

Original Article

EGFR activation results in enhanced expression of COX-2 and tumor growth through activation of β_2 -Adrenergic receptor in esophageal squamous cell carcinoma

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Received November 17, 2015; Accepted November 25, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: We previously reported that epidermal growth factor (EGF)-induced esophageal cancer cell proliferation required transactivation of β -adrenoceptor. In the present study, we further investigated whether β_2 -adrenoceptor was involved in the modulation of cyclooxygenase-2 (COX-2) expression and cell proliferation by EGF in esophageal squamous cell carcinoma (ESCC). Human ESCC cell line KYSE-30 was treated with EGF, EGFR inhibitor (AG1478), β_2 -selective antagonist (ICI-118,551) and highly selective cyclooxygenase-2 inhibitor (nimesulide). Cell survival was tested by MTT assay. The expression of COX-2 was detected by Western blot and real-time reverse transcription polymerase chain reaction (PCR). ESCC xenograft in nude mice was administered with EGF combining or not combining EGFR inhibitor, β_2 -selective antagonist and cyclooxygenase-2 inhibitor. Tumor growth was observed and COX-2 expression was detected by Western blot and PCR. EGFR, β_2 -adrenergic receptor and COX-2 was expressed in KYSE-30 cells. EGF stimulated KYSE-30 cell proliferation in a dose-dependent manner. AG1478, ICI-118,551, and nimesulide attenuated cell proliferation induced by EGF. AG1478 and ICI-118,551 also abrogated EGF-induced up-regulation of COX-2 expression in the mRNA and protein level. Animal model indicated that EGF significantly stimulated the growth of ESCC xenograft in nude mice, which was attenuated by AG1478, ICI-118,551, and nimesulide. Moreover, AG1478 and ICI-118,551 abrogated EGF-induced up-regulation of COX-2 expression in the tumor xenograft. These data provided the first evidence that EGFR activation resulted in enhanced expression of COX-2 and tumor growth through activation of β_2 -adrenergic receptor in ESCC. This novel finding shed new light on combination of EGFR blocker and COX-2 inhibitor for the treatment of ESCC.

Keywords: Esophageal Squamous Cell Carcinoma (ESCC), Epidermal Growth Factor Receptor (EGFR), β -Adrenergic Receptor, Cyclooxygenase-2 (COX-2)

Introduction

Esophageal squamous cell carcinoma (ESCC) is the sixth most frequent cause of cancer death in the world, with over 400000 new cases diagnosed each year. Based on histological classification, more than 90% of esophageal cancers are either squamous cell carcinomas or adenocarcinomas [1]. Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype in China, accounting for more than 90% of cases of esophageal cancer overall [2]. Most patients with ESCC are diagnosed at an advanced stage. The 5-year sur-

vival ranges from 5 to 20% for patients undergoing operations with curative intent. Recent advances in the diagnosis and treatment of ESCC only have improved cancer-specific outcomes in the last two decades [3]. The cellular and molecular mechanisms leading to the development of ESCC, however, are not completely comprehended.

Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, plays an important role in the development of cancer. The expression or activation of EGFR is commonly altered in many malignant tumors, in which EGFR acti-

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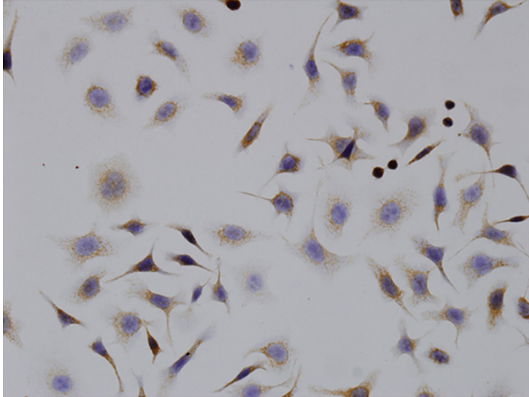


Figure 1. Immunocytochemistry revealed the expression of β_2 -adrenoceptor protein in the esophageal squamous-cell carcinoma cell line KYSE-30. β_2 -Adrenoceptor was predominantly located in the cell membrane and cell plasma.

vation with abnormally regulated kinase activity transduces excessive mitogenic signals to the cells, resulting in the uncontrollable growth and aggressive phenotype in these cells [4, 5]. Our previous study found that the proliferation of ESCC cells induced by EGFR activation can be inhibited by β -adrenoceptor blockers. Further mechanistic investigation demonstrated that the cellular release of epinephrine and the expression of tyrosine hydroxylase involved in the biosynthesis of epinephrine were induced by EGF. The expression of β -adrenoceptor and the downstream signal transducer protein kinase A was also up-regulated to promote cell proliferation. This study demonstrates that EGFR activation up-regulates β -adrenergic signaling at several levels thus mediating its mitogenic signal in ESCC [6].

β -Adrenoceptor, a G-protein-coupled receptor, is now identified as a cell surface catecholamine receptor that regulates a series of cellular processes such as cell proliferation, migration and apoptosis, especially in tumor cells. For instance, β -adrenergic stimulation has been shown to promote the growth of human breast, pulmonary, and colon cancer cells [7-9]. Existing research suggest that metabolism of arachidonic acid is involved in tumor promotion caused by β -adrenergic receptor activation [10]. Our previous studies confirmed that stimulation of β_2 -adrenergic receptor transactivates the extracellular signal-regulation kinase (ERK)/COX-2 pathway to promote the proliferation of ESCC cells. Stimulation of β_2 -adrenergic

receptor with epinephrine significantly increases the ESCC cell proliferation and COX-2 expression, which were blocked by β_2 -selective antagonist. COX-2 inhibitor also significantly inhibited ESCC cell proliferation induced by epinephrine [11].

Therefore, we hypothesized that EGFR activation could promote the expression of COX-2 and tumor growth through activation of β_2 -adrenergic receptor in ESCC cells. In the present study, we aimed to elucidate the role of β_2 -adrenergic receptor and COX-2 in the regulation of the growth of ESCC cells via EGFR activation.

Materials and methods

Reagents and drugs

EGF, AG1478 (EGFR tyrosine kinase inhibitor), ICI-118,551 (β_2 -adrenergic receptor inhibitor) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). COX-2 antibody was purchased from Cayman Chemical (Ann Arbor, MI, USA).

Cell Culture and drug treatment

KYSE-30 cell line was obtained from the American Type Culture Collection (Manassas, VA, United States). The cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) and antibiotics (100 U/mL penicillin G and 100 μ g/mL streptomycin) at 37°C, 95% humidity and 5% CO₂.

KYSE-30 cells were plated at a density of 1×10^4 cells/well in 96-well plates. After 24 h of incubation, the cells were starved in FBS-free medium for 12 h to synchronize the cell cycle. EGF at different concentrations was incubated with the cells for 24 h to study its proliferative effect. In order to examine the effects of various inhibitors, cells were pretreated with or without AG1478 (2.5 nM), ICI-118, 551 (50 μ M), or nimesulide (50 μ M) for 1 h before EGF treatment.

Immunocytochemistry

Most of KYSE-30 cells attached to the dishes after 24 h of primary culture. The 96-well plates were washed twice with 0.1 M PBS and cells were fixed with poly-formaldehyde at 4°C over-

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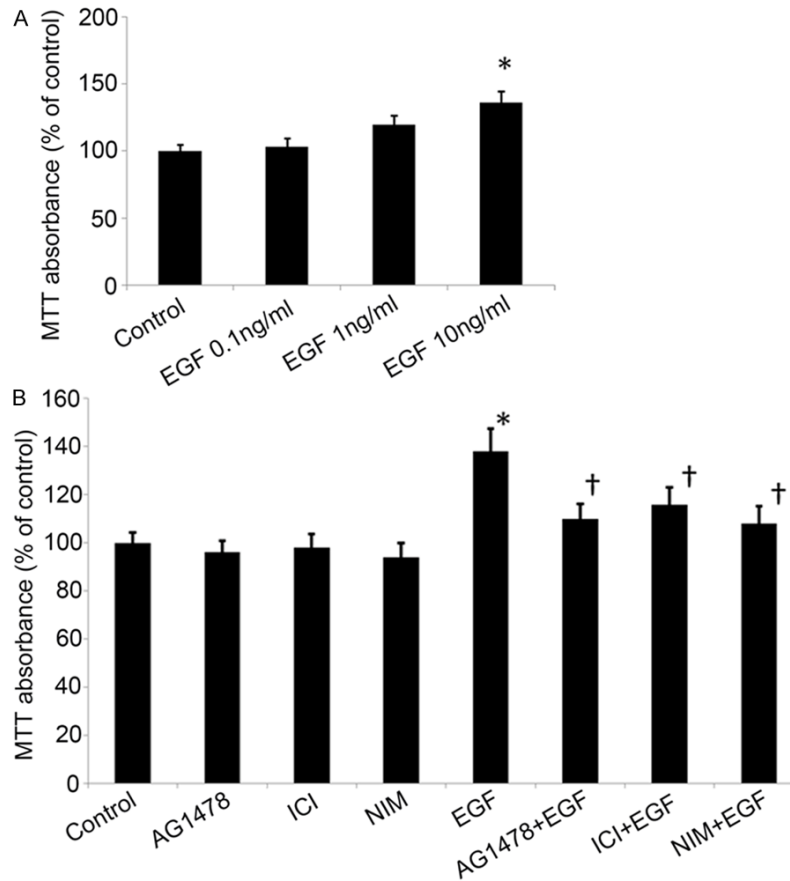


Figure 2. Involvement of β_2 -adrenoceptor and COX-2 in the mitogenic effect of EGF on KYSE-30 esophageal cancer cell proliferation. A. MTT assay showed that incubation of EGF for 24 h dose-dependently increased cell proliferation. B. The mitogenic effect of EGF (10 ng/ml) was significantly abolished in the presence of AG1478 (2.5 nM), ICI-118,551 (50 μ M), or nimesulide (50 μ M). At these concentrations, AG1478, ICI-118,551 and nimesulide had no effect on basal cell proliferation. *, $P < 0.05$, significantly different from the untreated control group. †, $P < 0.05$, significantly different from the EGF-treated group.

night. The following procedures were performed according to the instructions for the streptavidin/peroxidase kit. The cells were washed with PBS, then incubated with bovine serum albumin and reacted with primary antibody dissolved in PBS. After washing, the cells were incubated with peroxidase-conjugated second antibody, washed again and reacted with streptavidin/peroxidase for 20 min. Color reaction was developed by incubation with DAB. In the negative controls, PBS was used instead of primary antibody.

MTT cell proliferation assay

KYSE-30 cells were seeded onto 96-well culture plates and subjected to different treatments. Following treatment, the media were

removed and 20 μ l 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) was added to each well. The cells were further cultured at 37°C for 4 h, prior to the removal of the cell culture and the addition of 100 μ l dimethyl sulfoxide to dissolve formazan. The absorbance of formazan was determined at 546 nm by the microplate reader and the survival rate was evaluated. All experiments were repeated in triplicate.

Quantitative reverse transcription-polymerase chain reaction

The total cellular RNA was isolated from KYSE-30 cells using Trizol reagent. The RNA concentration was measured by GeneQuant II (Amersham Corporation) at 260 nm. Same amount of total RNA (2 μ g) was used to generate the first strand of cDNA by reverse transcription in accordance with the manufacturer's instructions (Invitrogen). The polymerase chain reaction (PCR) primers of COX-2 were as follows: 5'-CTCC-

TTCTTCTGCGAGCTGT-3' (sense), 5'-AGGATGGG-CAGGAAGGAC-3' (antisense). Quantitative PCR was carried out using SYBR Green PCR Master Mix (Bio-Rad) and Multicolor Real-Time PCR Detection System (Bio-Rad) as recommended by the manufacturer. Conditions for quantitative PCR were 94°C for 5 min, 50 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. The results were analyzed using the melting-curve analysis method with GAPDH as an internal control. The specificity of PCR product was confirmed by DNA gel electrophoresis.

Western blot analysis

KYSE-30 cells were harvested in radioimmuno-precipitation buffer [50 mM Tris-HCl (pH 7.5), 150 mM sodium chloride, 0.5% α -cholate acid,

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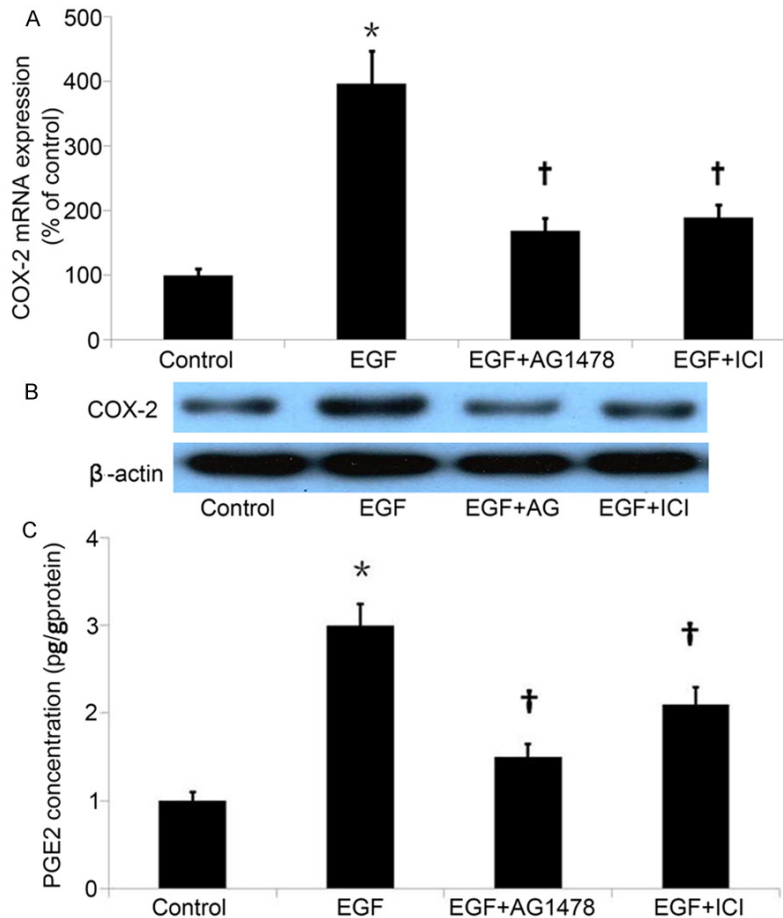


Figure 3. A. Expression of COX-2 mRNA was increased after 24 h treatment with EGF (10 ng/ml) as revealed by real-time PCR analysis. The effect was blocked by AG1478 (2.5 nM) or ICI-118,551 (50 μM). B. Western blot analysis revealed that COX-2 protein expression was increased by EGF (24 h, 10 ng/ml), which was abolished by AG1478 (2.5 nM) or ICI-118,551. The expression levels are expressed as ratio of COX-2 to β-actin. The results are representative of three independent experiments. C. EGF (24 h, 10 ng/ml) stimulation resulted in three-fold increase in PGE2 release in KYSE-30 cells. The induction of PGE2 release induced by EGF was abrogated by AG1478 (2.5 nM) or ICI-118,551. *, $P < 0.05$, significantly different from the untreated control group. †, $P < 0.05$ significantly different from the EGF-treated group.

0.1% SDS, 2 mM EDTA, 1% Triton X-100, and 10% glycerol] containing proteinase and phosphatase inhibitors. Protein was quantified using protein assay kit (Bio-Rad Laboratories, Hercules, USA). Equal amounts of protein (20 μg/lane) were resolved by SDS-PAGE, and transferred to Hybond C nitrocellulose membranes (Amersham Corporation, Arlington Heights, IL, USA). The membranes were probed with primary antibodies overnight at 4°C and incubated for 1 h with peroxidase-conjugated secondary antibodies. They were developed with an enhanced chemiluminescence system (Amersham Corporation, Arlington Heights, IL,

USA) and exposed to an X-ray film (FUJI Photo Film Co., Ltd., Tokyo, Japan). Quantitation was carried out with a video densitometer (ScanMaker III, Microtek, Taiwan).

Prostaglandin E2 assay

Prostaglandin E2 (PGE2) assay was performed according to the manufacturer's instructions. Cells were incubated in the absence or presence of EGF with or without different inhibitors for 1 h, then supernatant from each well was collected and PGE2 was measured by specific enzyme immunoassay (EIA) (Cayman Chemical Co.). PGE2 concentration was normalized to total cellular protein and expressed as pg/mg protein.

Animal experiments

The animal experiments were approved by the Institutional Animal Care and Use Committee. Male athymic nude mice were housed in our experimental animal facility under standard laboratory conditions with free access to food and water. Each mouse was inoculated in the flank region by subcutaneous injection with cells (1×10^6 , viability $\geq 95\%$) suspended in 0.2 ml of phosphate-buffered saline of the human ESCC cell line KYSE-30. The mice were then randomly assigned to the following treatment groups (6 mice per group):

Group 1: control; Group 2: 10 μg/kg of EGF daily by subcutaneous injection of tumor inoculation area every day; Group 3: 10 μg/kg of EGF daily by subcutaneous injection of tumor inoculation area + AG1478 (20 nmol/kg daily) by intraperitoneal injection every day; Group 4: 10 μg/kg of EGF daily by subcutaneous injection of

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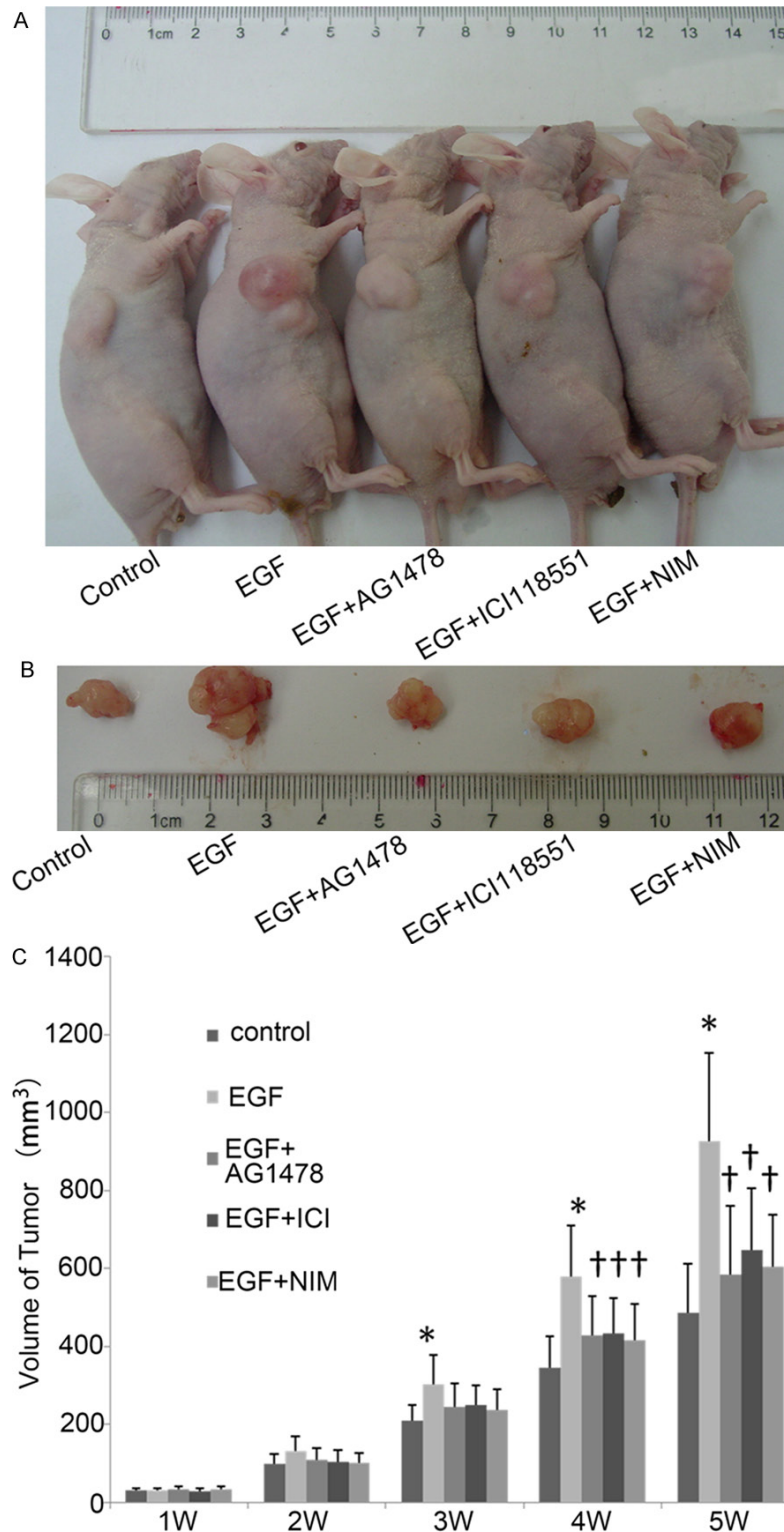


Figure 4. A. Mice implanted with KYSE-30 esophageal cells were randomized into five groups. Tumor volume was measured every 7 days after the implantation of the esophageal cancer cells. EGF promoted tumor growth of KYSE-30 esophageal cancer xenograft in nude mice. The effect was blocked by AG1478, ICI-118,551 or nimesulide. B. Representative nude mice from five groups at week 5. C. Representative tumor xenografts in nude mice of five groups at week 5. *, $P < 0.05$, significantly different from the untreated control group. †, $P < 0.05$ significantly different from the EGF-treated group.

tumor inoculation area + ICI-118,551 (20 $\mu\text{mol/kg}$ daily) by intraperitoneal injection every day; Group 5: 10 $\mu\text{g/kg}$ of EGF daily by subcutaneous injection of tumor inoculation area + nimesulide (20 $\mu\text{mol/kg}$ daily) by intragastric administration every day.

All animals were observed for 35 days while being monitored on a daily basis by a staff veterinarian certified in the laboratory animal science. Body weights were recorded once a week.

At the end of the 28-day observation period, the animals were euthanized by CO_2 inhalation. Xenograft volumes were calculated according to the formula: volume = (length/2) \times (width²), using two measured perpendicular diameters of each tumor. The tumors were excised and frozen in liquid nitrogen for analysis by quantitative RT-PCR and Western blotting.

Statistical analysis

Results were expressed as the mean \pm SEM for each sample for all paired statistical comparisons. Statistical analysis was performed with an analysis of variance followed by the Turkey's t-test. P values less than 0.05 were considered statistically significant.

Results

KYSE-30 cells expressed β_2 -Adrenoceptor

KYSE-30 cell line was established from a primary

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Table 1. Tumor volumes in nude mice

Group	Tumor volume (mm ³ , mean ± SD)				
	1 st week	2 nd week	3 rd week	4 th week	5 th week
A	30.22±9.34	98.44±26.10	209.09±43.94	344.65±89.33	486.5±189.51
B	29.88±8.67	130.6±33.73	302.43±73.89*	580.36±177.23*	998±265.35*
C	34.56±11.87	109.97±27.58	225.79±54.57	407.8±122 [#]	584.44±212.59 [#]
D	28.76±10.98	104.37±26.21	238.81±53.05	433.72±126.66 [#]	646.68±209.79 [#]
E	32.42±10.38	102.33±25.21	226.89±5.05	415.67±124.76 [#]	605.51±213.84 [#]

* $p < 0.05$ compared with the group A; [#] $p < 0.05$ compared with the blank group.

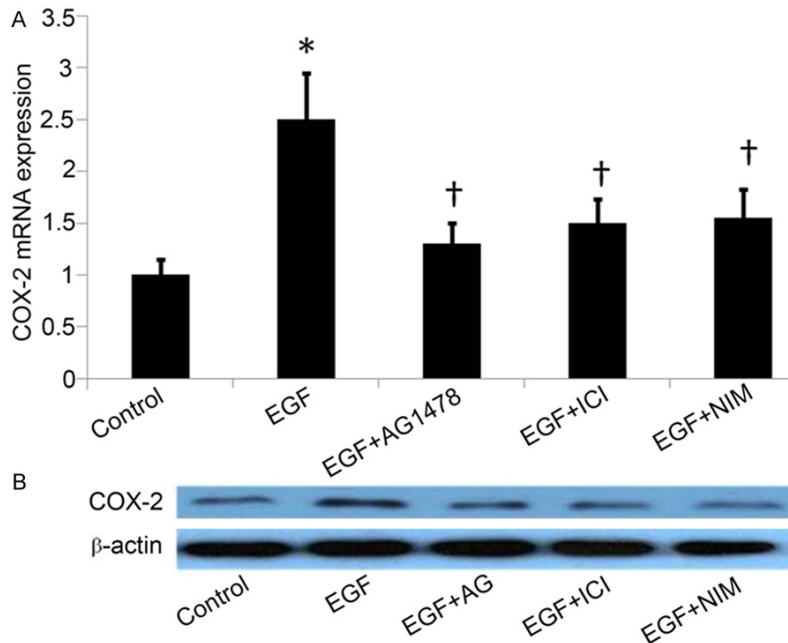


Figure 5. A. Real-time PCR analysis revealed that expression of COX-2 mRNA was increased by treatment with EGF in KYSE-30 esophageal cancer xenograft. The effect was blocked by AG1478, ICI-118,551 or nimesulide. B. Western blot analysis revealed that COX-2 protein expression in KYSE-30 esophageal cancer xenograft was increased by EGF, which was abolished by treatments with AG1478 or ICI-118,551. The expression levels are expressed as ratio of COX-2 to β-actin.

well differentiated squamous cell carcinoma of the esophagus from a 64-year-old Japanese patient. A p53 mutation at the splice acceptor site of intron 6 and a 12-fold amplification of c-ErbB has been reported in the KYSE-30 cell. KYSE-30 cells express a large number of epidermal growth factor receptor (EGFR). Thereafter, we chose KYSE-30 cells as the working cell line for further study in the subsequent experiments to examine the mechanism of EGF activation in cancer cell proliferation. Our previous study demonstrates that EGFR activation up-regulates β-adrenergic signaling at multiple levels and thus mediates its mito-

genic signal in ESCC. As stimulation of β-adrenoceptors has been shown to promote cancer cell proliferation, the expression of β₂-adrenoceptor was determined in KYSE-30 esophageal squamous-cell carcinoma cell lines by immunocytochemistry. It was found that the KYSE-30 cells expressed the proteins of β₂-adrenoceptor (Figure 1), which were predominantly located in the cell membrane and cell plasma.

EGF increased KYSE-30 cell proliferation

To study the effect of EGF on proliferation of esophageal squamous-cell carcinoma cells, we examined the cell proliferation by MTT assay in response to EGF in KYSE-30 cells. Treating the cells with EGF for 24 h increased the cell proliferation

in a dose-dependent manner, with the maximal stimulatory effect observed with the dose of 10 ng/ml (Figure 2A). To determine the mechanism of EGF-induced cell proliferation, EGFR tyrosine kinase inhibitor AG1478 was used to confirm that stimulatory action was mediated through EGFR. The result showed that AG1478 significantly dampened EGF-induced cell proliferation, indicating that the mitogenic action is mediated through EGFR (Figure 2B). To elucidate whether β₂-adrenoceptor was involved in the mitogenic action of EGF, KYSE-30 cells were treated with β₂-adrenoceptor antagonist ICI-118,551 in the

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absence or presence of EGF. Results showed that ICI-118,551 by itself has no effect on basal cell proliferation but significantly reduces DNA synthesis induced by EGF, signifying the involvement of β -adrenoceptor in this stimulatory action (**Figure 2B**). To further elucidate whether COX-2 was involved in the mitogenic action of EGF, KYSE-30 cells were treated with COX-2-selective inhibitor nimesulide in the absence or presence of EGF. Results showed that nimesulide also significantly reduced DNA synthesis induced by EGF, signifying the involvement of COX-2 in this stimulatory action (**Figure 2B**).

EGF increased COX-2 expression

Our previous studies showed that activation of arachidonic acid cascade was involved in the stimulation of cell proliferation induced by β_2 -adrenoceptor activation. COX-2-selective inhibitor significantly dampened EGF-induced cell proliferation, indicating that the mitogenic action of EGF is mediated through COX-2. We therefore determined whether COX-2 expression was increased upon EGF treatment. The results showed that EGF significantly increased the mRNA level of COX-2 in KYSE-30 cells, which were abolished by EGFR tyrosine kinase inhibitor (**Figure 3A**). To elucidate whether β_2 -adrenoceptor was involved in the EGF-induced COX-2 expression, KYSE-30 cells were treated with β_2 -adrenoceptor antagonist ICI-118,551 in the absence or presence of EGF. The results showed that inhibition of β_2 -adrenoceptor significantly abolished the stimulatory effect of EGF on COX-2 mRNA expression, signifying the involvement of β_2 -adrenoceptor in this stimulatory action (**Figure 3A**). In parallel, the protein level of COX-2 was increased by about 50% in the EGF-treated KYSE-30 cells, which was also reversed by EGFR tyrosine kinase inhibitor or β_2 -adrenoceptor inhibitor (**Figure 3B**). Prostaglandin E2 (PGE2) is the product of COX-2 activation, which plays key roles in influencing the tumorigenesis. Our results showed that EGF significantly increased the production of PGE2 in KYSE-30 cells, which was abolished by EGFR tyrosine kinase inhibitor, β_2 -adrenoceptor antagonist, or COX-2 selective inhibitor (**Figure 3C**).

EGF promoted tumor growth in ESCC xenograft in nude mice

Seven days after inoculation with KYSE-30 cells, palpable xenografts were detected in all

mice. The mice were treated with EGF alone or EGF plus three different inhibitors for 35 days. All mice survived until the end of the 35-day intervention period. Body weights of untreated mice and mice from treatment groups were not statistically different (**Figure 4B**). None of the animals demonstrated any abnormalities in behavior.

On day 35, tumor volumes in the EGF-treated animals were significantly larger than those in the untreated group (**Table 1** and **Figure 4C**). To study the involvement of β_2 -adrenoceptor in the promoting actions of EGF on ESCC tumor growth, β_2 -adrenoceptor antagonist ICI-118,551 was injected intraperitoneally every day into nude mice bearing KYSE-30 tumor xenograft. ICI-118,551 significantly attenuated the stimulatory effect of EGF on tumor growth (**Figure 4A**). To study the involvement of COX-2 in the promoting actions of EGF on ESCC tumor growth, COX-2 selective inhibitor nimesulide was administered intragastrically every day into nude mice bearing KYSE-30 tumor xenograft. The results showed that nimesulide also significantly attenuated the stimulatory effect of EGF on tumor growth (**Figure 4A**).

β_2 -Adrenoceptor antagonist attenuated EGF-induced COX-2 expression in tumor xenograft

The stimulatory effect of EGF on tumor growth was abolished by COX-2 selective inhibitor. We therefore determined whether COX-2 expression was increased upon EGF treatment in ESCC xenograft. Results showed that EGF significantly increased the mRNA level of COX-2, which was abolished under the action of EGFR tyrosine kinase inhibitor. Results also showed that inhibition of β_2 -adrenoceptor significantly diminished the stimulatory effect of EGF on COX-2 mRNA expression in tumor xenograft (**Figure 5A**), signifying the involvement of β_2 -adrenoceptor in this stimulatory action, which was similar to the reaction in KYSE-30 cell study. In parallel, experiments on the animal models also indicated that COX-2 protein expression was increased in the EGF-treated KYSE-30 cell xenograft, which was abrogated by EGFR tyrosine kinase inhibitor or β_2 -adrenoceptor inhibitor (**Figure 5B**). We found that COX-2-selective inhibitor nimesulide also attenuated the increase of COX-2 mRNA and protein expression by EGF stimulation in tumor xenograft.

Discussion

The EGFR signaling pathway is fundamental to carcinogenesis, as it plays a key role in the control of cell proliferation. Proteins involved in the EGFR signaling now became attractive and promising targets in the pathway-directed cancer therapy. Results of clinical trials demonstrate that EGFR-directed therapies can be valuable in the treatment of various cancers including the cancer of esophagus [12]. COX-2 plays a regulatory role in the metabolism of arachidonic acid and its conversion to inflammatory prostaglandins, especially PGE₂. It is well documented that expression of COX-2 contributes to increased anti-apoptotic, pro-angiogenic and metastatic potentials of cancer cells [13, 14]. Our previous study has indicated that COX-2 plays a crucial role in the carcinogenesis of human ESCC by increasing cell proliferation and resistance to apoptosis and thus enhancing tumorigenesis [15, 16].

Activations of EGFR and COX-2 have been implicated in the promotion of esophageal squamous cancer cell proliferation, but the connection between these two molecules is not completely understood. Our previous study demonstrates that EGFR activation up-regulates β -adrenergic signaling to mediate its mitogenic signal in ESCC. The findings of the present study demonstrate that the esophageal cancer cell line KYSE-30 expresses the protein of β_2 -adrenoceptor and increases cell proliferation in response to EGF stimulation. In this study, EGF-induced cell proliferation is attenuated by EGFR kinase inhibitor or β_2 -adrenoceptor antagonists. These results indicate that β_2 -adrenoceptor is transactivated to mediate the mitogenic effect of EGF in ESCC cells.

The metabolism of arachidonic acid has been proved to be involved in tumor promotion caused by β -adrenergic receptor activation [17, 18]. Our results show EGF stimulation increase the expression of COX-2 mRNA and protein, and EGF-induced cell proliferation is attenuated by COX-2 inhibitor. One goal of this study was to identify signaling pathways that mediate COX-2 induction after EGFR activation. By using specific inhibitor of β_2 -adrenergic receptor, we establish that β_2 -adrenergic receptor involves induction of COX-2 via EGFR activation. In animal study, β_2 -adrenergic receptor inhibitor also

abrogates EGF-induced COX-2 expression and tumor growth.

Both COX-2 and EGFR pathways are activated in many human cancers, including ESCC. Clear understanding of how these two pathways coordinate tumor progression will provide a significant advance in the field of cancer research. Multiple lines of evidence demonstrate that a cross-talk of COX-2 and EGFR pathways synergistically promotes tumor progression and metastasis [19]. It is well documented that EGFR is a downstream target of COX-2. For instance, it has been reported that prostaglandin E₂ regulates cancer cell proliferation and migration via the intracellular activation of EGFR [20]. Meanwhile, it has been recognized that EGF signaling up-regulates COX-2 expression and activity. Activation of EGFR induces COX-2 expression through various pathways, including STAT5, PKC, and P38 MAPK [21, 22]. Our study found that β -adrenergic receptor can also mediate COX-2 expression induced by activation of EGFR.

Specific targeting of EGFR is already used in the clinics to treat patients with metastatic colon cancer and lung cancer, for example by administering cetuximab [23]. Researchers have been investigating whether inhibiting both the EGFR and COX-2 signaling pathways could yield additive effects on blocking tumor growth and the spread of metastatic disease [24]. In this study, we show that transactivation of adrenoceptor is required for EGF-induced COX-2 expression. It is conceivable that combination therapy with EGFR blocker and a COX-2 inhibitor may yield better clinical outcome in cancer prevention and treatment.

To conclude, the present study not only demonstrates that EGFR activation can promote the COX-2 expression and tumor growth via β_2 -adrenergic receptor pathways in ESCC cells and nude mouse model, but also opens up a novel therapeutic opportunity for the use of combination therapy with EGFR blocker and a COX-2 inhibitor for ESCC treatment.

Acknowledgement

This work was supported by National Natural Science Foundation of China (30900680). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

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