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Reverse effect of curcumin on CDDP-induced drug-resistance via Keap1/p62-Nrf2 signaling in A549/CDDP cell

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ABSTRACT

Objective: To assess the effect of curcumin on CDDP-induced drug resistance and explore the underlying molecular mechanism through Nrf2 system and autophagy pathway.

Methods: A drug-resistant cell model was established by exposing A549/CDDP cell to 2 μg/mL CDDP. A549/CDDP cell was treated with 20 μg/mL CDDP and 10 μM curcumin. The cell viability and apoptosis level, the signals of Keap1/P62-Nrf2 and autophagy pathway were analyzed.

Results: CDDP induction promoted drug-resistant phenotype in A549/CDDP cell and activated autophagy as well as Nrf2 signals in A549/CDDP cell. Meanwhile, curcumin combination attenuated autophagy and Nrf2 activation induced by CDDP, and reversed the drug-resistant phenotype. Notably, curcumin combination augmented Keap1 transcription. Furthermore, Keap1 ablation with short hairpin RNAs hampered the efficacy of curcumin, suggesting Keap1 played a crucial role on reversal effect of curcumin.

Conclusions: The present findings demonstrate that CDDP promotes abnormal activation of Nrf2 pathway and autophagy, leading to drug resistance of A549/CDDP cell. Curcumin attenuates this process and combat drug-resistance through its potent activation on Keap1 transcription, which is essential for interplay between oxidative stress induced Nrf2 activation and autophagy/apoptosis switch.

1. Introduction

Chemoresistance proves a tough obstacle to CDDP cisplatin (CDDP) based regimen, the backbone of chemotherapy for human tumors including lung cancer [1,2]. Inspiringly, the ongoing development of natural polyphenols as novel chemosensitizers has opened up new opportunities to combat chemoresistance for us [3]. Curcumin, a natural polyphenolic compound derived from spice turmeric (*Curcuma longa*) and favored by Asian as dietary ingredients, had been demonstrated to enhance chemotherapy efficacy through regulating nuclear factor erythroid 2-related factor 2 (Nrf2) activation [3,4], which regulates reactive oxygen species (ROS) to response to cellular redox by activating transcription of antioxidant genes, including phase II detoxifying enzymes and other stress response proteins.

Recently, this polyphenol has also been revealed to induce autophagic cell death [5–7]. Moreover, both autophagy and Nrf2 activation were essential for cancer cells to survive against oxidative stress and to promote chemoresistance [8,9]. Importantly, Nrf2-ARE system activation had been associated with deregulation of autophagy induced by oxidative stress [10,11].

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Curcumin might sensitize CDDP through repression of Nrf2-ARE system activation, and its relation with autophagy has also been revealed. Therefore, we hypothesized that curcumin might reverse chemoresistance by regulating cell responses to oxidative stress and autophagy. In our study, we employed CDDP-induced human lung adenocarcinoma cell lines (A549/CDDP) as a model to investigate autophagy and Nrf2 signaling, and observe the effects of curcumin on drug-resistant phenotype.

2. Materials and methods

2.1. Cell culture

The human lung adenocarcinoma cell A549 and the CDDP-resistant clones A549/CDDP cell line were cultured at 37 °C in a 5% CO_2 and 95% air atmosphere, in DMEM (HyClone, USA) supplemented with 10% FBS (HyClone), 100 U/mL penicillin and 100 U/mL streptomycin. A549/CDDP cell was maintained with 2 μg/mL CDDP (Sigma–Aldrich, St. Louis, MO, USA). Curcumin (curcuminoid content ≥ 94%, curcumin ≥80%), and 3-Methyladenine (3-MA, M9281) were obtained from Sigma–Aldrich.

In order to investigate the autophagy signaling of A549/CDDP cell exposed to CDDP induction, time course of conversion of microtubule-associated protein light-chain 3 (LC3) which is a biochemical marker for autophagy was detected. A549/CDDP cell was exposed to 20 μg/mL CDDP for 4, 12, 24, and 48 h, respectively. A549/CDDP cell were treated with different doses of curcumin for 24 h, 10 μM was identified as a low concentration of curcumin based on the cell viability assay. To clarify the effect of curcumin on CDDP induced drug-resistance, A549/CDDP cells were treated with increasing doses of CDDP for 24 h, in combination with 10 μM curcumin, and with pretreatment of autophagy inhibitor 3-MA (2 mM), respectively. For pathway study, A549/CDDP cell was treated with DMEM as control, CDDP (20 μg/mL unless otherwise stated), curcumin (10 μM unless otherwise stated), CDDP + curcumin, CDDP + 3-MA (2 mM), respectively.

2.2. Cell viability assays

The cell viability was measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. In brief, exponentially growing cells were plated in 96-well plates (1×10^4 cells per well). Then indicated concentrations of CDDP were added, and incubation proceeded for 24 h. To each well, 20 μL MTT (Beyotime Biotech, Beijing, China) was added followed by incubation for 4 h, and then 150 μL dimethyl sulfoxide (Beyotime) was added to dissolve the formazan crystals. Absorbance was measured in Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland) at a wavelength of 570 nm. The inhibition rate was calculated as follows: $(1 - A_{exp\ group}/A_{control}) \times 100\%$.

2.3. Real time quantitative PCR determination

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription of 2 μg of RNA was done using oligo (dT) primers. Real time quantitative PCR were determinated on StepOne Plus Real time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with 20 ng of cDNA, together with QuantiNova SYBR Green PCR Master mix (Qiagen, Germany) and specific primers (Table 1). The expression of housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was measured for comparative reference. The mRNA relative quantitation was calculated using the ΔΔCt method.

2.4. Western blot analysis

Cells were washed and lysed in ice-cold radio-immunoprecipitation lysis buffer (Beyotime). Cell lysates were sonicated and centrifuged to remove debris. Protein concentrations were determined using the Protein Assay Kit (Beyotime).

For western blot analysis, protein samples (30 μg) were separated on a 10% w/v SDS-PAGE gel by electrophoresis, then transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). Membranes were incubated with the desired primary antibody overnight at 4 °C, and then with corresponding secondary antibody. Antibodies against Keap1, Nrf2, p62/SQSTM1(P62), LC3-I, LC3-II, NQO1, Lamin B, and actin were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). Immunoreactive bands were visualized using the diaminobenzene coloration method. The representative bands were determined with Chemiscope 3000 gel image system (Clinx Science Instruments, Shanghai, China) and analyzed.

2.5. Keap1 knockdown by lentivirus short hairpin RNA (shRNA)

In order to explore the role of Keap1 in synergistic effect of curcumin, cultured cells were transfected with designed shRNA for Keap1, three of four independently designed shRNAs (Table 2) against Keap1. Lentivirus shRNA targeting human Keap1 (GenBank accession NM_203500) of A549/CDDP were screened and constructed by Genechem (Shanghai, China). Knockdown with lentivirus was performed according to the manufacturer's instruction. In brief, A549/CDDP cells were seeded into 6-well plates, and infected on the following day with quantitative lentivirus granula. The cell infection rate was identified to above 80% according to green fluorescent protein fluorescence intensity observed under fluorescence microscope after 3 days of infection. Cells were harvested and whole cell lysates were prepared for western blot. For the apoptosis level assay, infected cells were disposed on combination of CDDP and curcumin for 24 h and subjected to the flow cytometry assay to determine cell apoptosis level.

Table 1
Primer sequences used in real-time PCR assays.

Gene	Sequence (5′–3′)
Nrf2	(F) ATAGCTGAGCCCAGTATC
	(R) CATGCACGTGAGTGCTCT
Keap1	(F) CTGGTATCTGAAACCCGTCTA
	(R) TGGCTTCTAATGCCCTGA
NQO1	(F) CAGTGGTTTGGAGTCCCTGCC
	(R) TCCCCGTGGATCCCTTGCAG
P62	(F) ATCGGAGGATCCGAGTGT
	(R) TGGCTGTGAGCTGCTCTT
GAPDH	(F) AGAAGGCTGGGGCTCATTTG
	(R) AGGGGCCATCCACAGTCTTC

Table 2
Target sequences and structures of shRNA for Keap1.

No.	Target Seq	ShRNA Seq
sh#1	GAATGATCACAGCAATGAA	ccgggcGAATGATCACAGCAATGAActcgagTTCATTGCTGTGATCATTCgcttttttg
		aattcaaaaagcGAATGATCACAGCAATGAActcgagTTCATTGCTGTGATCATTCgc
sh#2	GGCGAATGATCACAGCAAT	ccgggtGGCGAATGATCACAGCAATctcgagATTGCTGTGATCATTCGCCacttttttg
		aattcaaaaagtGGCGAATGATCACAGCAATctcgagATTGCTGTGATCATTCGCCac
sh#3	CTTAATTCAGCTGAGTGTT	ccgggcCTTAATTCAGCTGAGTGTTctcgagAACACTCAGCTGAATTAAGgcttttttg
		aattcaaaaagcCTTAATTCAGCTGAGTGTTctcgagAACACTCAGCTGAATTAAGgc
sh#4	GGAGTACATCTACATGCAT	ccggcgGGAGTACATCTACATGCATctcgagATGCATGTAGATGTACTCCcgttttttg
		aattcaaaaacgGGAGTACATCTACATGCATctcgagATGCATGTAGATGTACTCCcg

2.6. Flow cytometry analyses

Following exposure to specialized experimental conditions, cells were trypsinized and stained with propidium iodide and annexin V-FITC (Invitrogen, Carlsbad, CA) for 15 min at 37 °C. Data were then analyzed for apoptosis level in a Novocyte 2040R flow cytometer (ACEA Biosciences, Hangzhou, China).

2.7. Statistical analyses

Experiments were performed on three or five occasions, and data were presented as the mean ± SD. Comparisons between treatments were examined using one-way ANOVA; differences between treatment means were examined with Dunnett's test. GraphPad Prism software, version 5.0 was adopted (GraphPad Software, Inc., San Diego, California).

3. Results

3.1. Deregulation of Keap1/P62-Nrf2 signaling in A549/CDDP cell by CDDP

A549/CDDP cell was markedly more resistant to CDDP than normal A549 cell (Figure 1A). The calculated IC_{50} value of A549/CDDP cell was 67.9 μg/mL, significantly higher than that of normal A549 cell (14.7 μg/mL). As for the apoptosis level, compared with control, it did not increase in 20 μg/mL of CDDP treated cells, which indicated CDDP-induced resistance (Figure 2B). These observations verified that CDDP could induce drug-resistant phenotype in A549/CDDP cell.

The protein level of LC3-II was enhanced in a time dependent manner in A549/CDDP cell (Figure 1B). At the same time, the expression of total Nrf2 protein also increased in similar time course of autophagy activation in A549/CDDP cell treated with CDDP.

The Keap1/P62-Nrf2 signaling was then investigated in A549/CDDP cell treated with CDDP for 24 h. Keap1, the degrading modulator of Nrf2, was markedly decreased by CDDP in A549/CDDP cell. Nrf2 and NADPH quinone oxidoreductase 1 (NQO1), a typical Nrf2 promoted gene, increased coherently (Table 3). P62/SQSTM1 protein (sequestosome 1, hereafter referred to as P62) was believed to participate in both autophagy and Nrf2 activation.

Furthermore, qRT-PCR was conducted to detect the mRNA levels of *Keap1/P62-Nrf2* signaling. CDDP induction was found to significantly reduce the mRNA level of *Keap1*, and associated with concomitant activation of *Nrf2-ARE* gene (Table 3). Meanwhile, the mRNA levels of *P62* were less influenced. Hence, the activation of *Nrf2* signaling by CDDP might be attributed to *Keap1* transcription suppression.

3.2. Reverse effect of curcumin combination on CDDP-induced drug resistance by Keap1/P62-Nrf2 pathway

Compared with CDDP treatment alone, curcumin combination or 3-MA pretreatment inhibited A549/CDDP cell proliferation evidently (Figure 2A). Furthermore, consistent with cell viability assay, the results of flow cytometry analysis indicated curcumin or 3-MA significantly augmented CDDP-induced apoptotic cell death, as evidenced by increased apoptosis ratio (Figure 2B).

In addition, the expressing changes in Keap1/P62-Nrf2 signaling following curcumin combination was observed. Combination of curcumin brought about efficient up-regulation

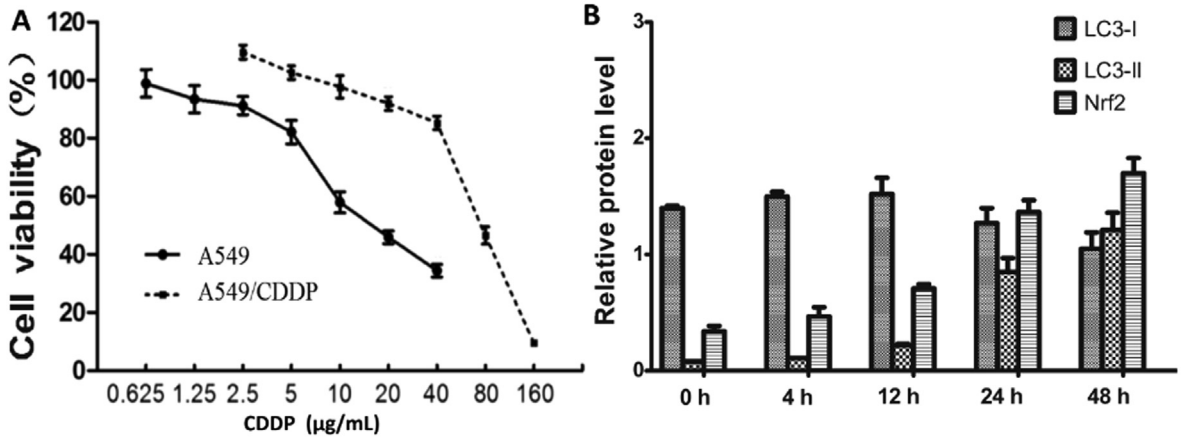

Figure 1. Deregulation of Keap1/P62-Nrf2 signaling in A549/CDDP cell by CDDP.
(A) Cell viability of A549 and A549/CDDP cell on different concentrations of CDDP for 24 h.
(B) Time course of LC3-II conversion and Nrf2 expression in A549/CDDP cell induced by CDDP (20 μg/mL).

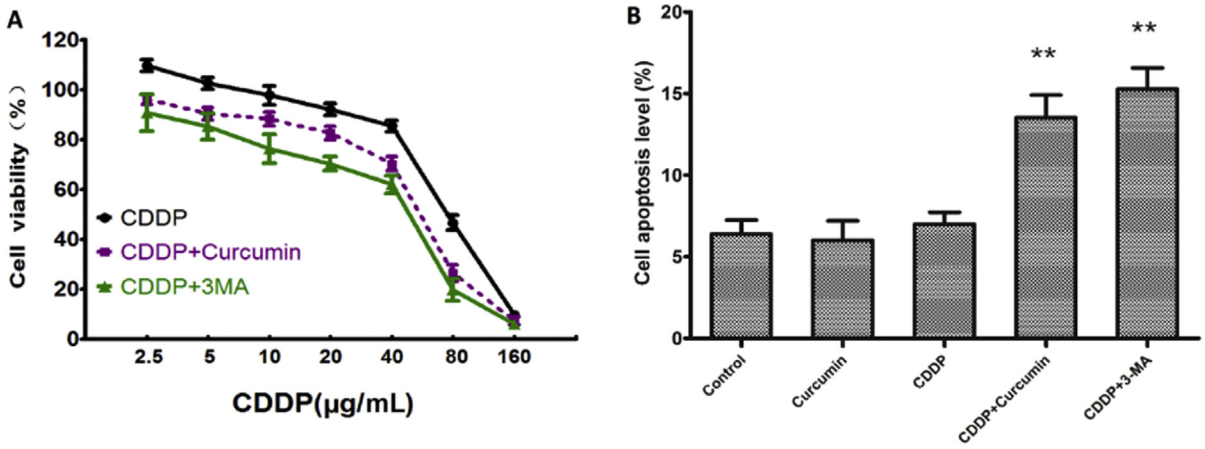

Figure 2. Reverse effect of curcumin combination on CDDP-induced drug resistance by Keap1/P62-Nrf2 pathway.
(A) Cell viability.
(B) Cell apoptosis level. **: $P < 0.01$, compared to the control.

Table 3
Effect of 20 μg/mL CDDP on protein and mRNA expressions of Keap1/P62-Nrf2 pathway in A549/CDDP cell ($n = 3$, mean ± SD).

Items		Control	CDDP
mRNA	*Keap1*	1.000 ± 0.021	0.420 ± 0.114**
	P62	1.010 ± 0.015	1.170 ± 0.087**
	Nrf2	1.000 ± 0.024	1.920 ± 0.025**
	NQO1	1.000 ± 0.034	1.880 ± 0.136**
Protein	Keap1	0.320 ± 0.014	0.100 ± 0.005**
	P62	3.540 ± 0.099	1.270 ± 0.053**
	Nrf2	1.330 ± 0.187	1.970 ± 0.179**
	NQO1	2.340 ± 0.383	4.270 ± 0.399**

**: $P < 0.01$ compared with control group.

of Keap1, which led to negative regulating on Nrf2 signaling. Consequently, autophagy and Nrf2 system activated by CDDP was suppressed (Table 4).

Interestingly, curcumin combination strikingly up-regulated Keap1 and down-regulated downstream target: *NQO1* transcription, compared to CDDP treatment alone (Table 5). However, curcumin combination was associated with less change in *P62* or *Nrf2* mRNA levels (Table 5). Therefore, the Keap1/P62-Nrf2 signaling in response to curcumin might be mediated by activation of Keap1 transcription.

3.3. Keap1 mediated reverse effect of curcumin on A549/CDDP cell

The designed shRNAs demonstrated efficient Keap1 suppression (Figure 3A), and sh#2 displayed better knockdown efficiency. Western blot analysis also confirmed the successful ablation of Keap1 protein in A549/CDDP cell (Table 6). The shRNA was used to ablate Keap1 by lentivirus for further experiments.

Then the experiment was performed to test whether Keap1 depletion conferred resistance to chemotherapeutic drug treatment. Suppression of Keap1 significantly restored CDDP-resistant phenotype of A549/CDDP cell regardless of the presence of curcumin, as determined by flow cytometry analysis (Figure 3B). The effects of such knockdown on autophagy were

Table 4
Effect of curcumin combination on protein expression of Keap1/P62-Nrf2 pathway in A549/CDDP cells ($n = 3$, mean ± SD).

Protein	Control	CDDP	Curcumin	CDDP + Curcumin
Keap1	0.340 ± 0.008	0.100 ± 0.004**	0.430 ± 0.049*##	0.800 ± 0.081##
P62	3.540 ± 0.059	1.280 ± 0.042**	3.990 ± 0.271*##	6.490 ± 0.632##
Nrf2	1.390 ± 0.158	2.190 ± 0.155**	1.760 ± 0.223##	1.070 ± 0.073*##
Nrf2-N	0.730 ± 0.059	1.360 ± 0.102**	0.390 ± 0.045*##	0.080 ± 0.016##
NQO1	2.170 ± 0.176	4.060 ± 0.305**	1.830 ± 0.016*##	1.380 ± 0.269##
LC3-I	0.890 ± 0.014	0.740 ± 0.038**	0.780 ± 0.032**##	0.590 ± 0.019##
LC3-II	0.280 ± 0.003	0.640 ± 0.075**	0.330 ± 0.037##	0.130 ± 0.001##

*: $P < 0.05$, **: $P < 0.01$, compared to the control; ##: $P < 0.01$ compared to the CDDP treatment alone.

Table 5
Effect of curcumin combination on mRNA expression of Keap1/P62-Nrf2 pathway in A549/CDDP cells ($n = 3$, mean ± SD).

mRNA	Control	CDDP	Curcumin	CDDP + Curcumin
Keap1	1.000 ± 0.023	0.390 ± 0.087**	1.530 ± 0.052**##	3.340 ± 0.221**##
P62	1.010 ± 0.034	1.240 ± 0.042**	1.890 ± 0.021**##	2.490 ± 0.172**##
Nrf2	1.000 ± 0.047	1.980 ± 0.045**	1.750 ± 0.053**##	1.070 ± 0.073##
NQO1	1.000 ± 0.028	1.920 ± 0.076**	1.730 ± 0.014**##	0.370 ± 0.009**##

**: $P < 0.01$, compared to the control; ##: $P < 0.01$ compared to the CDDP treatment alone.

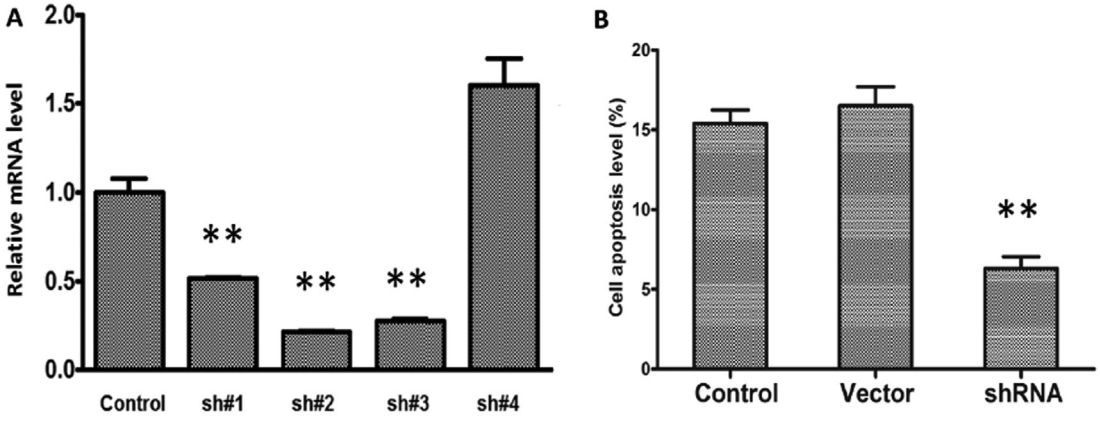

Figure 3. Abrogation of reverse effect of curcumin on A549/CDDP cell by Keap1 ablation.
(A) Validation of suppression efficacy by shRNA. **: $P < 0.01$, compared to the control.
(B) Abrogation of curcumin induced apoptosis by Keap1 ablation. **$P < 0.01$, compared to the control.

Table 6
Effect of Keap1 ablation on protein expression of Keap1/P62-Nrf2 pathway in A549/CDDP cells ($n = 5$, mean ± SD).

Protein	Control	Vector	shRNA
Keap1	0.740 ± 0.010	0.750 ± 0.035	0.110 ± 0.024**
P62	5.540 ± 0.133	5.760 ± 0.172	4.930 ± 0.283*
Nrf2	1.230 ± 0.045	1.250 ± 0.050	2.760 ± 0.200**
NQO1	1.320 ± 0.063	1.350 ± 0.074	2.730 ± 0.111**
LC3-I	0.590 ± 0.005	0.610 ± 0.023	0.520 ± 0.031*
LC3-II	0.140 ± 0.009	0.160 ± 0.008	0.820 ± 0.022**

*: $P < 0.05$, **: $P < 0.01$, compared to the control.

confirmed by reducing LC3-I/II conversion levels. Consistently, Keap1-depleted cells showed increased Nrf2 protein level and expression of NQO1, indicating that both autophagy and Nrf2 system might be driven by Keap1.

The data showed Keap1 inactivation abrogated curcumin mediated CDDP-sensitization, and Keap1 depletion also led to a marked increase of P62 and Nrf2 levels in A549/CDDP cell. Therefore, Keap1 ablation caused P62-related Nrf2 activation and deregulated autophagy impairment. In conclusion, these results further demonstrated that Keap1 mediates sensitization effect of curcumin on CDDP through Keap1/P62-Nrf2 signaling.

4. Discussion

In the present study, a chemoresistant cell model was established by exposing cells to CDDP to simulate oxidative stress, which demonstrated Keap1/P62-Nrf2 dysfunction as a dominant trigger for drug resistance in A549/CDDP cell. It was likely that CDDP induction induced Nrf2 signaling as well as autophagy activation. The data also indicated curcumin might serve as a nontoxic chemosensitizer by mediating cross-talks between oxidative stress and autophagy signals in cancer cells. Moreover, a distinct mechanism was identified whereby curcumin inhibited hyperactivated Nrf2 and autophagic signaling induced by CDDP.

Both Nrf2 and autophagy activation have been reported to play protective roles in CDDP-induced apoptotic cell death [10,11]. In general, Nrf2 was ubiquitinated through Keap1 and degraded continuously. Upon exposure to ROS, Keap1 was inactivated and Nrf2 was stabilized and translocated into nucleus. Consequently, a series of cytoprotective genes encoding detoxifying enzymes and antioxidant proteins were activated and transcripted. However, it has been reported that Nrf2 was constantly stabilized in lung cancers with poor prognosis [12,13]. Coherently, ROS levels were much higher in tumor than normal cells, which might be attributed to mitochondrial and metabolic dysfunction. Hence, it was supposed that tumors could hijack the Nrf2 activation to protect themselves from drug induced cell death, leading to intrinsic or acquired chemoresistance [14–16].

Although the role of autophagy in cell death and survival was still debatable, there was an increasing understanding that autophagy plays a prosurvival function in cells under stress status, including oxidative stress and DNA damage, which leads to resistance of cancer cells to chemotherapy. Therefore, inhibition of autophagy has been recognized as a potential therapeutic method to combat drug-resistance [17]. In this study, 3-MA, a pharmacological inhibitor of autophagy, was employed to be the positive control of autophagy inhibition.

P62 is a selective autophagy ingredient binding directly with LC3-II, it up-regulated Nrf2 by competitively binding to Keap1 [10,14]. Multiplicative P62 was an indication of potential autophagy impairment, while decrease of P62 could be applied to recognize autophagic flux [18]. CDDP significantly decreased P62 accumulation in A549/CDDP cell, which could be associated with autophagic flux promotion.

Altogether, it is reasonable to assume that CDDP facilitated autophagy-flux and enhanced Nrf2 activation by suppressing Keap1 transcription. On the other hand, curcumin combination augmented Keap1 transcription, leading to Keap1/P62 up-regulation, which in turn inactivated Nrf2 signaling, and switched cell from autophagy to apoptosis via P62-related autophagy impairment.

More importantly, this study clarified the individual contributions of Keap1 and P62 in Keap1/P62-Nrf2 system, and Keap1 was indispensable for curcumin-mediated sensitization to CDDP, suggesting the sensitization of curcumin was largely dependent on the Keap1 status. Therefore Keap1 was identified as a negative regulator of the P62-Nrf2 pathway, which likely caused the sensitization of curcumin. These results also indicated that Keap1 was a major determinant in the response of

tumors to CDDP, and served as an inhibitor for autophagy and Nrf2 system, which could be attributed to its regulation on Keap1/P62 level. Keap1 may bridge the feedback loop between ROS-regulated Nrf2 system and P62-regulated autophagy, and exert great effect on the switch of autophagy/apoptosis [8,19,20]. Accordingly, the transgenation of Keap1 that hampers its connection with Nrf2 had been reported to bring about hyperactivation of Nrf2 in a series of human tumors [15,16].

Data from the current study also expand our knowledge on the bioactivity of curcumin, which had been researched through multiple methods [21–24]. The results of the study prove that curcumin attenuates CDDP induced drug-resistance by repression of autophagy and Nrf2 activation as well as transcriptional activation of Keap1. Correspondingly, curcumin has been reported to promote autophagy in cancer cells in previous researches [19,25].

A large number of studies demonstrate that curcumin was a very promising chemosensitizer through the induction of ROS dependent cell death and the modulation of oxidative stress [3,22,26]. For instance, a curcumin analog could engender autophagy by a biphasic mechanism in HepG2 cell. Low concentrations (<10 μM) evoked autophagy with invertible and mild cytoplasmic vacuolization, while high concentrations stimulated autophagic flux, and generated an arrested autophagy progress with irreversible and intensive cytoplasmic vacuolization.

It should be noted that 10 μM curcumin treatment alone did not initiate activation of Keap1 transcription in A549/CDDP cell. Therefore, curcumin combination might help rebalance the feedback loop of Keap1/P62-Nrf2 system, which might help cancer cells to survive in apoptosis induced by antitumor drugs.

Keap1 activation resulting from curcumin combination might cooperate with other oxidative stress and autophagy signaling pathways. For instance, a recent study reported that curcumin inhibited fork head box O3 (Foxo3a) nuclear translocation in human neuroblastoma cells [27]. Foxo inactivation was previously reported to promote tumorigenesis and drug resistance by Keap1-Nrf2 pathway [28]. On this basis, it can be assumed that curcumin may restore the dysregulated AKT/Foxo pathway, which may drive chemoresistance progression by downregulated Keap1/P62-Nrf2 signaling in A549/CDDP cell.

In conclusion, the interplay between oxidative stress and autophagy might protect caner cells during chemotherapy, attenuate the efficacy of antitumor drugs, and ultimately result in poor sensitivity of lung caner to chemotherapy [17,19]. Moreover, this study demonstrated that curcumin combination activates the expression of Keap1 and Keap1/P62-Nrf2 signaling in A549/CDDP cell. Further study is required to identify the underlying molecular mechanisms of curcumin on autophagy inhibition and Nrf2 activation by regulating Keap1 transcription. We hope present study may inspire more developments of curcumin and other natural polyphenols as novel chemosensitizers to combat chemoresistance.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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