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Combination of cyclooxygenase-2 inhibitors and oxaliplatin increases the growth inhibition and death in human colon cancer cells

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Abstract

The cyclooxygenase-2 (COX-2) protein is highly expressed in a variety of human cancers and has been reported to promote tumor growth. Non-steroidal anti-inflammatory drugs such as etodolac and celecoxib have been shown to inhibit COX-2 activity and may play a role in the chemoprevention of cancer. Oxaliplatin is a third-generation platinum compound that exhibits a different spectrum of activity compared with cisplatin. Other cisplatin-resistant tumors can still respond to oxaliplatin. However, the anticancer ability of the combination of COX-2 inhibitors and oxaliplatin is still unknown. In this study, we investigated the effects of combination of COX-2 inhibitors and oxaliplatin on the cell growth and survival in human colon cancer cells. Treatments with etodolac (0.3–0.5 mM) or celecoxib (20–80 μM) for 24 h concentration-dependently induced the cytotoxicity in the RKO colon carcinoma cells. Etodolac and celecoxib did not alter the COX-2 protein levels but inhibited its enzyme activity to reduce prostaglandin E_2 production. Furthermore, the cell survival was concentration-dependently decreased following oxaliplatin (1–100 μM, 24 h) treatment. Combination of oxaliplatin and etodolac additively increased the death and growth inhibition of RKO cells. Survivin, an inhibitor protein of apoptosis, mediates anti-apoptosis and promotes cell division in cancer cells. Oxaliplatin or COX-2 inhibitors significantly decreased the levels of survivin proteins. Moreover, survivin proteins were markedly diminished following co-treatment with oxaliplatin and etodolac. Together, this is the first report that combination of COX-2 inhibitors and oxaliplatin can increase the reduction of survivin protein expression, growth inhibition, and death in human colon cancer cells.

Keywords: Colon cancer; Celecoxib; COX-2; Etodolac; Oxalipatin; Survivin

1. Introduction

The cyclooxygenase-2 (COX-2) was highly expressed in a variety of human cancers including colon, lung, prostate, and many other solid cancers [1–4]. COX-2 has been associated with tumor growth, angiogenesis, invasion, and metastasis [5–7]. Overexpression of COX-2 may increase the resistance of apoptosis in cancer cells [8,9]. Thus, the reduction of the COX-2 enzyme activity or protein expression may inhibit the cell survival and growth in cancer cells. Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit COX enzymes and may be employed for the chemoprevention of cancer [10–14]. Some NSAIDs such as etodolac and celecoxib exhibit high selectivity for the COX-2 enzyme, but demonstrate little activity for the COX-1 enzyme [11–13,15]. The anti-inflammatory and anti-angiogenic effects of celecoxib have been explored for the cancer therapy and the drug is currently under clinical trials for this purpose [16]. Furthermore, COX-2 inhibitors in combination with other anticancer drugs including doxorubicin, bleomycin, vincristine, and 5-fluorouracil, have been evaluated for the anticancer activity in human cancers [17,18].

Oxaliplatin, a clinical anticancer drug, is a third-generation platinum compound that confers a different spectrum of activity compared with cisplatin [19–21]. Some

Abbreviations: COX-2, cyclooxygenase-2; NSAID, non-steroidal anti-inflammatory drug; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; FITC, fluorescein isothiocyanate; FBS, fetal bovine serum; PBS, phosphate-buffered saline; ERK, extracellular signal-regulated kinase; F-actin, actin filament; PG, prostaglandin

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cisplatin-resistant tumors such as colon cancer can still response to oxaliplatin [19–21]. The action of oxaliplatin is due to the formation of intrastrand cross links between two adjacent guanine residues or a guanine and an adenine of DNA that result in the blockage of replication and transcription [22]. It has been shown that oxaliplatin can induce apoptosis in cancer cells [23,24]. The combination of oxaliplatin with a variety of anticancer drugs has been intensely evaluated for cancer therapy in recent years. For example, combination of oxaliplatin with 5-fluorouracil and leucovorin have been suggested as the first line therapy for patients with colon and ovarian cancers [20,21,25]. However, the anticancer activity of the combination of COX-2 inhibitors and oxaliplatin remains unknown.

It has been shown that survivin is expressed in a variety of human cancer cells, but is undetectable in normal adult cells [26–28]. Survivin has been found to inhibit apoptosis and to promote cell division in cancer cells [27–32]. Moreover, the survivin proteins can be stabilized by the COX-2 overexpression leading to the reduction of apoptosis in the human lung cancer cells [8,9]. The expression of survivin may reduce cancer cell death resulting from treatments with anticancer agents [31,33–35]. Thus, survivin has been proposed as a radio- and chemo-resistance factor [29,35,36]. Moreover, survivin has been correlated with decreased survival, unfavorable prognosis, and accelerated rates of recurrences in cancer therapy [35]. Inhibitors of the survivin have been intensely investigated for cancer therapy in recent years because the depletion of survivin expression will block the resistance of therapy, anti-apoptosis, and proliferation of cancer cells.

In this study, we investigated the COX-2 inhibitors (etodolac and celecoxib) and oxaliplatin on the cytotoxicity and cell growth in human colon carcinoma cells. The level of survivin proteins in colon carcinoma cells was examined following treatment with COX-2 inhibitors and oxaliplatin. Treatments of etodolac, celecoxib, and oxaliplatin decreased the level of survivin proteins, induced cytotoxicity, and inhibited cell growth. Moreover, the combination of etodolac and oxaliplatin additively increased the reduction of survivin proteins, growth inhibition, and death of the human colon carcinoma cells.

2. Materials and methods

2.1. Materials and reagents

Etodolac, Hoechst 33258, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and the Cy3-labeled mouse anti-β-tubulin (c-4585) were purchased from Sigma Chemical Co. (St. Louis, MO). Celecoxib was purchased from Toronto Research Chemical, Inc. (North York, Canada). BODIPY FL phallacidin was purchased from Molecular Probes Co. (Eugene, OR). Anti-COX-2 (H-62), and anti-ERK-2 (C-14) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The Cy5-labeled goat anti-rabbit IgG was purchased from Amersham Pharmacia Biotech (Little Chalfont Buckinghamshire, UK). Celecoxib and etodolac were dissolved in 80% ethanol, and the concentration of ethanol was <0.8% in the control and drug-containing media.

2.2. Cell culture

RKO (ATCC number: CRL-2577) was a poorly differentiated colon carcinoma cell line that expressed the wild-type p53 proteins [37]. RKO cells were maintained in DMEM medium (Invitrogen Co., Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, L-glutamine (0.03%, w/v), and sodium bicarbonate (3.7 g/l). RKO cells were cultured at 5% CO_2 and 37 °C in a humidified incubator (310/Thermo, Forma Scientific, Inc., Marietta, OH).

2.3. Cytotoxicity MTT assay

The cytotoxicity following treatment with drugs was determined by the MTT colorimetric assay [38]. RKO cells were plated in 96-well plates at a density of 1×10^4 cells/well for 12 h. Then the cells were treated with drugs for 24 h in complete DMEM medium. After drug treatment, the cells were washed twice with phosphate-buffered saline (PBS), and were re-cultured in fresh DMEM medium for 2 days. Subsequently, the medium was replaced and the cells were incubated with 500 μg/ml of MTT in complete DMEM medium for 4 h. The surviving cells converted MTT to formazan that generates a blue-purple color [38]. Finally, the cells were dissolved in dimethyl sulfoxide and the plates were gently rotated for 5 min. The intensity of formazan was measured at 565 nm using a plate reader (Molecular Dynamics, OPTImax) for enzyme-linked immunosorbent assays, and the relative percentage of survival was calculated by dividing the absorbance of treated cells by that of the control in each experiment.

2.4. Prostagladin E_2 production assay

RKO cells were plated at a density of 5×10^5 cells/p60 Petri dish in complete DMEM medium for 12 h. Then the cells were treated with or without 20–80 μM celecoxib or 0.1–0.3 mM etodolac for 24 h. After drug treatment, supernatant (culture medium) was collected and centrifuge briefly. The amount of prostagladin E_2 in the medium was measured using a commercial ELISA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions.

2.5. Cell growth assay

RKO cells were plated at a density of 7×10^5 cells per p100 Petri dish in complete DMEM medium for 12 h. Then

the cells were treated with or without 0.3 mM etodolac, 1 μM oxaliplatin, or etodolac plus oxaliplatin for 24 h in complete DMEM medium. At the end of treatment, the cells were washed twice with PBS and re-cultured in fresh DMEM medium. Subsequently, the cells were incubated for various times before they were counted by a hemocytometer.

2.6. Indirect immunofluorescence and confocal microscopy

RKO cells were cultured on coverslips, which were kept in a p60 Petri dish for 12 h before treatment. At the end of treatment, the cells were washed with isotonic PBS (pH 7.4), fixed in 4% paraformaldehyde solution in PBS for 1 h at 37 °C. Then the coverslips were washed three times with PBS, and non-specific binding sites were blocked in PBS containing 10% normal bovine serum, 0.3% Triton X-100 for 1 h. The cells were incubated with rabbit anti-COX-2 (1:250) or anti-survivin (1:250) antibodies in PBS containing 0.3% Triton X-100 and 10% normal bovine serum for overnight at 4 °C, and washed three times with 0.3% Triton X-100 in PBS. Then the cells were incubated with goat anti-rabbit Cy5 (1:250) in PBS containing 0.3% Triton X-100 and 10% normal bovine serum for 2–3 h at 37 °C, and washed three times with 0.3% Triton X-100 in PBS. The

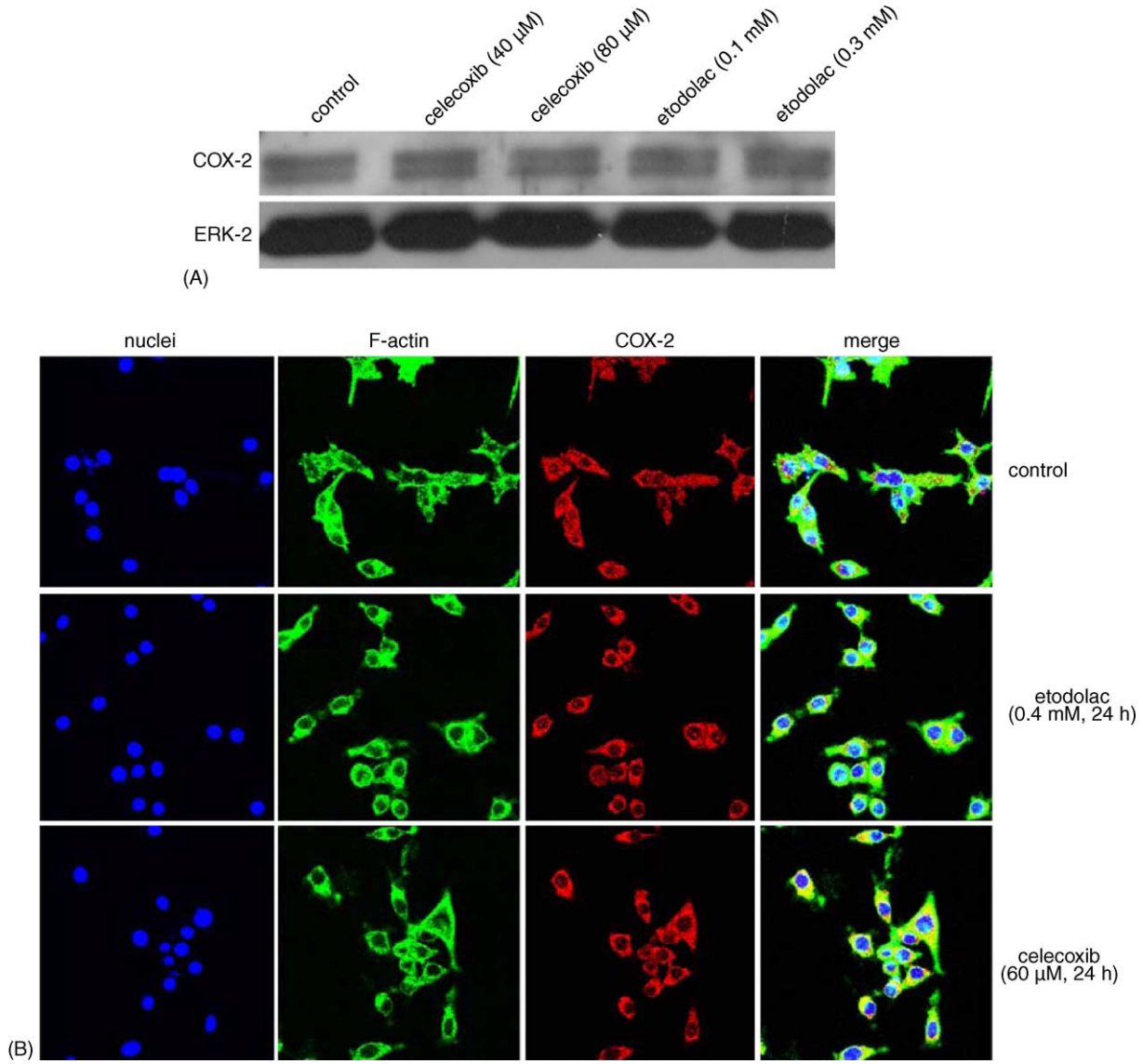

Fig. 1. Effects of COX-2 inhibitors on the COX-2 protein expression and PGE_2 production in RKO cells. (A) RKO cells were treated with 40 and 80 μM celecoxib or 0.1 and 0.3 mM etodolac for 24 h. The total protein extracts were subjected to Western blot analysis using anti-COX-2 and anti-ERK-2 antibodies. (B) The cells were treated with or without 60 μM celecoxib or 0.4 mM etodolac for 24 h. The cells were incubated with rabbit anti-COX-2 antibody and then incubated with goat anti-rabbit Cy5. The F-actin and nuclei were stained with the BODIPY FL phallacidin and Hoechst 33258, respectively. Representative immunofluorescence and Western blot data were shown from one of three separate experiments with similar findings. (C) The cells were plated at a density of 5×10^5 cells/p60 Petri dish for 12 h. Then the cells were treated with 20 μM celecoxib or 0.1 mM etodolac for 24 h. After incubation, the PGE_2 level in the culture medium was measured by a PGE_2 EIA kit according to the manufacturer's protocol. The means of three independent experiments in each treatment were shown.

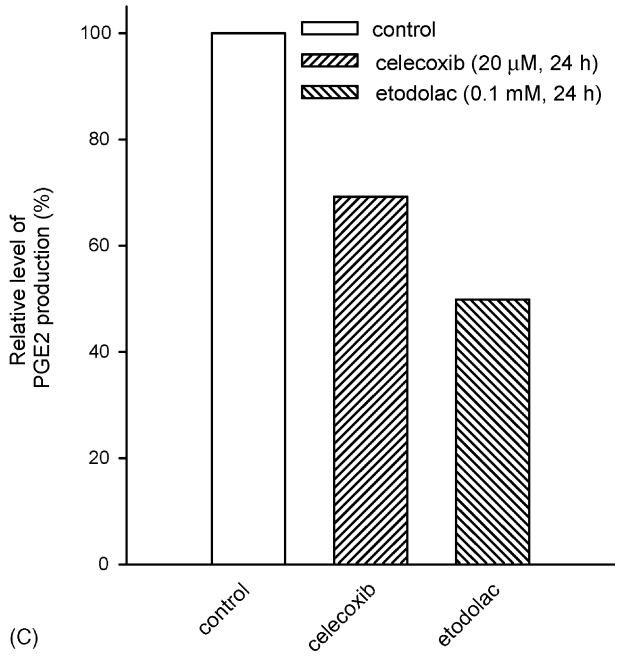

Fig. 1. (*Continued*).

actin filament (F-actin) and β-tubulin were stained with 20 U/ml BODIPY FL phallacidin and anti-β-tubulin Cy3 (1:50) for 30 min at 37 °C, respectively. The nuclei were stained with 2.5 μg/ml Hoechst 33258 for 30 min. Finally, the samples were observed under a Leica confocal laser scanning microscope (Mannheim, Germany) that equipped with an UV laser (351/364 nm), an Ar laser (457 nm/488 nm/514 nm), and a HeNe laser (543 nm/633 nm).

2.7. Western blot analysis

At the end of treatment, RKO cells were lysed in the ice-cold cell extract buffer (pH 7.6) containing 0.5 mM DTT, 0.2 mM EDTA, 20 mM HEPES, 2.5 mM $MgCl_2$, 75 mM NaCl, 0.1 mM Na_3VO_4, 50 mM NaF, 0.1% Triton X-100, 1 μg/ml aprotinin, 0.5 μg/ml leupeptin, and 100 μg/ml 4-(2-aminoethyl)benzenesulfonyl fluoride. The protein concentrations were determined by the BCA protein assay kit (Pierce, Rockford, IL). The protein extracts (40–80 μg/well) were subjected to electrophoresis using 10–12% sodium dodecyl sulfate-polyacrylamide gels. Following electrophoretic transfer of proteins onto polyvinylidene difluoride membranes, they were sequentially hybridized with primary antibody and followed with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Finally, the protein bands were visualized using the enhanced chemiluminescence detection system (NEN, Boston, MA).

2.8. Statistical analysis

Data from the population of cells treated with different conditions were analyzed using paired Student's *t*-test or ANOVA test (a comparison of multiple groups), and p value of <0.05 was considered statistically significant in the experiments.

3. Results

3.1. COX-2 inhibitors inhibit the production of PGE_2 but are without effect on the COX-2 protein expression in human colon cancer cells

To examine the expression and location of COX-2 proteins in human colon cancer cells, RKO cells were subjected to Western blot and immunofluorescence staining. As shown in Fig. 1A, the immunoblot indicated that COX-2 proteins were expressed in RKO cells. The level of ERK-2 proteins was used as an internal control. Moreover, the red fluorescence (Cy5) exhibited by COX-2 proteins was observed in the cytoplasma of RKO cells (Fig. 1B). Celecoxib and etodolac have been known as a selective COX-2 inhibitor in several studies [4,11,12,15]. However, treatment with 40–80 μM celecoxib or 0.1–0.3 mM etodolac for 24 h did not significantly alter the level of COX-2 proteins in RKO cells (Fig. 1A). Also, the fluorescence (Cy5) exhibited by survivin was not altered when cells were exposed to 0.4 mM etodolac or 60 μM celecoxib for 24 h (Fig. 1B). To further determine the effect of COX-2 inhibitors on the COX-2 enzyme activity, the cells were treated with celecoxib or etodolac, and PGE_2 production was measured. The average of PGE_2 levels in the control from three independent experiments was 0.72 ng/ml. Compared with the control, the PGE_2 production was reduced to about 70 and 50% after the celecoxib (20 μM, 24 h) and etodolac (0.1 mM, 24 h) treatments, respectively (Fig. 1C). Higher concentrations of celecoxib (40–80 μM) and etodolac (0.3–0.5 mM) also significantly inhibited the PGE_2 production in RKO cells (data not shown).

3.2. COX-2 inhibitors decrease the cell survival in human colon carcinoma cells

To investigate the effect of COX-2 inhibitors on the cell survival of colon cancer cells, RKO cells were treated with etodolac or celecoxib, and subjected to MTT assay. As shown in Fig. 2A, treatment with 0.3–0.5 mM etodolac for 24 h significantly induced the cytotoxicity in RKO cells. Also, celecoxib (40–80 μM, 24 h) decreased the cell survival via a concentration-dependent manner (Fig. 2C). The cell morphology was observed under a phase contrast microscope following treatment with etodolac or celecoxib. Higher concentrations of etodolac (0.5 mM, 24 h) and celecoxib (80 μM, 24 h) significantly induced the cell disruption and death in RKO cells (Fig. 2B and D).

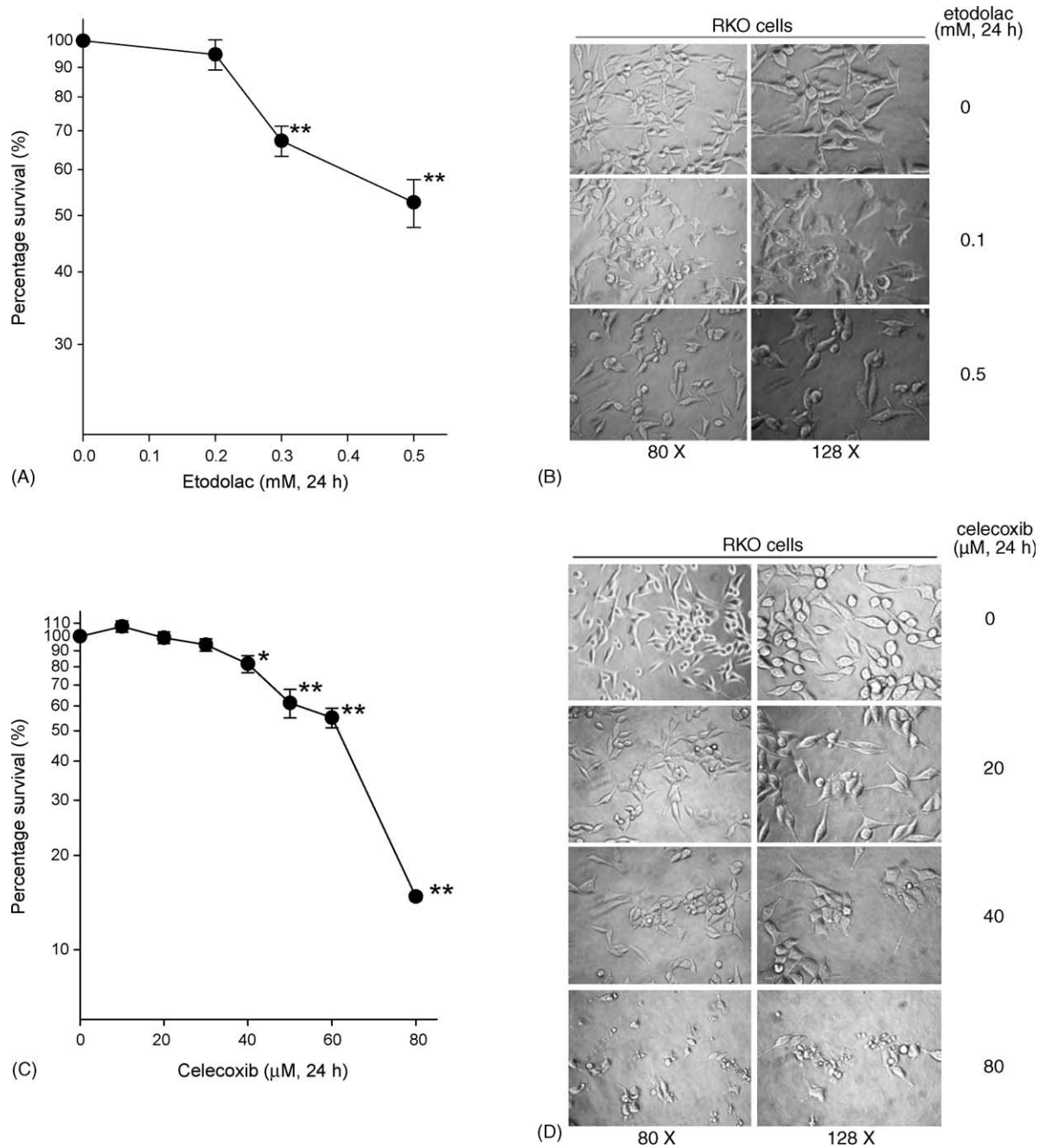

Fig. 2. Effects of COX-2 inhibitors on the cell survival in RKO cells. (A and C) The cells were treated with 0–0.5 mM etodolac or 0–80 μM celecoxib for 24 h. The cell survival was measured by MTT assay. Results were obtained from 5 to 12 experiments and the bar represents the mean ± S.E. $^{*}p < 0.05$ and $^{**}p < 0.01$, indicate between untreated and COX-2 inhibitors treated samples. (B and D) The cells were treated with 0–0.5 mM etodolac or 0–80 μM celecoxib for 24 h and the cell morphology was observed under an inverted phase contrast microscope.

3.3. Co-treatment with oxaliplatin and COX-2 inhibitors increases the cell growth inhibition and cell death in colon carcinoma cells

As shown in Fig. 3A, oxaliplatin induced the cytotoxicity of RKO cells in a concentration-dependent manner. Higher concentrations of 50–100 μM oxaliplatin for 24 h treatment almost completely inhibited the cell survival (Fig. 3A). Furthermore, oxaliplatin (5–50 μM, 24 h) significantly induced the cell disruption and death (Fig. 3B). To further determine the effects of co-treatment of oxaliplatin and COX-2 inhibitors on the cell survival and growth in colon carcinoma cells, the cells were subjected to cytotoxicity and cell growth assays. As shown in Fig. 4, co-treatment with 1 μM oxaliplatin and 0.3–0.5 mM etodolac for 24 h additively increased the cytotoxicity in RKO cells. Furthermore, the concentration of 0.2 mM etodolac enhanced the oxaliplatin-induced cytotoxicity from ∼20% to ∼40% (Fig. 4). In addition, celecoxib additively increased the oxaliplatin-induced cell death in RKO cells

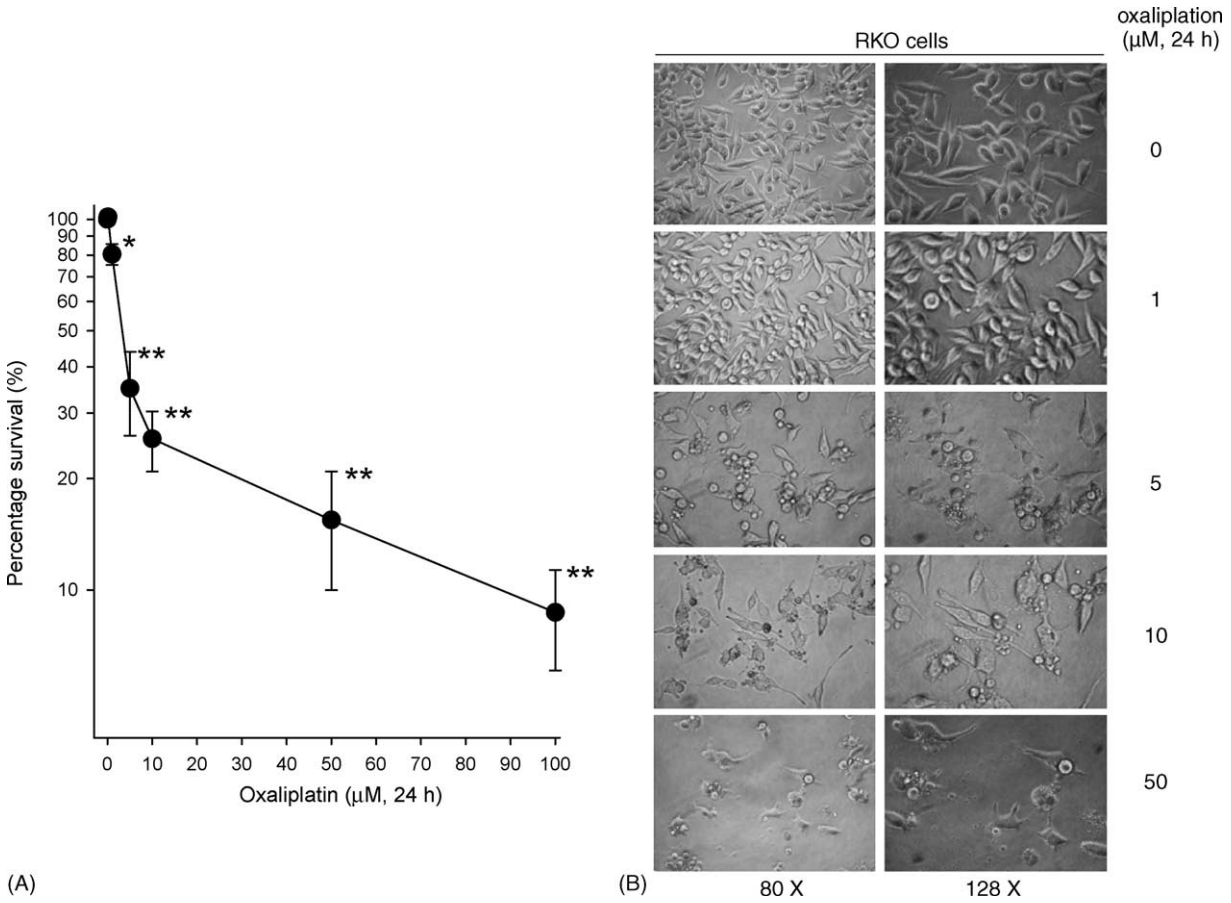

Fig. 3. Effects of oxaliplatin on the cell survival in RKO cells. (A) The cells were treated with 0–100 μM oxaliplarin for 24 h. The cell survival was measured by MTT assay. Results were obtained from 5 to 10 experiments and the bar represents the mean ± S.E. $^{*}p < 0.05$ and $^{**}p < 0.01$, indicate between untreated and oxaliplatin treated samples. (B) The cells were 0–50 μM oxaliplatin for 24 h and the cell morphology was observed under an inverted phase contrast microscope.

(data not shown). We have further investigated the effect of combination of oxaliplatin and etodolac on the cell growth in RKO cells. RKO cells were treated with 1 μM oxaliplatin or 0.3 mM etodolac for 24 h. After treatment, the drugs were replaced and then re-cultured in fresh medium for various times before the cell number was counted. Oxaliplatin or etodolac alone inhibited the cell growth after cells re-cultured for 7 days (Fig. 5). Moreover, Co-treatment with oxaliplatin and etodolac additively induced the cell growth inhibition in colon carcinoma cells (Fig. 5).

3.4. Oxaliplatin and COX-2 inhibitors decrease the level of survivin proteins in colon carcinoma cells

Previously, we have found that survivin is highly expressed in human colon cancer cells [31]. To further investigate the expression and location of survivin proteins in colon carcinoma cell, RKO cells were subjected to immunofluorescence staining and confocal microscopy. As shown in Fig. 6, the red fluorescence (Cy5) exhibited by survivin that was highly expressed in mitotic phase, was co-localized with chromosomes at prophase and metaphase, and was concentrated on the midbodies at anaphase and cytokinesis (Fig. 6, arrows). To study the effect of COX-2 inhibitors and oxaliplatin on the survivin protein expression, the cells were treated with drugs and subjected to immunoblot analysis. Treatment with 80 μM celecoxib or 0.3 mM etodolac for 24 h decreased the level of survivin proteins (Fig. 7A and C). Also, oxaliplatin (1–10 μM, 24 h) decreased the survivin protein expression via a concentration-dependent manner in RKO cells (Fig. 7B). Moreover, co-treatment with 5 μM oxaliplatin and 0.3 mM etodolac for 24 h additively diminished the level of survivin proteins (Fig. 7C).

4. Discussion

Combination of a variety of anticancer agents in cancer therapy has been intensively evaluated and applied in recent years [17,18,20,21]. COX-2 proteins were highly expressed in a variety of human cancers [1–4,8,9] that mediated tumor survival, angiogenesis, invasion, and metastasis [5–7]. COX-2 was overexpressed in approximately 80% human colorectal cancers relative to normal epithelium mucosa [14,39]. Thus, a reduction of the func-

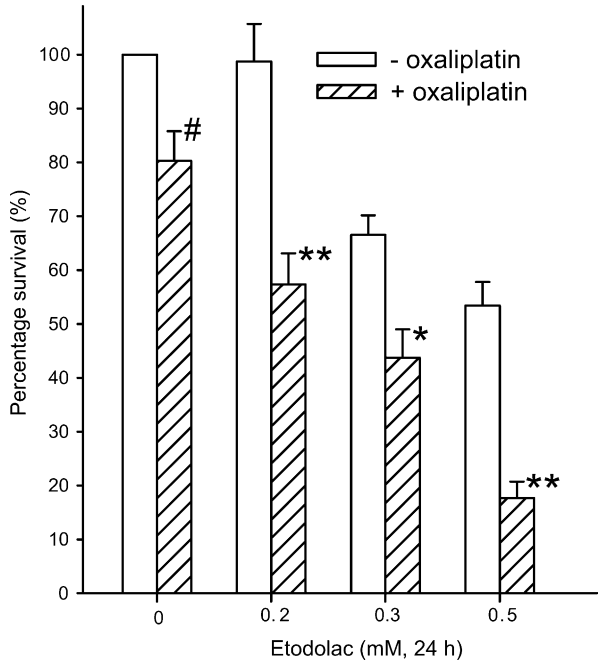

Fig. 4. Co-treatment with etodolac and oxaliplatin on the cell survival in RKO cells. The cells were co-treated with 1 μM oxaliplatin and 0–0.5 mM etodolac for 24 h. Then the cell survival was measured by MTT assay. Results were obtained from 4 to 13 experiments and the bar represents ±S.E. $^{\#}p < 0.05$, indicate between untreated and oxaliplatin treated sample. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with among the controls (either with or without oxaliplatin) and co-treatments with etodolac and oxaliplatin.

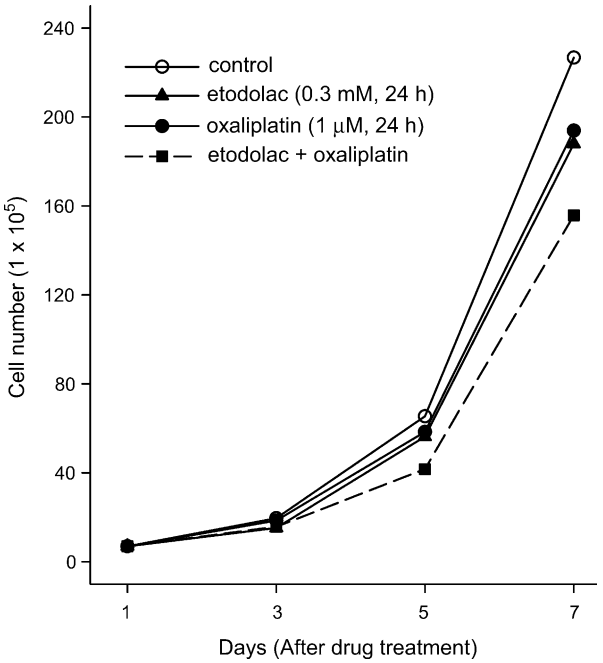

Fig. 5. Co-treatment with etodolac and oxaliplatin on the cell growth in RKO cells. The cells were plated at a density of 7×10^5 cells/p100 Petri dish for 12 h. After drug treatment, the cells were washed twice with PBS, and incubated for various times before they were counted by a hemocytometer. Results were obtained from the average of three experiments.

tion by the elimination of COX-2 enzyme activity and protein expression may inhibit the cell survival and growth in cancer cells, providing an important strategy in the colon cancer therapy. Moreover, combination of oxaliplatin with other anticancer drugs such as fluorouracil and leucovorin has been recommended as the first-line therapy for patients with colon cancer [20,21]. Accordingly, we investigated the anticancer effects of combination of COX-2 inhibitors (etodolac and celecoxib) and oxaliplatin on the growth inhibition and death of the human colon cancer cells. Both etodolac and celecoxib significantly induced the cytotoxicity and cell growth inhibition in the RKO colon carcinoma cells. The cell survival and cell growth were also inhibited in the oxaliplatin-treated cells. Moreover, the combination of oxaliplain and etodolac additively increased the cytotoxicity and cell growth inhibition in RKO cells. Therefore, our results indicate that combination of COX-2 inhibitors and oxaliplatin can enhance the growth inhibition and death in human colon cancer cells, providing a new strategy in the colon cancer therapy.

Meanwhile, etodolac and celecoxib did not alter the level of COX-2 proteins but inhibited the PGE_2 production in RKO cells. COX enzymes can catalyze the synthesis of prostaglandins from arachidonic acid [14,40]. PGE_2 is a key product of the catalysis of COX-2 [14,40]. The COX-1 proteins were constitutively expressed in most normal tissues for the maintenance of homeostasis [14,40]. In contrast, COX-2 proteins were overexpressed in the inflammatory tissues and neoplasms [14]. NSAIDs including etodolac and celecoxib have been shown to inhibit COX enzymes [10–14]. Moreover, etodolac and celecoxib have been developed that are highly selective for the COX-2 enzyme, but do not inhibit the COX-1 enzyme [4,11,12,15]. It has been shown that etodolac and celecoxib can inhibit the PGE_2 production in human cancer cells [41,42]. The overexpression of COX-2 as well as treatment with PGE_2 increased the survival of the human lung cancer cells [8,9]. In addition, treatment with exogenous PGE_2 increased the stability of the anti-apoptosis protein survivin in cancer cells [8]. Thus, these data indicate that the etodolac- and celecoxib-mediated cell growth inhibition and death may inhibit the activity of COX-2 enzyme but not alter the protein expression.

The anticancer drugs may induce chemo-resistance during cancer therapy in patients. Survivin has been demonstrated to inhibit apoptosis and promotes the mitotic progression in cancer cells [30,43]. The treatments with anticancer drugs may increase the survivin expression to resist apoptosis in cancer cells [29]. Furthermore, survivin has been associated with reduced survival, unfavorable prognosis, and accelerated rates of recurrences in cancer therapy [35]. It has been proposed that survivin is a radio- and chemo-resistance factor [29,35,36]. Accordingly, the inhibition of survivin would block the survival, resistance of therapy, and growth of cancer cells, providing important strategy in cancer therapy. In this study, both etodolac and

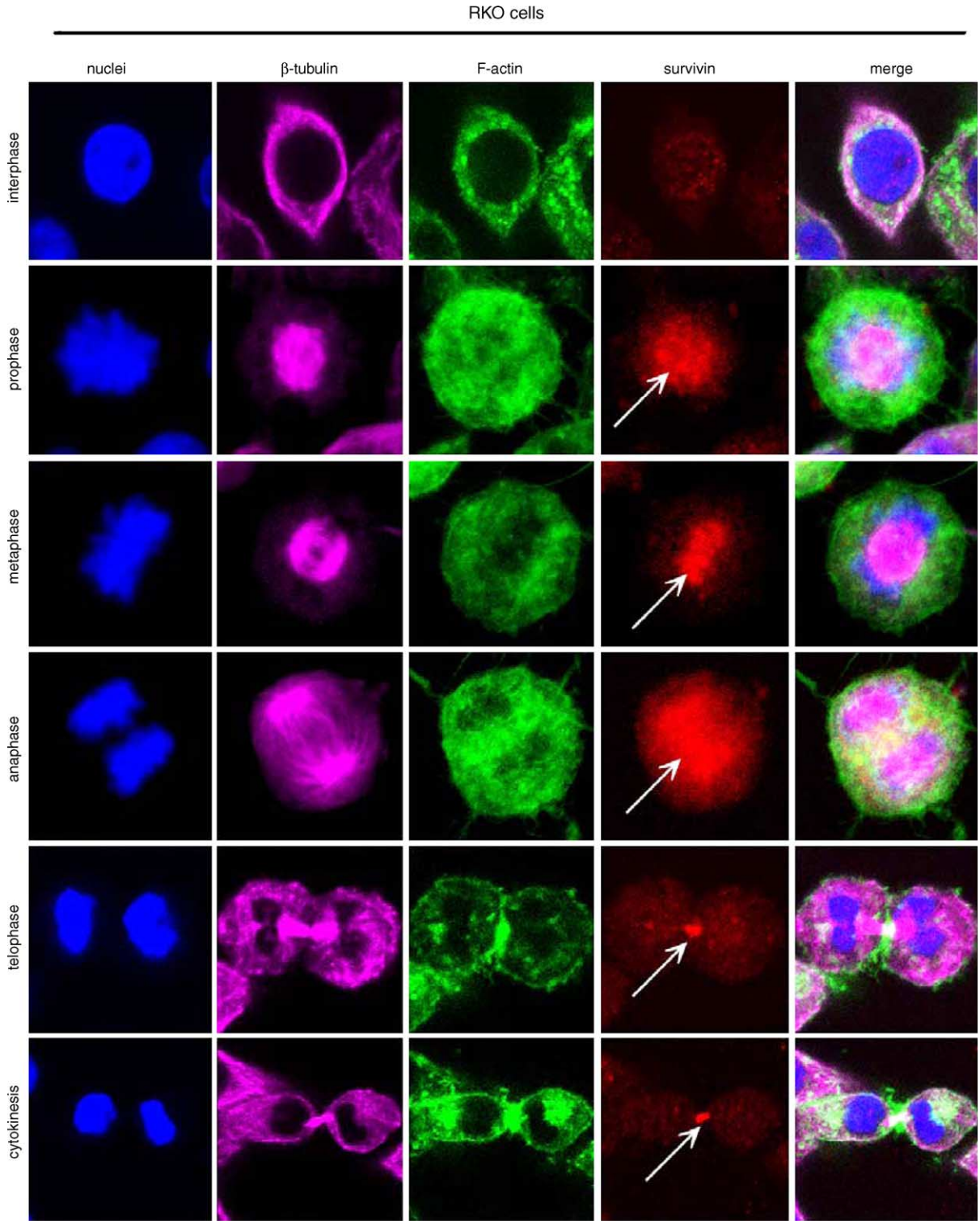

Fig. 6. The expression and localization of survivin proteins in RKO cells. The cells were incubated with rabbit anti-survivin antibody and then incubated with goat anti-rabbit Cy5. The actin filament (F-actin) and β-tubulin were stained with BODIPY FL phallacidin and anti-β-tubulin Cy3, respectively. The nuclei were stained with Hoechst 33258. The arrows indicated the localization of survivin.

celecoxib decreased the levels of survivin proteins in colon carcinoma cells. Consistently, COX-2 has been shown to stabilize the survivin protein to resist apoptosis in cancer cells [8,9]. Therefore, we suggest that the inhibition of COX-2 function may cause the loss of survivin function on the anti-apoptosis and cell division to mediate the death and growth inhibition of cancer cells. Moreover, the protein expression of survivin was repressed by oxaliplatin. Interestingly, co-treatment of oxaliplain and etodolac additively decreased the levels of survivin proteins in RKO cells.

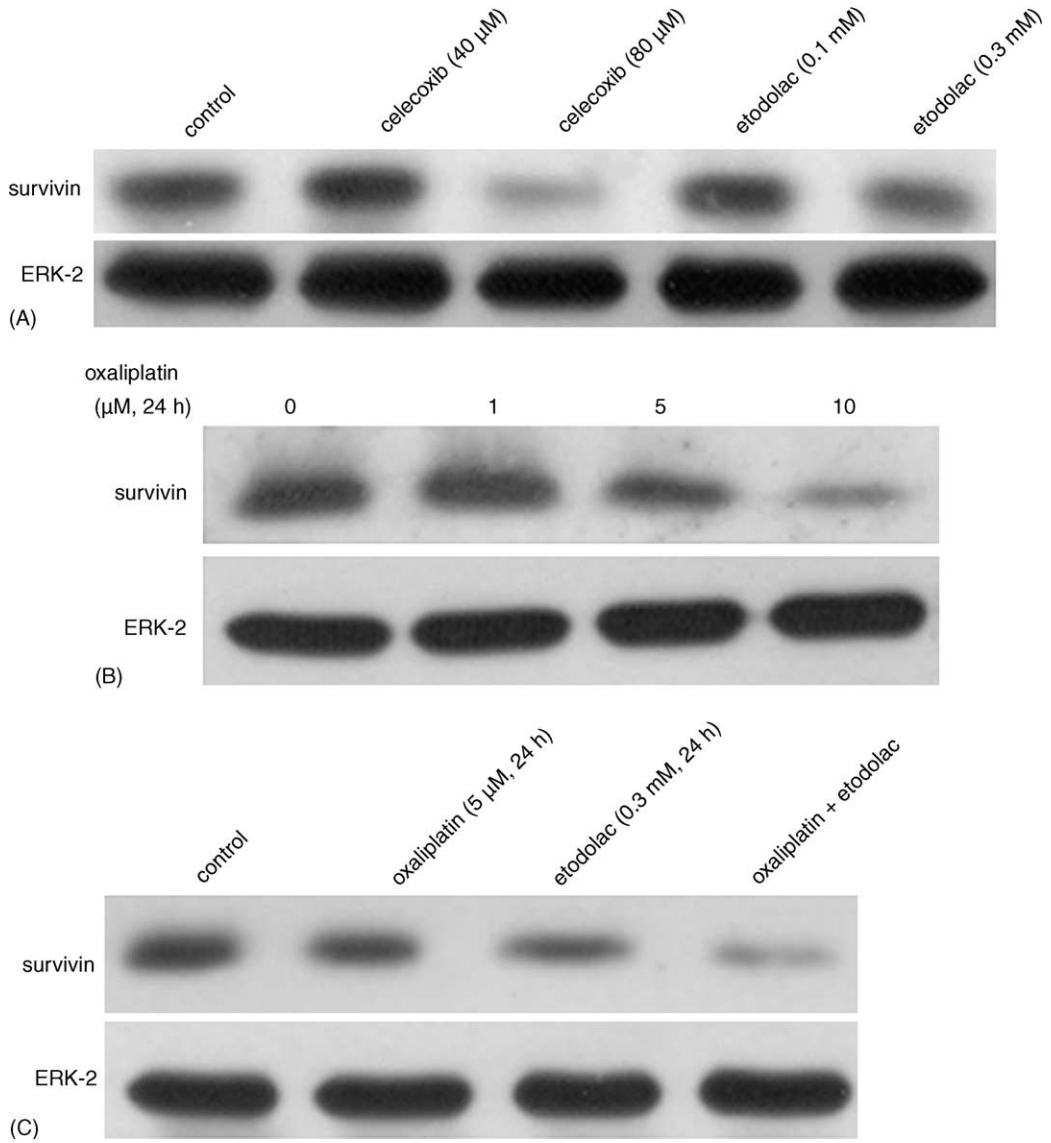

Fig. 7. Effects of COX-2 inhibitors and oxaliplatin on the level of survivin proteins in RKO cells. (A) RKO cells were treated with 40 and 80 μM celecoxib or 0.1 and 0.3 mM etodolac for 24 h. (B) RKO cells were treated with 0–10 μM oxaliplatin for 24 h. (C) The cells were co-treated with 5 μM oxaliplatin and 0.3 mM etodolac for 24 h. The total protein extracts were subjected to Western blot analysis using anti-survivin and anti-ERK-2 antibodies. Representative Western blot data were shown from one of three separate experiments with similar findings.

Together, our data suggest that the co-treatment with oxaliplatin and COX-2 inhibitors may mediate cell growth inhibition and cell death that are likely to result from the inhibition of survivin protein expression in human colon cancer cells. However, the precise mechanism of the inhibition of survivin protein expression in the oxaliplatin- and COX-2 inhibitor-treated cells needs further investigation.

In conclusion, it is the first report that the combination of COX-2 inhibitors and oxaliplatin can additively increase the growth inhibition and cell death of the human colon carcinoma cells. Moreover, the combination of COX-2 inhibitors and oxaliplatin decreases the level of survivin proteins in colon carcinoma cells. This study may provide a new strategy that combination of COX-2 inhibitors and oxaliplatin can be used in the therapy of human colon cancers.

References

[1] Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, et al. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J Clin Invest 2001;108:1657–65.

[2] Brabender J, Park J, Metzger R, Schneider PM, Lord RV, Holscher AH, et al. Prognostic significance of cyclooxygenase 2 mRNA expression in non-small cell lung cancer. Ann Surg 2002;235:440–3.

[3] Shariat SF, Kim JH, Ayala GE, Kho K, Wheeler TM, Lerner SP. Cyclooxygenase-2 is highly expressed in carcinoma in situ and T1 transitional cell carcinoma of the bladder. J Urol 2003;169:938–42.

[4] Chen WS, Liu JH, Wei SJ, Liu JM, Hong CY, Yang WK. Colon cancer cells with high invasive potential are susceptible to induction of apoptosis by a selective COX-2 inhibitor. Cancer Sci 2003;94:253–8.

[5] Tabernero A, Reimund JM, Chasserot S, Muller CD, Andriantsitohaina R. Cyclooxygenase-2 expression and role of vasoconstrictor

prostanoids in small mesenteric arteries from patients with Crohn's disease. Circulation 2003;107:1407–10.
[6] Chen WS, Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK. Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. Int J Cancer 2001;91:894–9.
[7] Han C, Leng J, Demetris AJ, Wu T. Cyclooxygenase-2 promotes human cholangiocarcinoma growth: evidence for cyclooxygenase-2-independent mechanism in celecoxib-mediated induction of p21waf1/cip1 and p27kip1 and cell cycle arrest. Cancer Res 2004; 64:1369–76.
[8] Krysan K, Merchant FH, Zhu L, Dohadwala M, Luo J, Lin Y, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 2004;18:206–8.
[9] Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 2004;64:6359–62.
[10] Evans JF, Kargman SL. Cancer and cyclooxygenase-2 (COX-2) inhibition. Curr Pharm Des 2004;10:627–34.
[11] Keller JJ, Giardiello FM. Chemoprevention strategies using NSAIDs and COX-2 inhibitors. Cancer Biol Ther 2003;2:S140–9.
[12] Church RD, Fleshman JW, McLeod HL. Cyclo-oxygenase 2 inhibition in colorectal cancer therapy. Br J Surg 2003;90:1055–67.
[13] Koki AT, Masferrer JL. Celecoxib: a specific COX-2 inhibitor with anticancer properties. Cancer Contr 2002;9:28–35.
[14] Sinicrope FA, Gill S. Role of cyclooxygenase-2 in colorectal cancer. Cancer Metastasis Rev 2004;23:63–75.
[15] Kismet K, Akay MT, Abbasoglu O, Ercan A. Celecoxib: a potent cyclooxygenase-2 inhibitor in cancer prevention. Cancer Detect Prev 2004;28:127–42.
[16] Marx J. Angiogenesis. A boost for tumor starvation. Science 2003; 301:452–4.
[17] Hashitani S, Urade M, Nishimura N, Maeda T, Takaoka K, Noguchi K, et al. Apoptosis induction and enhancement of cytotoxicity of anticancer drugs by celecoxib, a selective cyclooxygenase-2 inhibitor, in human head and neck carcinoma cell lines. Int J Oncol 2003;23: 665–72.
[18] Chen WS, Liu JH, Liu JM, Lin JK. Sequence-dependent effect of a cyclooxygenase-2 inhibitor on topoisomerase I inhibitor and 5-fluorouracil-induced cytotoxicity of colon cancer cells. Anticancer Drugs 2004;15:287–94.
[19] Sandor V. Chronotherapy with 5-fluorouracil, oxaliplatin, and folinic acid in colorectal cancer. Lancet 1997;350:1325–6.
[20] Hochster H, Chachoua A, Speyer J, Escalon J, Zeleniuch-Jacquotte A, Muggia F. Oxaliplatin with weekly bolus fluorouracil and low-dose leucovorin as first-line therapy for patients with colorectal cancer. J Clin Oncol 2003;21:2703–7.
[21] Ramanathan RK, Clark JW, Kemeny NE, Lenz HJ, Gococo KO, Haller DG, et al. Safety and toxicity analysis of oxaliplatin combined with fluorouracil or as a single agent in patients with previously treated advanced colorectal cancer. J Clin Oncol 2003;21:2904–11.
[22] Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, et al. In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 1997;57:1841–5.
[23] Gourdier I, Del Rio M, Crabbe L, Candeil L, Copois V, Ychou M, et al. Drug specific resistance to oxaliplatin is associated with apoptosis defect in a cellular model of colon carcinoma. FEBS Lett 2002; 529:232–6.
[24] Arango D, Wilson AJ, Shi Q, Corner GA, Aranes MJ, Nicholas C, et al. Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells. Br J Cancer 2004;91:1931–46.
[25] Pectasides D, Pectasides M, Farmakis D, Gaglia A, Koumarianou A, Nikolaou M, et al. Oxaliplatin plus high-dose leucovorin and 5-fluorouracil (FOLFOX 4) in platinum-resistant and taxane-pretreated ovarian cancer: a phase II study. Gynecol Oncol 2004;95:165–72.
[26] Ambrosini Q, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3:917–21.
[27] Li F, Ambrosini Q, Chu EY, Plescia J, Tognin S, Marchisio PC, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998;396:580–4.
[28] Chao JI, Kuo PC, Hsu TS. Down-regulation of survivin in nitric oxide-induced cell growth inhibition and apoptosis of the human lung carcinoma cells. J Biol Chem 2004;279:20267–76.
[29] Wall NR, O'Connor DS, Plescia J, Pommier Y, Altieri DC. Suppression of surviving phosphorylation on Thr34 by flavopiridol enhances tumor cell apoptosis. Cancer Res 2003;63:230–5.
[30] O'Connor DS, Grossman D, Plescia J, Li F, Zhang H, Villa A, et al. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci USA 2000;97:13103–7.
[31] Kuo PC, Liu HF, Chao JI. Survivin and p53 modulate quercetin-induced cell growth inhibition and apoptosis in human lung carcinoma cells. J Biol Chem 2004;279:55875–85.
[32] Kawamura K, Sato N, Fukuda J, Kodama H, Kumagai J, Tanikawa H, et al. Survivin acts as an antiapoptotic factor during the development of mouse preimplantation embryos. Dev Biol 2003;256:331–41.
[33] Chakravarti A, Zhai GG, Zhang M, Malhotra R, Latham DE, Delaney MA, et al. Survivin enhances radiation resistance in primary human glioblastoma cells via caspase-independent mechanisms. Oncogene 2004;23:7494–506.
[34] Lu B, Mu Y, Cao C, Zeng F, Schneider S, Tan J, et al. Survivin as a therapeutic target for radiation sensitization in lung cancer. Cancer Res 2004;64:2840–5.
[35] Altieri DC. The molecular basis and potential role of survivin in cancer diagnosis and therapy. Trends Mol Med 2001;7:542–7.
[36] Rodel C, Haas J, Groth A, Grabenbauer GG, Sauer R, Rodel F. Spontaneous and radiation-induced apoptosis in colorectal carcinoma cells with different intrinsic radiosensitivities: survivin as a radioresistance factor. Int J Radiat Oncol Biol Phys 2003;55:1341–7.
[37] Bhat MK, Yu C, Yap N, Zhan Q, Hayashi Y, Seth P, et al. Tumor suppressor p53 is a negative regulator in thyroid hormone receptor signaling pathways. J Biol Chem 1997;272:28989–93.
[38] Plumb JA, Milroy R, Kaye SB. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. Cancer Res 1989;49:4435–40.
[39] Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–8.
[40] Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–82.
[41] Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S, Kawai S. Selective cyclooxygenase-2 inhibitors show a differential ability to inhibit proliferation and induce apoptosis of colon adenocarcinoma cells. FEBS Lett 2002;531:278–84.
[42] Cheng J, Imanishi H, Liu W, Nakamura H, Morisaki T, Higashino K, et al. Involvement of cell cycle regulatory proteins and MAP kinase signaling pathway in growth inhibition and cell cycle arrest by a selective cyclooxygenase 2 inhibitor, etodolac, in human hepatocellular carcinoma cell lines. Cancer Sci 2004;95:666–73.
[43] Deveraux QL, Reed JC. IAP family proteins—suppressors of apoptosis. Genes Dev 1999;13:239–52.