributes to UBE1L induction by curcumin, EGCG and resveratrol in Beas-2B cells.

Nrf2 IS INVOLVED IN UBE1L EXPRESSION

Nrf2 is a redox-sensitive transcription factor that regulates the expression of antioxidative enzymes, which confers cytoprotection against oxidative stress [Surh et al., 2008]. As mentioned above, JNK is upstream kinase of Nrf2. When JNK expression was inhibited by JNK inhibitor SP600125, Nrf2 protein level was decreased (Fig. 6A). When Beas-2B cells were transfected with three single siRNAs targeting Nrf2, the UBE1L protein level was decreased (Fig. 6B). These results further suggest that Nrf2 is responsible for UBE1L expression.

DISCUSSION

Because UBE1L is considered to be a potential tumor suppressor and also plays an essential role in anti-viral activities, it is important to determine the mechanism of regulation of UBE1L. To our knowledge, this is the first report to uncover that the chemopreventive polyphenols curcumin, EGCG and resveratrol can influence intracellular redox status, which in turn can regulate UBE1L expression in human bronchial epithelial cells. JNK/Nrf2 pathway is responsible for ROS levels in UBE1L expression. These data also suggest a novel chemoprevention mechanism for polyphenols.

In this study, lower dose of curcumin, EGCG and resveratrol upregulate UBE1L, while high dose of curcumin, EGCG, and resveratrol downregulate UBE1L level. We found that intracellular ROS was decreased or increased when the cells were treated with lower or higher concentrations of curcumin, EGCG, and resveratrol. Increased ROS may lead to a decrease in cellular viability. The mechanism of the dual action of the polyphenols is linked to the phenolic concentration, cell type, and/or time of treatment [Juan et al., 2005]. Resveratrol-mediated heme oxygenase-1 induction occurred strongly in a concentration-dependent manner at

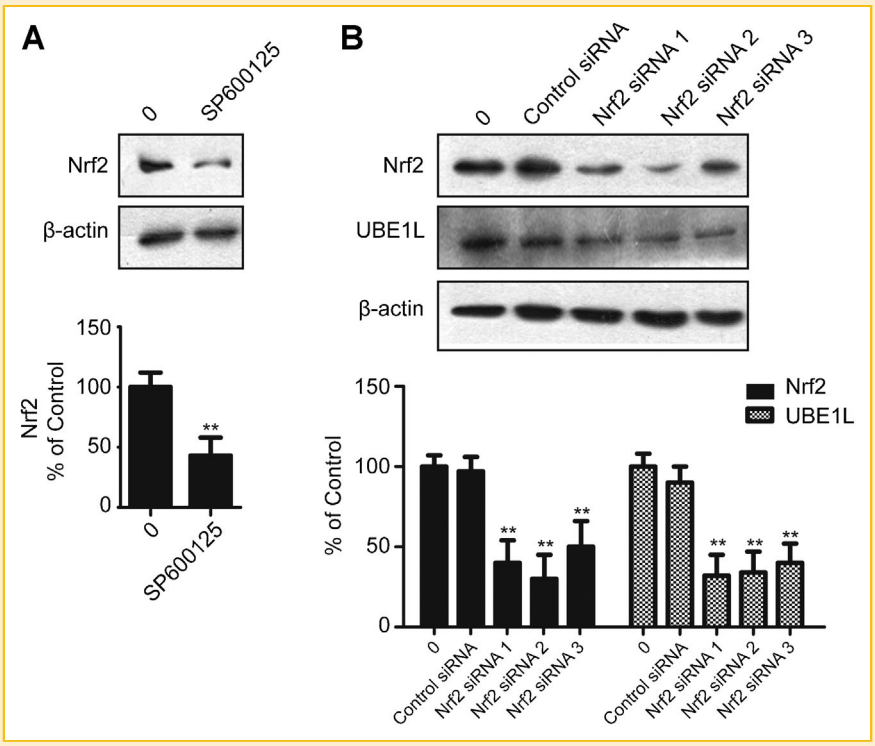

Fig. 6. Effect of Nrf2 on regulation of UBE1L. A: Beas-2B cells were treated with 30 μM JNK inhibitor SP600125 for 24 h. The Nrf2 levels were evaluated by western blot analysis. B: Beas-2B cells were transfected with three single siRNA targeting Nrf2 or control siRNA for 24 h. The Nrf2 and UBE1L levels were evaluated by Western blot analysis. β-Actin was used as loading control. Each blot represents three separate experiments. Data were expressed as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

concentrations of less than 10 μM. Nevertheless, resveratrol, at concentrations of 20 μM and above, diminished heme oxygenase-1 expression as well as its promoter activity [Juan et al., 2005]. Although the chemopreventive polyphenols are well-known anti-oxidants, several reports have demonstrated that they are also pro-oxidant agents at higher doses. Low dose of curcumin depressed intracellular ROS levels induced by Cu(II). However, high doses of curcumin failed to decrease Cu (II)-induced oxidative stress [Huang et al., 2011]. EGCG had a protective effect on DNA at low concentrations (2–30 μM), but it enhanced the DNA oxidative damage at higher concentrations (>60 μM) thus exhibiting a pro-oxidant effect on DNA [Tian et al., 2007].

Nrf2 plays an important role in the maintenance of intracellular ROS levels [Surh et al., 2008]. Knockdown of Nrf2 expression in lung cancer cells induces generation of ROS [Singh et al., 2008]. MAPKs are serine-threonine protein kinases that play an essential role in signal transduction from the cell membrane to the nucleus. Activation of MAPKs induces Nrf2-dependent ARE activity and Nrf2-related antioxidant enzymes [Li et al., 2013]. It is interesting that the UBE1L level is negatively correlated with the intracellular ROS level. Among the three major MAPK kinases, only JNK has been found to contribute to UBE1L induction by low doses of curcumin, EGCG and resveratrol in Beas-2B cells. Nrf2 has been shown to be activated by JNK signaling pathway [Liang et al., 2013]. We speculate that JNK triggers the expression of Nrf2 and then leads to reduction of the ROS level, which is responsible for UBE1L expression. How JNK affects Nrf2 and thereby regulates UBE1L needs to be verified in future.

UBE1L is known to be induced by certain stresses such as type I IFNs [de Veer et al., 2001], chemotherapy drugs (doxorubicin and camptothecin) [Jeon et al., 2012] and LPS [Kim et al., 2005]. Under the stress of bone marrow transplantation, hematopoietic cells from UBE1L-deficient mice exhibit an impaired proliferation defect [Cong et al., 2010]. UBE1L deficiency causes a block of the cell cycle at phase G2/M in hematopoietic multipotential progenitors [Cong et al., 2010]. These results indicate that UBE1L plays a significant role during interferon related stress responses, such those that occur after bone marrow transplantation. *UBE1L* is also a retinoic acid-induced gene [Kitareewan et al., 2002; Pitha-Rowe et al., 2004]. It is hypothesized that all-*trans*-retinoic acid (ATRA) elevates the levels of UBE1L through the IFN signaling pathway. Treatment with retinoic acid leads to an increased secretion of type I IFNs into culture media. A blockade of the type I IFN receptor with a neutralizing antibody prevents retinoic acid mediated accumulation of ISG15 and its conjugates [Dao et al., 2006].

Curcumin, EGCG and resveratrol are well studied natural anticancer agents. Poor bioavailability of these polyphenols is a major issue for therapeutic use. An increasing number of recent studies have aimed to design novel formulations to overcome these barriers. Bioavailability of curcumin, EGCG and resveratrol has been improved using different nanotechnology approaches [Aras et al., 2014]. For example, 48 h after oral administration of Cur-SLNs

(curcumin solid lipid nanoparticles), curcumin plasma concentrations were still higher than 2 μg/ml, whereas it was undetectable after 24 h for curcumin suspension in rats model [Ji et al., 2014]. The area under the concentration–time curve from time zero to time ($AUC_{0\rightarrow t}$) value of Cur-SLNs (124.51 ± 14.53 (μg/ml) h) was 12.27 times greater than that of the curcumin (10.14 ± 0.61(μg/ml) h) suspension [Ji et al., 2014]. Here, the concentration of 5 μM of curcumin, 10 μM of EGCG and 5 μM of resveratrol showed antioxidant properties. We found that lower concentrations of curcumin, EGCG and resveratrol were able to decrease intracellular ROS through increasing JNK phosphorylation, resulting in the induction of UBE1L. Because of the low bioavailability of polyphenols, it is likely that curcumin, EGCG and resveratrol exert their chemopreventive actions through UBE1L induction with a moderate daily diet. With novel formulations, it is hopeful that these polyphenols can be developed as therapeutic drugs in the future.

In conclusion, the chemopreventive polyphenol-mediated regulation of UBE1L is through changes in the intracellular ROS status, which may be regulated through the JNK/Nrf2 signal pathway. Because UBE1L plays important roles in tumor suppression and stress responses, such as during viral infections, the mechanism of UBE1L regulation reported in this study may provide a new strategy for cancer chemoprevention or therapy.

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