A New Cyclooxygenase-2 Inhibitor, (1*E*,4*E*)-1,5-Bis(2-bromophenyl)penta-1,4-dien-3-one (GL63) Suppresses Cyclooxygenase-2 Gene Expression in Human Lung Epithelial Cancer Cells: Coupled mRNA Stabilization and Posttranscriptional Inhibition

Jian XIAO,^{a,b} Yi TAN,^{b,c} Yunbao PAN,^a Guang LIANG,^b Changju QU,^a Xie ZHANG,^b Yi ZHANG,^b Xiaokun LI,^{*,b} and Huiling YANG^{*,a}

^aDepartment of Pathophysiology, Sun Yat-Sen University; Guangzhou 510080, P.R. China; ^bKey Laboratory of Biotechnology Pharmaceutical Engineering, Wenzhou Medical College, School of Pharmaceutical Science; Wenzhou 325035, P.R. China; and ^cDepartment of Pediatrics, University of Louisville; Louisville, Kentucky 40202, U.S.A.

Received January 26, 2010; accepted April 7, 2010; published online April 28, 2010

Lung cancer is a leading cause of morbidity and mortality worldwide. Cyclooxygenase-2 (COX-2) expression is upregulated in lung carcinomas and is considered an attractive therapeutic target. In this study, the effect of curcumin and curcumin analogues on COX-2 expression induced by phorbol 12-myristate 13-acetate (PMA) were investigated. We found that a novel curcumin analogue (GL63) inhibited PMA-induced COX-2 mRNA and protein levels in H460 cells to a greater degree than curcumin. To understand the molecular mechanisms governing COX-2 regulation, the effect on COX-2 mRNA degradation was examined; we found that GL63 significantly decreased COX-2 mRNA stability by reducing cytoplasmic localization and protein abundance of human antigen R (HuR). The 3'-untranslated region (3'-UTR) report gene assay also showed GL63 substantially reduced the 3'-UTR green fluorescent protein values, indicating that the destabilizing effect on COX-2 mRNA may be coupled with the posttranscriptional inhibition of COX-2. Taken together, our results provide evidence that the novel curcumin analogue can effectively inhibit PMA-induced COX-2 expression in H460 cells, a mechanism associated with COX-2 mRNA stability and post-transcriptional regulation.

Key words curcumin analogue; cyclooxygenase-2; human antigen R; posttranscription

Curcumin is a widely used food additive and constituent of traditional medicine, which has long been used to treat various inflammatory diseases throughout the Asia-Indian subcontinent.¹⁾ It has a surprisingly wide range of beneficial properties, including antiinflammatory, antioxidant, chemopreventive, and chemotherapeutic activities, and has been used to treat several different cancers, including lung carcinomas.²⁾ However, preclinical and clinical studies have indicated that the poor bioavailability and pharmacokinetic profile of curcumin, due to its instability under physiologic conditions, have limited its application in anticancer therapy.^{3–5)}

Recently, much research effort has been focused on the chemical modifications of curcumin to generate potential analogues with superior bioavailability and enhanced antitumor activities.^{6–10)} To this end, we have designed and synthesized a series of monocarbonyl analogues of curcumin by deleting the reactive β -diketone moiety.^{11,12)}

Cyclooxygenase-2 (COX-2) is the key enzyme in the conversion of arachidonic acid to prostaglandins (PGs) and other bioactive lipids and contributes to the regulation of normal growth responses and aberrant cellular growth. COX-2 overexpression has been associated with poor prognosis in resected lung cancer patients; moreover, lung cancer incidence has been found to be reduced among users of nonsteroidal antiinflammatory drugs.¹³⁾ There has also been recent evidence reported that supports COX-2 as a promoter of tumor proliferation, invasive angiogenesis, and resistance to apoptosis in lung cancer.^{14–16)} COX-2 inhibitors have been characterized as capable of impeding tumor growth in animal models and of eliciting responses when combined with conventional therapy in phase II clinical trials; furthermore, they have been effectively used as radiosensitizers under chemo-

radiation conditions.^{16,17)}

Phorbol 12-myristate 13-acetate (PMA), found in croton oil from the *Croton tiglium* plant, functions as an environmental tumor promoter and has been reported to upregulate COX-2 expression in human lung cancer cells.¹⁸⁾ Due to the likely correlation between malignancy and COX-2, inhibition of PMA-induced COX-2 expression has become a particularly attractive option for cancer prevention and tumor treatment. Some reports have also indicated that curcumin was able to inhibit PMA-induced COX-2 expression in mouse osteoblastic cells¹⁹⁾ and gastrointestinal epithelial cells.²⁰⁾

Since PMA-induced COX-2 expression may facilitate tumor formation and progression, curcumin or curcumin analogue mediated inhibition of PMA-induced COX-2 expression merits detailed investigation, especially in relation to human cancer cells. Among the statistics calculated for cancer-related deaths, lung cancer continues to be the leading cause of cancer deaths wide world.²¹⁾ The development of innovative new therapeutics for the management of lung cancer is especially urgent.

In this study, we synthesized a new COX-2 inhibitor, (1*E*,4*E*)-1,5-bis(2-bromophenyl)penta-1,4-dien-3-one (GL63), an analogue of curcumin. We also found evidence that GL63 is able to suppress PMA-induced COX-2 expression in a human lung epithelial cancer cell line, H460, more effectively than curcumin. Moreover, we report that GL63 regulates COX-2 expression and is involved in COX-2 mRNA stabilization via the RNA binding protein human antigen R (HuR) subcellular localization and HuR abundance. In conclusion, we generated a new COX-2 inhibitor with potential for clinical therapeutic applications in lung cancer patients.

* To whom correspondence should be addressed. e-mail: xiaokunli@163.net; hlyangsums@hotmail.com

MATERIALS AND METHODS

Materials Human lung epithelial H460 cancer cells, were purchased from the American Type Culture Collection (Manassas, VA, U.S.A.). Cell culture reagents and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Antibodies against COX-2, HuR, Lamin B, and horseradish peroxidase (HRP)-conjugated donkey anti-goat immunoglobulin G (IgG) were from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Bio-Rad protein assay reagent, HRP-conjugated goat anti-rabbit IgG, and Precision Plus Protein Kaleidoscope Standards were from Bio-Rad (Hercules, CA, U.S.A.). Western Lightning Chemiluminescence Plus reagent was from PerkinElmer Life Sciences (Boston, MA, U.S.A.). BD ApoAlert Annexin V kit was from BD Biosciences (Palo Alto, CA, U.S.A.). The RNAqueous total RNA isolation kit was from Ambion (Austin, TX, U.S.A.). The High-Capacity cDNA archive kit and gene expression kit for human COX-2 were from Applied Biosystems (Foster City, CA, U.S.A.). Curcumin, dimethyl sulfoxide (DMSO), PMA, and 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT) were from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.).

Synthesis of GL63 The general procedure used for the synthesis of GL63 is described below.²² An aliquot of 7.5 mmol of acetone was added to a solution of 15 mmol of arylaldehyde in MeOH (10 ml). The solution was stirred at room temperature for 20 min, followed by dropwise addition of NaOCH₃/CH₃OH (1.5 ml; 7.5 mmol). When the reaction was finished, the residue was poured into saturated NH₄Cl solution and filtered. The resulting precipitate was washed with water and cold ethanol and dried in a vacuum. The solid product was purified by chromatography over silica gel using CH₂Cl₂/CH₃OH (9:1) as the eluent to afford the pure product (yield: 59%). Single crystal was checked by thin-layer chromatography (TLC) and ¹H-NMR (Fig. 1).

Cell Culture and Treatment H460 cells were cultured in RPMI-1640 medium containing 10% heat-inactivated FBS, penicillin 100 U/ml and streptomycin 100 µg/ml (Mediatech Inc., Manassas, VA, U.S.A.) at 37°C with 5% CO₂ (Steri-Cult CO₂ Incubator; Thermo Forma Co., Waltham, MA, U.S.A.). Cells were subcultured upon reaching 100% confluence. GL63 and curcumin were dissolved in DMSO. H460 cells were pretreated with PMA (50 nM) for 1 h to induce COX-2 expression and subsequently treated with GL63 and curcumin, which were added the culture medium to final concentrations of 5, 10, or 20 µM.

Fig. 1. Schematic Representation of the Synthesis of GL63

(1*E*,4*E*)-1,5-Bis(2-bromophenyl)penta-1,4-dien-3-one (GL63). Orange powder, 59% yield, mp 97°C. ¹H-NMR (CDCl₃) δ: 7.03 (2H, d, *J* = 16 Hz, C=CH-CO×2), 7.22–7.63 (8H, m, Ar-H), 8.09 (2H, d, *J* = 16 Hz, Ar-CH=C×2). ESI-MS *m/z*: 393.10 (M+1)⁺, Calcd for C₁₇H₁₂Br₂O: 392.08.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay H460 cells were grown in a 96-well plate for 24 h and then treated with different doses of GL63 (5, 10, 20 µM) and curcumin for 24 h or 48 h, followed by MTT treatment (5 mg/ml) of the cells in each well for 4 h at 37°C. MTT was aspirated and 100 µl of DMSO was added to each well. Absorbance at 570 nm was read in a plate reader. Each treatment was carried out in triplicate, and the results are expressed as percentages of the respective control.

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction Total cellular RNA was isolated from H460 cells after treatment with GL63, curcumin, or vehicle control for 24 h using an Ambion RNAqueous kit. Total RNA (10 µg) was used for first-strand cDNA synthesis using the High-Capacity cDNA archive kit. The mRNA levels of COX-2 were quantified using the specific gene expression assay kits for human COX-2 on a DNA Engine Opticon2 Sequence Detection System (MJ Research, Inc. Waltham, MA, U.S.A.). The mRNA values of COX-2 in H460 cells were quantified using the following *TaqMan* primers: forward 5'-TGCTGCCTGGTCTGATGATG-3'; and reverse 5'-GTG-GTAACCGCTCAGGTGTTG-3'. iQTM SYBR Green Supermix (Bio-Rad) was used as the fluorescent dye to detect the presence of double-stranded DNA. The mRNA values for each gene were normalized to internal control β-actin mRNA. The ratio of the normalized mean value for each treatment group to the vehicle control group (DMSO) was calculated.

Assessment of COX-2 mRNA Stability H460 cells were pretreated with PMA for 1 h to induce COX-2 production, then GL63 (20 µM) and curcumin (20 µM) or vehicle were added and incubation was carried out for 2 h before the addition of dactinomycin (5 mg/ml) (time 0). Total cellular RNA was extracted 0, 1, 2, 3, and 4 h after dactinomycin addition. COX-2 mRNA levels were determined using real-time polymerase chain reaction (RT-PCR) as described above, and the results are expressed as the percentage of mRNA amount at the time of actinomycin D addition.

Western Blot Analysis After drug treatment, the culture medium was collected, and the cells were washed with ice-cold phosphate buffered saline (PBS). Whole cell lysates were prepared using lysis buffer containing NaCl 150 mM, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), Tris 50 mM, pH 8.0, and protease and phosphatase inhibitors. Nuclear and cytosolic extracts were isolated as previously described.²³ The protein concentration was measured using Bio-Rad protein assay reagent. The total cell lysate (50 µg of protein), cytoplasmic proteins (30 µg) or nuclear proteins (15 µg) were resolved on 10% Bis-Tris Criterion XT gels (Bio-Rad) and transferred onto nitrocellulose membranes. Immunoblots were blocked with 5% nonfat milk in Tris-buffered saline (TBS) for 1 h at room temperature and then incubated with primary antibodies against COX-2, HuR, Lamin B, or actin at 4–8°C overnight. Immunoreactive bands were detected using HRP-conjugated secondary antibodies with the Western Lightning Chemiluminescence Plus reagent. The density of the immunoreactive bands was digitally analyzed using ImageJ computer software (rsbweb.NIH.gov).

COX-2-3'-Untranslated Region (3'-UTR) Reporter Gene Assay For the reporter activity assay, plasmids that

contained the full-length COX-2 3'-UTR fused to the green fluorescent protein (GFP) gene (pEGFP-C3 vector) were employed as pEGFP-COX-2-3'-UTR. H460 cells were transfected following the instructions of the manufacturer. A total of 5×10^5 cells were seeded in 24-well plates. Following the transfection, incubation was continued for 24 h, followed by individual stimulation with either GL63 or curcumin. GFP activity was determined at the indicated time points. GFP values were normalized with sample protein concentration.

Statistical Analysis All assays were repeated at least three times and levels of all parameters were expressed as mean $\pm$ standard error (S.E.). Statistical comparisons between cells treated with GL63 and curcumin *versus* control were performed with the *t*-test and one-way ANOVA. Graphpad Prism 5.0 was used for all statistical tests. Statistical significance was considered to have been reached at $p < 0.05$.

RESULTS

Effect of GL63 and Curcumin on COX-2 Protein Expression and mRNA Level in H460 Cells Endogenous COX-2 expression was very low in H460 cells, and PMA 50 nM induced COX-2 expression in H460 cells in a time-dependent manner (Fig. 2A). We tested the effects of GL63 and curcumin on PMA-induced COX-2 mRNA levels and its protein expression. We found that GL63 significantly reduced the level of COX-2 protein at concentrations of 10 and 20 μ M, whereas curcumin treatment did not cause any change in COX-2 expression, even when used at 20 μ M (Figs. 2B, D). We also found that GL63 inhibited COX-2 expression in a time-dependent manner (Figs. 2C, E). RT-PCR was used to verify the effects of GL63 on PMA-induced COX-2 mRNA expression. Under identical experimental conditions employed in the protein study, GL63 significantly reduced the

mRNA level of COX-2 in H460 cells at the concentration of 10 μ M, while curcumin only slightly and not significantly reduced the mRNA level of COX-2 treatment up to 20 μ M (Fig. 3A). The time course of COX-2 mRNA level also showed the same result after treatment for 6 h (Fig. 3B). Collectively, these results indicate that GL63, as a new analogue of curcumin, can potentially inhibit the expression of COX-2 at both the mRNA and protein levels.

Effect of GL63 and Curcumin on Cell Proliferation in H460 Cells To determine the effects of the COX-2 inhibitor GL63 on H460 cells, cell viability was examined in the MTT assay. H460 cells were pretreated with PMA 50 nM for 1 h, followed by GL63 or curcumin treatment for 24 h. As shown in Fig. 4A, no significant cytotoxicity was observed in the presence of PMA 50 nM with or without curcumin or GL63 in H460 cells. Figure 4B shows that GL63 and curcumin 20 μ M did not inhibit H460 cells proliferation compared with DMSO control. We also counted the cell numbers in controls with or without PMA treatment for 24 h in 96-well plates and they totaled $1.7 \pm 0.3 \times 10^4$ and $1.6 \pm 0.3 \times 10^4$, respectively.

GL63 Decreases COX-2 mRNA Stability in H460 Cells We measured the effects of GL63 and curcumin on COX-2 mRNA degradation in the dactinomycin assay.²⁴⁾ H460 cells were exposed to PMA with GL63 or curcumin for 4 h, and thereafter an inhibitor of transcription, actinomycin D (1 μ g/ml), or solvent was added the culture. RT-PCR analysis revealed that when transcription was blocked with dactinomycin, the levels of COX-2 mRNA decreased more rapidly in GL63-treated cells than in curcumin-treated cells. The half-life of COX-2 mRNA was approximately 3 h in cells treated with curcumin, but it was reduced to less than 1.5 h in cells treated with GL63 (Fig. 5).

GL63 Reduced Cytoplasmic HuR Protein in H460 Cells Previous studies showed that the 3'-UTR of COX-2

Fig. 2. GL63 Inhibited PMA-Induced COX-2 Protein Expression

(A) COX-2 protein measured by Western blotting in H460 cells treated with PMA 50 nM for 0–24 h. (B) COX-2 protein in H460 cells pretreated with PMA 50 nM for 1 h, with subsequent DMSO, GL63 (5, 10, 20 μ M), or curcumin (5, 10, 20 μ M) for 24 h. (C) COX-2 protein in H460 cells pretreated with PMA 50 nM for 1 h, then DMSO, GL63 (20 μ M) or curcumin (20 μ M), for 6, 12, or 24 h. Actin was used as a protein loading control and for band density normalization. (D) Bar diagram of the effects of different concentrations of GL63 and curcumin on COX-2 protein from three separate treatments for 24 h. (E) Bar diagram of the effects of different times of GL63 (20 μ M) and curcumin (20 μ M) on COX-2 protein from three separate treatments. * $p < 0.05$ compared with DMSO control; # $p < 0.05$ compared with curcumin at the same concentration or same time.

Fig. 3. GL63 Inhibited PMA-Induced COX-2 mRNA Levels

(A) Bar diagram of the effect of GL63 (5, 10, 20 μ M) and curcumin (5, 10, 20 μ M) on COX-2 mRNA levels for 24 h from three RT-PCR from three separate treatments. (B) Bar diagram of the effects of GL63 (20 μ M) and curcumin (20 μ M) on COX-2 mRNA levels for 0, 3, 6, 12, and 24 h from three RT-PCR from three separate treatments. * $p < 0.05$ compared with the DMSO control; # $p < 0.05$ compared with curcumin at the same concentration or same time.

Fig. 4. Effects of GL63 and Curcumin on Cell Growth in H460 Cells

(A) H460 cells were pretreated with PMA 50 nM for 1 h, then DMSO, GL63 (5, 10, 20 μM), or curcumin (5, 10, 20 μM) for 24 h. Cell viability was determined in the MTT assay. Percentage of control was quantitated from three independent experiments performed in triplicate. The mean of the three values was determined, and the results are expressed as percentage of control. Data are mean ± S.E. (B) Representative phase-contrast images of H460 cells treated with DMSO, GL63 (20 μM), or curcumin (20 μM) with PMA for 24 h (original magnification ×200).

Fig. 5. Effects of GL63 and Curcumin on COX-2 mRNA Stability in H460 Cells

H460 cells were pre-treated with PMA 50 nM for 1 h, then DMSO, GL63 (20 μM) or curcumin (20 μM). After 2 h incubation, dactinomycin was added to the cell culture to stop transcription, and incubations were terminated at the indicated time points (0–4 h). Total RNA was extracted and analyzed using real-time RT-PCR. COX-2 mRNA levels were normalized against β-actin from three separate treatments.

mRNA contains AU-rich elements (ARE) that can bind the regulatory protein HuR, a stabilizer of COX-2 mRNA. HuR primarily resides in the nucleus of resting cells, but translocates to the cytoplasm upon COX-2 translocation. We initially investigated whether the different effects of GL63 and curcumin on COX-2 release were correlated with the different effects on the intracellular localization of HuR. As shown in Fig. 6, GL63 decreased cytoplasmic HuR levels and generated a corresponding increase in nuclear HuR levels; however, total cellular HuR levels showed almost no change. In contrast, curcumin had no significant effect on HuR translocation.

Fig. 6. Effects of GL63 and Curcumin on HuR Cellular Distribution in H460 Cells

H460 cells were pretreated with PMA 50 nM for 1 h, then DMSO, GL63 (20 μM), or curcumin (20 μM) for 24 h. The cytoplasmic (A), nuclear (B), and total cell lysate (C) proteins were probed using Western blot analysis. Lamin B was used as the loading control for nuclear protein, and actin as the loading control for both the total and cytoplasmic proteins. Three experiments were performed and showed similar results.

Fig. 7. Effects of GL63 and Curcumin on the COX-2 3'-UTR in H460 Cells

pEGFP-COX-2-3'-UTR plasmid was transfected into H460 cells. H460 cells were pretreated with PMA 50 nM for 1 h, then DMSO, GL63 (5, 10, 20 μM), or curcumin (5, 10, 20 μM) were added and incubation was performed for another 24 h. GFP values were determined. GFP values were normalized with sample protein concentration from three separate treatments. * $p < 0.05$ compared with the DMSO control; # $p < 0.05$ compared with curcumin at the same concentration.

GL63 Reduced COX-2-3'-UTR in H460 Cells Taking into account that mRNA stabilization is an important feature of COX-2 regulation, we performed GFP reporter assays using the COX-2-3'-UTR GFP reporter plasmids. In these plasmids, the full-length COX-2-3'-UTR region was fused to the GFP gene. GFP expression was therefore anticipated to be indicative of increased or decreased 3'-UTR mediated COX-2 mRNA stabilization. H460 cells transfected with pEGFP-C3 plasmid plus COX-2-3'-UTR and treated with PMA, GL63, and curcumin revealed a dose-dependent decrease compared with controls. In contrast, curcumin produced no significant effects on the expression of GFP in this *in vitro* reporter system (Fig. 7). We concluded that the decreased COX-2 translation and expression might attribute to a COX-2-3'-UTR-dependent mRNA stabilization mechanism in H460 cells.

DISCUSSION

Preclinical data strongly suggest that overexpression of COX-2 plays a critical role in tumor-mediated angiogenesis, invasion, metastasis, and chemoresistance. The roles of COX-2 in lung carcinogenesis have been extensively studied both *in vitro* and *in vivo*.^{16,17} COX-2 inhibition in the context of chemoprevention of lung cancer has garnered a special amount of research interest; recent clinical studies have demonstrated that the use of selective COX-2 inhibitors results in a significant risk reduction for lung cancer (79% reduction), suggesting that COX-2-blocking agents have great potential for the chemoprevention of lung cancer.^{25,26}

Curcumin-mediated regulation of COX-2 expression has been demonstrated as an important mechanism by which curcumin prevents a number of disease processes, including cancer.²⁷ Moreover, curcumin has been shown to inhibit COX-2 protein in the lung cancer cell line A549, albeit at relatively high concentrations of up to 50 μM .²⁸ Preclinical and clinical studies have indicated that the poor bioavailability and pharmacokinetic profiles of curcumin, mainly due to its instability under physiologic conditions, have limited its applicability to anticancer therapies.^{3–5} More potent curcumin analogues that can inhibit COX-2 at lower dosages are needed to treat lung cancer more effectively.

Recently, substantial research efforts have been put into the chemical modification of curcumin to generate novel analogues with superior bioavailability and antitumor activities.^{6–10,29} We have designed and synthesized a series of monocarbonyl analogues of curcumin by deleting the reactive β -diketone moiety. Our preliminary studies indicated that some monocarbonyl analogues not only were able to enhance stability and antitumor activities *in vitro* but also have better pharmacokinetic profiles *in vivo*.^{11,12,22,30} In this study, we demonstrated that a new analogue, GL63, is more potent than curcumin in inhibiting PMA-induced COX-2 mRNA and protein expression (Figs. 2, 3). COX-2 is positively correlated with tumor migration and invasion,¹⁷ although we did not observe significant cytotoxicity of GL63 and curcumin in H460 cells (Fig. 4). We suggest that GL63 20 μM is not sufficient to inhibit H460 cell proliferation, while GL63 may have inhibitory effects on the migration and invasion of H460 cells mediated through the suppression of COX-2 expression, which needs further research. It was also reported that the anticancer active constituent dihydroartemisinin (DHA) at 50 μM had no cytotoxicity against human fibrosarcoma HT-1080 cells, but DHA at 50 μM inhibited the PMA-induced invasion of HT-1080 cells.³¹

COX-2 expression is regulated at both the transcriptional and posttranscriptional levels, depending on the type of stimulus, cellular context, and presence of transcription factors, such as nuclear factor kappa-B (NF- κ B) and cAMP-response element binding protein.³² In addition, evidence was presented indicating that COX-2 regulation occurs at the level of mRNA stability. COX-2 mRNA possesses a long 3'-UTR containing multiple ARE that mediate its stability by interaction with various RNA binding proteins, such as ARE/poly(U)-binding/degradation factor-1, tristetraprolin, and HuR.^{33,34} HuR has been reported to be involved in the regulation of COX-2 mRNA stability in tumors from colon, uterine/cervical, and ovarian tissues.^{35,36} However, whether

COX-2 inhibitors can also regulate the stability of COX-2 mRNA in non-small cell lung carcinoma has not been reported. In the present study, we found that GL63 treatment facilitated COX-2 mRNA degradation, as evidenced by the mRNA degradation assay. The half-life of COX-2 mRNA was reduced from 3 h to less than 1.5 h in cells treated with GL63, while curcumin only slightly reduced the half-life of COX-2 as compared with the DMSO-treated group (Fig. 5).

Although the precise mechanisms regulating HuR expression are still unclear, its localization in the cytoplasm or nucleus is crucial for its function. Under normal conditions, HuR is predominantly present in the nucleus; in response to various stimuli, HuR can be shuttled between the cytoplasm and nucleus.^{36,37} Because GL63 reduced COX-2 mRNA stability, we measured the effects of GL63 on HuR protein levels. Our data demonstrated that GL63, but not curcumin, decreased the translocation of HuR from the nucleus to cytoplasm (Fig. 6). It has been confirmed that, as an mRNA-stabilizing factor, HuR is able to bind the 3'-UTR of COX-2 mRNA, which in turn promotes COX-2 expression. Sequestration of HuR in the cytoplasm may explain, at least in part, the ability of GL63 to destabilize COX-2 mRNA. Furthermore, the COX-2-3'-UTR GFP reporter gene confirmed the activity of GL63. GL63 decreased PMA-induced COX-2-3'-UTR GFP values in a dose-dependent manner, as compared with curcumin, a finding that also indicates that decreasing COX-2 translation and expression might contribute to a 3'-UTR of COX-2-dependent mRNA stabilization mechanism in H460 cells (Fig. 7).

In conclusion, we described a new monocarbonyl analogue of curcumin, GL63, and defined its potent function in inhibiting PMA induced-COX-2 expression in the human lung epithelial cancer cell line H460; these findings suggest that GL63 has promising potential for application as a therapeutic or chemopreventative agent for cancer, particularly of the lung.

Acknowledgments This work was supported by the National Natural Science Funding of China (30901819), the Science Foundation of Zhejiang Province of China (Y2090668), Science and Technology Grant of Wenzhou city (Y20090175), Department of Education Science Grant of Zhejiang province (20070992), and funds from the Zhejiang Provincial Extremely Key Subject Building Project "Pharmacology and Biochemical Pharmaceutics 2008."

REFERENCES

- 1) Strimpakos A. S., Sharma R. A., *Antioxid. Redox. Signal*, **10**, 511–545 (2008).
- 2) Hatcher H., Planalp R., Cho J., Torti F. M., Torti S. V., *Cell. Mol. Life Sci.*, **65**, 1631–1652 (2008).
- 3) Anand P., Kunnumakkara A. B., Newman R. A., Aggarwal B. B., *Mol. Pharm.*, **4**, 807–818 (2007).
- 4) Appiah-Opong R., de Esch I., Commandeur J. N., Andarini M., Vermeulen N. P., *Eur. J. Med. Chem.*, **43**, 1621–1631 (2008).
- 5) Sharma R. A., Steward W. P., Gescher A. J., *Adv. Exp. Med. Biol.*, **595**, 453–470 (2007).
- 6) Wang X., Lin J., Chen Y., Zhong W., Zhao G., Liu H., Li S., Wang L., Li S., *Bioorg. Med. Chem.*, **17**, 1898–1904 (2009).
- 7) Subramaniam D., May R., Sureban S. M., Lee K. B., George R., Kuppusamy P., Ramanujam R. P., Hideg K., Dieckgraefe B. K., Houchen C. W., Anant S., *Cancer Res.*, **68**, 1962–1969 (2008).
- 8) Selvam C., Jachak S. M., Thilagavathi R., Chakraborti A. K., *Bioorg.*

- Med. Chem. Lett.*, **15**, 1793—1797 (2005).
- 9) Ohori H., Yamakoshi H., Tomizawa M., Shibuya M., Kakudo Y., Takahashi A., Takahashi S., Kato S., Suzuki T., Ishioka C., Iwabuchi Y., Shibata H., *Mol. Cancer Ther.*, **5**, 2563—2571 (2006).
- 10) Mosley C. A., Liotta D. C., Snyder J. P., *Adv. Exp. Med. Biol.*, **595**, 77—103 (2007).
- 11) Liang G., Yang S. L., Shao L. L., Zhao C. G., Xiao J., Lv Y. X., Yang J., Zhao Y., Li X. K., *J. Asian Nat. Prod. Res.*, **10**, 957—965 (2008).
- 12) Liang G., Shao L., Wang Y., Zhao C., Chu Y., Xiao J., Zhao Y., Li X., Yang S., *Bioorg. Med. Chem.*, **17**, 2623—2631 (2009).
- 13) Petkova D. K., Clelland C., Ronan J., Pang L., Coulson J. M., Lewis S., Knox A. J., *Resp. Med.*, **98**, 164—172 (2004).
- 14) Benish M., Bartal I., Goldfarb Y., Levi B., Avraham R., Raz A., Ben-Eliyahu S., *Ann. Surg. Oncol.*, **15**, 2042—2052 (2008).
- 15) Zhu Y. M., Azahri N. S., Yu D. C., Woll P. J., *BMC Cancer*, **8**, 218 (2008).
- 16) Krysan K., Reckamp K. L., Sharma S., Dubinett S. M., *Anticancer Agents Med. Chem.*, **6**, 209—220 (2006).
- 17) Sandler A. B., Dubinett S. M., *Semin. Oncol.*, **31**, 45—52 (2004).
- 18) Chang M. S., Chen B. C., Yu M. T., Sheu J. R., Chen T. F., Lin C. H., *Cell. Signal*, **17**, 299—310 (2005).
- 19) Miyahara T., Katoh T., Watanabe M., Mikami Y., Uchida S., Hosoe M., Sakuma T., Nemoto N., Takayama K., Komurasaki T., *Toxicology*, **200**, 159—167 (2004).
- 20) Zhang F., Altorki N. K., Mestre J. R., Subbaramaiah K., Dannenberg A. J., *Carcinogenesis*, **20**, 445—451 (1999).
- 21) Hecht S. S., Kassie F., Hatsukami D. K., *Nat. Rev. Cancer*, **9**, 476—488 (2009).
- 22) Liang G., Yang S., Jiang L., Zhao Y., Shao L., Xiao J., Ye F., Li Y., Li X., *Chem. Pharm. Bull.*, **56**, 162—167 (2008).
- 23) Williams K., Rao Y. P., Natarajan R., Pandak W. M., Hylemon P. B., *Biochem. Pharmacol.*, **67**, 255—267 (2004).
- 24) Leclerc G. J., Leclerc G. M., Barredo J. C., *Cancer Cell. Int.*, **2**, 1 (2002).
- 25) Han S., Roman J., *Int. J. Cancer*, **116**, 536—546 (2005).
- 26) Han S., Roman J., *Lung Cancer*, **51**, 283—296 (2006).
- 27) Harris R. E., Beebe-Donk J., Alshafie G. A., *Subcell. Biochem.*, **42**, 193—212 (2007).
- 28) Lee J., Im Y. H., Jung H. H., Kim J. H., Park J. O., Kim K., Kim W. S., Ahn J. S., Jung C. W., Park Y. S., Kang W. K., Park K., *Biochem. Biophys. Res. Commun.*, **334**, 313—318 (2005).
- 29) Liang G., Li X., Chen L., Yang S., Wu X., Studer E., Gurley E., Hylemon P. B., Ye F., Li Y., Zhou H., *Bioorg. Med. Chem. Lett.*, **18**, 1525—1529 (2008).
- 30) Liang G., Yang S., Zhou H., Shao L., Huang K., Xiao J., Huang Z., Li X., *Eur. J. Med. Chem.*, **44**, 915—919 (2009).
- 31) Hwang Y. P., Yun H. J., Kim H. G., Han E. H., Lee G. W., Jeong H. G., *Biochem. Pharmacol.*, **10**, 1714—1726 (2010).
- 32) Harper K. A., Tyson-Capper A. J., *Biochem. Soc. Trans.*, **36**, 543—545 (2008).
- 33) Sengupta S., Jang B. C., Wu M. T., Paik J. H., Furneaux H., Hla T., *J. Biol. Chem.*, **278**, 25227—25233 (2003).
- 34) Subbaramaiah K., Marmo T. P., Dixon D. A., Dannenberg A. J., *J. Biol. Chem.*, **278**, 37637—37647 (2003).
- 35) Lim S. J., Kim H. J., Kim J. Y., Park K., Lee C. M., *Int. J. Gynecol. Pathol.*, **26**, 229—234 (2007).
- 36) Oyesanya R. A., Lee Z. P., Wu J., Chen J., Song Y., Mukherjee A., Dent P., Kordula T., Zhou H., Fang X., *FASEB J.*, **22**, 2639—2651 (2008).
- 37) Young L. E., Sanduja S., Bemis-Standoli K., Pena E. A., Price R. L., Dixon D. A., *Gastroenterology*, **136**, 1669—1679 (2009).