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Nicotine Promotes Colon Tumor Growth and Angiogenesis through β-Adrenergic Activation

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Cigarette smoking is a putative environmental risk factor for colon cancer. Nicotine, an active alkaloid in tobacco, has been implicated in carcinogenesis. In the present study, we demonstrated that oral nicotine administration (50 or 200 μg/ml) for 25 days stimulated growth of human colon cancer xenograft in nude mice. It also increased vascularization in the tumors and elevated cotinine and adrenaline plasma levels. β-Adrenoceptors, cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), and vascular endothelial growth factor (VEGF) in tumor tissues were also increased by nicotine. I.p. injection of β_1-selective antagonist (atenolol, 5 or 10 mg/kg) or β_2-selective antagonist (ICI 118,551, 5, or 10 mg/kg) blocked the nicotine-stimulated tumor growth dose dependently, in which β_2-selective antagonist produced a more prominent effect. β-Adrenoceptors blockade also abrogated the stimulatory action of nicotine on microvessel densities as well as cell expression of COX-2, PGE_2, and VEGF, in which β_2-selective antagonist produced a significant effect. These findings provide a direct evidence that nicotine can enhance colon tumor growth mediated partly by stimulation of β-adrenoceptors, preferentially the β_2-adrenoceptors. Activation of β-adrenoceptors and the subsequent stimulation of COX-2, PGE_2, and VEGF expression is perhaps an important mechanism in the tumorigenic action of nicotine on colon tumor growth. These data suggest that β-adrenoceptors play a modulatory role in the development of colon cancer and partly elucidate the carcinogenic action of cigarette smoke.

Key Words: **colon cancer; nicotine; β-adrenoceptors; smoking.**

Colon cancer is a leading cause of cancer and cancer deaths in the Western world (Jemal *et al*., 2006) and is increasing in Asian countries. Cigarette smoking has been suggested as one of the risk factors for colon cancer. Although recent study reported the inconsistent association between cigarette smoking and colon cancer (Vineis *et al*., 2004), early epidemiologic studies have consistently shown that smoking increased risk of colon cancer, especially in long-term smoking (Giovannucci, 2001; Heineman *et al*., 1994; Sturmer *et al*., 2000). Cigarette smoking is also related to adenomatous colon polyps and the duration of smoking is positively correlated to the risk of larger adenomatous colon polyps (Reid *et al*., 2003; Terry *et al*., 2002). However, the molecular and cellular mechanisms involved in the pathogenesis of smoking-related colon cancer remain understudied.

Cigarette smoke contains over 3000 chemicals (Brunnemann and Hoffmann, 1991). Many of them have been identified as carcinogens and are known to initiate and promote tumorigenesis (Wu and Cho, 2004). Nicotine, one of the main components in cigarette smoke, plays a dominant role in mediating the biochemical, pharmacological, and psychological effects of cigarette smoking. It has also been shown to play a role in carcinogenesis through the nicotinic acetylcholine receptors (nAChRs) (Minna, 2003; Schuller *et al*., 2000; Ye *et al*., 2004). There are also growing evidences supporting that nicotine may act via β-adrenoceptors (Jin *et al*., 2004; Park *et al*., 1995; Schuller *et al*., 1999). In particular, nicotine suppressed apoptosis of lung cancer cells by stimulating the phosphorylation of B-cell lymphoma 2–associated death promoter protein via the upstream β-adrenoceptors but not the α7-nAChR (Jin *et al*., 2004). Nicotine has been shown to induce secretion of adrenaline from adrenal glands (Li and Forsberg, 1996; Narita *et al*., 1973). The other line of evidence also suggests that adrenaline, a β-agonist, plays a contributory role in carcinogenesis and tumor progression (Entschladen *et al*., 2005).

Activation of β-adrenoceptors has been linked to the arachidonic acid cascade, especially the cyclooxygenase-2 (COX-2) activation in relation to cancer growth (Schuller *et al*., 1999; Weddle *et al*., 2001). β-Adrenoceptors have also been shown to play a role in the progression of ovarian cancer by stimulation of the production of vascular endothelial growth factor (VEGF) (Lutgendorf *et al*., 2003). Expression of β-adrenoceptors has been shown in both human colon cancer cell lines and tumor tissues (Odore *et al*., 2003; Palm *et al*., 2006). We have previously demonstrated that HT-29 (colon adenocarcinoma) cells express both β_1- and β_2-adrenoceptors (Wu *et al*., 2005). Whether these receptors are involved in colon tumor growth *in vivo* is not known. In the present study, we aimed to delineate whether nicotine could indeed promote colon tumor growth in

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a colon cancer xenograft and study further the modulatory role of β-adrenoceptors (both β_1 and β_2) in this process and the possible underlying mechanisms involved. This study could provide additional insights about the pathogenesis of colon cancer in particularly related to cigarette smoking.

MATERIALS AND METHODS

Reagents and drugs. Nicotine, atenolol (β_1-selective antagonist), ICI 118,551 (β_2-selective antagonist), and antibody for β-actin were purchased from Sigma (St Louis, MO). Antibodies for COX-2 and VEGF were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The cotinine enzyme immunoassay kit was obtained from Orasure Technologies (Bethlehem, PA). The adrenaline enzyme immunoassay kit was obtained from Immuno-Biological Laboratories (Hamburg, Germany). The prostaglandin E_2 (PGE_2) enzyme immunoassay kit was obtained from R&D Systems (Minneapolis, MN).

Cell culture and viability assay. The human colon cancer cell line HT-29 was obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cell viability was determined by trypan blue exclusion assay. In brief, cells were trypsinized and stained with 0.4% trypan blue. Viable cells were counted with a hemocytometer.

Tumorigenicity assays in nude mice. HT-29 cells were trypsinized and collected by centrifugation. The cells were >95% viability as determined by typan blue staining. The cells (3×10^6) were suspended in 0.2 ml of phosphate-buffered saline and implanted s.c. into the right flank of 4- to 6-week-old female BALB/c nu/nu mice (Laboratory Animal Unit, The University of Hong Kong) under sterile conditions. Mice were randomized to three treatment groups and received oral administration of (1) tap water, (2) 50 μg/ml nicotine, or (3) 200 μg/ml nicotine in tap water for 25 days. The doses of nicotine used mimicked the daily intakes of cigarettes in smokers (Lawson *et al*., 1998). To further determine the involvement of β-adrenoceptors in the process, mice implanted with HT-29 cells as described above were treated according to the following groups: (1) tap water, (2) tap water and atenolol (5 mg/kg), (3) tap water and atenolol (10 mg/kg), (4) tap water and ICI 118,551 (5 mg/kg), (5) tap water and ICI 118,551 (10 mg/kg), (6) 200 μg/ml nicotine, (7) 200 μg/ml nicotine and atenolol (5 mg/kg), (8) 200 μg/ml nicotine and atenolol (10 mg/kg), (9) 200 μg/ml nicotine and ICI 118,551 (5 mg/kg), or (10) 200 μg/ml nicotine and ICI 118,551 (10 mg/kg). The mice received tap water or nicotine administration after implantation of tumor cells. Both β_1- or β_2-selective antagonists were injected i.p. thrice per week. Tumors were measured in two dimensions at right angles to one another with a caliper. Tumor volume was calculated by the following formula: volume= (length/2) × (width^2). Body weight and solution consumed were monitored throughout the experiments. At the end of the experiments (day 25), mice were sacrificed, and blood samples were collected for enzyme immunoassays. The tumors were excised and fixed in 4% formalin and paraffin embedded or frozen in liquid nitrogen and stored at − 80°C until further assays.

Measurement of cotinine and adrenaline levels. Blood samples were centrifuged for 15 min at 5000 × g at 4°C. The resultant plasma was prepared, and cotinine levels were measured by enzyme immunoassay kit according to manufacturer's instructions. Absorbance was measured at 450 nm in microplate reader (Dynes Technologies, Microtiter Co., Chantilly, VA). A standard curve of cotinine concentrations (0–1000 ng/ml) was included in each assay. The plasma concentrations of cotinine were expressed as nanogram per milliliter of plasma. Adrenaline levels in plasma were determined by enzyme immunoassay kit as previously described (Guo *et al*., 2005). The plasma concentrations of adrenaline were expressed as nanogram per milliliter of plasma.

RT-PCR analysis. Total RNA was isolated from colon tumor tissues using TRIZOL Reagent (Life Technologies BRL, Gaithersburg, MD). Five micrograms of the total RNA was used to generate the first strand of cDNA by reverse transcription (Life Technologies BRL) according to manufacturer's instructions. The primers and the PCR conditions used were as previously described (Wu *et al*., 2005). The PCR products were electrophoresed on 1.5% (wt/vol) agarose gels containing 0.5 μg/ml ethidium bromide. Gel photographs were then analyzed semiquantitatively in a multianalyzer (Bio-Rad Laboratories, Hercules, CA).

TABLE 1

Effects of Oral Nicotine Administration on Various Parameters in Nude Mice Bearing HT-29 Colon Cancer Xenografts. Nicotine Treatment Did Not Affect the Average Body Weight and Total Drinking Solution Consumed per Mouse over the 25-Day Period. Cotinine Levels in Plasma Were Significantly Elevated in Nicotine-Treated Groups When Compared to the Tap Water–Treated Group. Exposure to 200 μg/ml Nicotine Also Significantly Increased Plasma Levels of Adrenaline. *$p < 0.05$; **$p < 0.005$, Significantly Different from the Tap Water–Treated Group

Treatment group	Average body weight (g)	Solution consumed (ml)	Cotinine level (ng/ml)	Adrenaline level (ng/ml)
Tap water	20.3 ± 0.55	162	8.7 ± 0.66	7.1 ± 0.83
50 μg/ml nicotine	19.2 ± 0.61	161	43.2 ± 7.10*	9.4 ± 1.14
200 μg/ml nicotine	19.7 ± 0.90	164	169.4 ± 12.21**	19.5 ± 1.68*

Western blot analysis. Tumors tissues were homogenized on ice in radioimmunoprecipitation assay buffer (50 mmol/l Tris-HCl [pH 7.5], 150 mmol/l sodium chloride, 0.5% α-cholate acid, 0.1% sodium dodecyl sulfate [SDS], 2 mmol/l EDTA, 1% Triton X-100, and 10% glycerol) containing 1.0 mmol/l phenylmethylsulfonyl fluoride and 1 μg/ml aprotinin. Samples were then centrifuged for 20 min at 12,000 rpm at 4°C, the supernatant was collected, and protein concentration was determined by protein assay kit (Bio-Rad) using bovine serum albumin as standard. Fifty micrograms of protein samples were resolved on SDS-polyacrylamide gel electrophoresis and transferred to Hybond

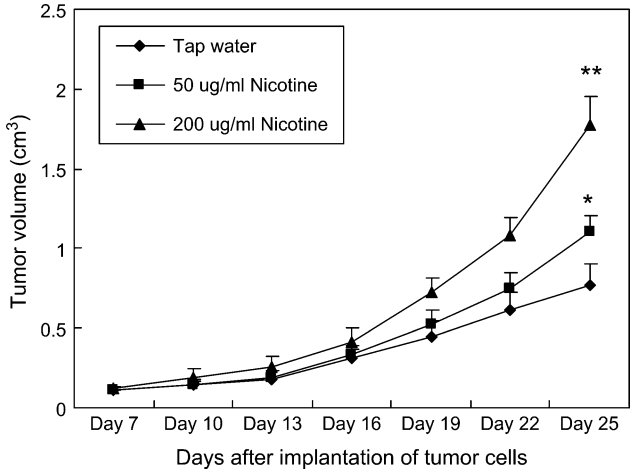

FIG. 1. Nicotine promoted tumor growth of HT-29 colon cancer xenograft in nude mice in a dose-dependent manner. Mice implanted with HT-29 colon cancer cells were randomized to receive 0, 50, or 200 μg/ml nicotine in drinking water. Tumor volume was measured 7 days after the implantation of the colon cancer cells. *$p < 0.05$; **$p < 0.005$, significantly different from the tap water–treated group.

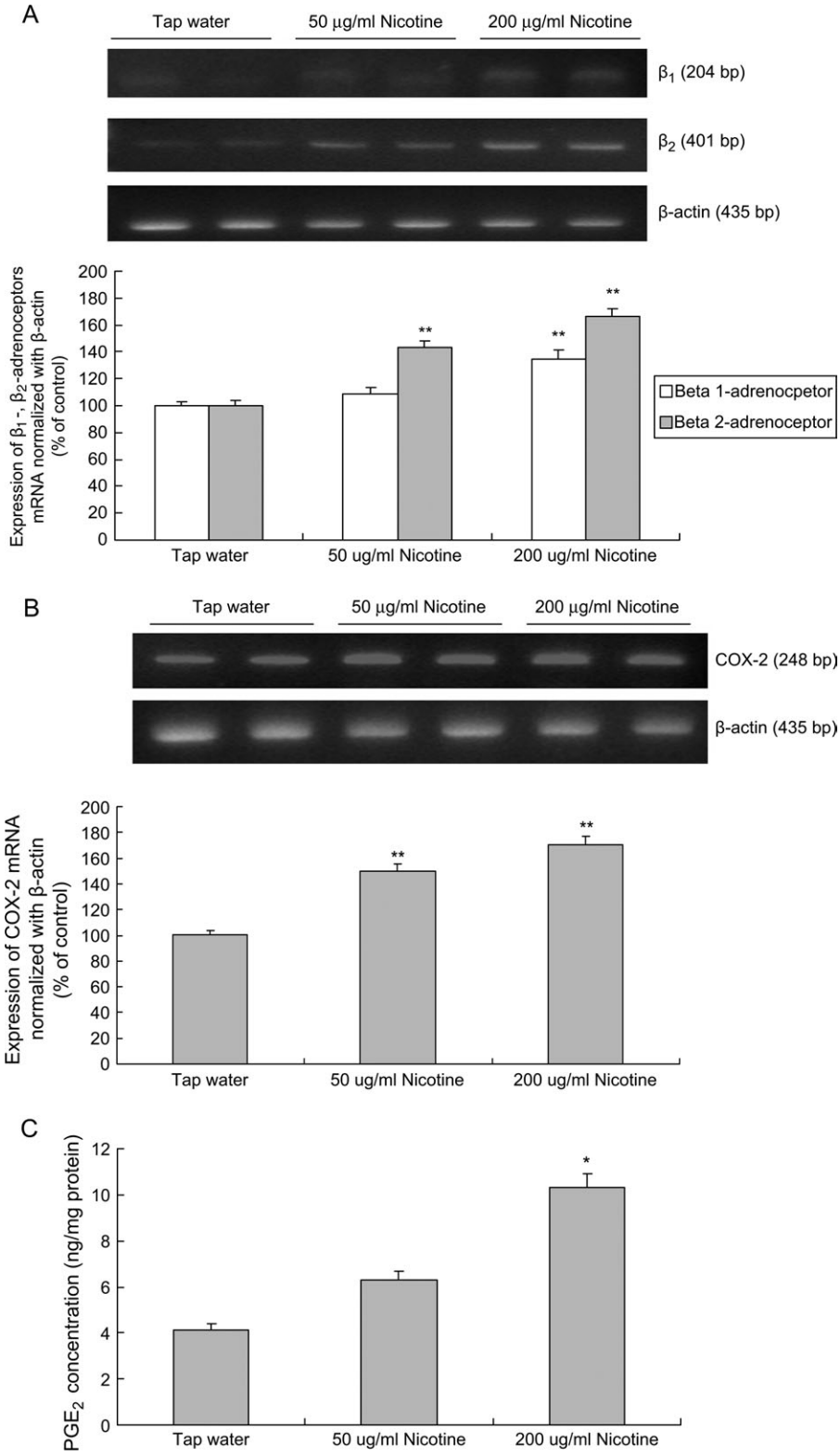

FIG. 2. Expression of β_1- and β_2-adrenoceptors (A) and COX-2 (B) mRNA in colon tumor tissues were increased in nicotine-treated groups dose dependently as revealed by RT-PCR. PGE_2 concentrations (C) were significantly elevated in 200 μg/ml nicotine–treated group. $^*p < 0.05$; $^{**}p < 0.005$, significantly different from the tap water–treated group.

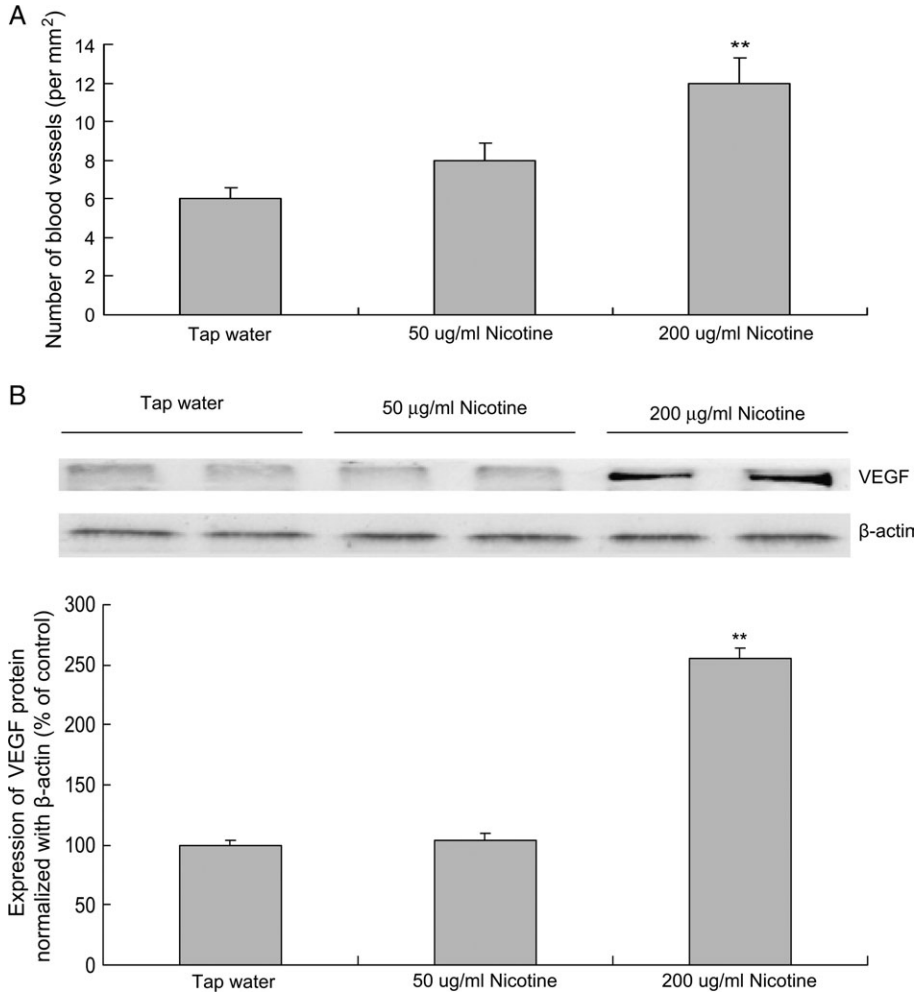

FIG. 3. Nicotine increased microvessel densities (A) in association with VEGF protein expression (B) in colon tumor tissues. Mice treated with 200 µg/ml nicotine resulted in significant increase in microvessel densities and VEGF expression. $^{**}p < 0.005$, significantly different from the tap water–treated group.

C nitrocellulose membranes (Amersham Corporation, Arlington Heights, IL). The membrane was probed with COX-2, VEGF, or β-actin antibodies overnight at 4°C and incubated for 1 h with secondary antibodies conjugated with peroxidase. The signals on the membrane were visualized by enhanced chemiluminescence (Amersham Corporation, Arlington Heights, IL) and exposed on x-ray film (Fuji Photo Film Co., Ltd, Tokyo, Japan). Quantification of the protein bands was carried out by video densitometry (Scan Marker III, Microtek, Carson, CA).

Measurement of PGE_2. Tumor tissues were homogenized on ice in an enzyme immunoassay buffer (50 mmol/l Tris–HCl [pH 7.4], 100 mmol/l sodium chloride, 1 mmol/l calcium chloride, 1 mg/ml D-glucose, and 28 µmol/l indomethacin). Samples were then centrifuged for 15 min at 10,000 rpm at 4°C. The supernatant was used for the determination of PGE_2 using enzyme immunoassay kit according to the manufacturer's instructions. Absorbance was measured at 405 nm in a microplate reader (Dynex Technologies, Microtiter Co., Chantilly, VA). A standard curve of PGE_2 concentrations (39–5000 pg/ml) was included in each assay. Protein concentration was determined as described above. The concentrations in the samples were expressed as nanogram per milligram of protein.

Immunohistochemical staining for microvessel density. The microvessels in tumors were identified by immunohistochemical staining with von Willebrand factor antibody (DAKO, Carpinteria, CA). Tissues were fixed and then followed by deparaffinization and hydration. After incubation with 0.3% H_2O_2/methanol, sections were digested with trypsin for 30 min and then blocked by the addition of normal serum in 0.05 mol/l Tris-HCl–buffered saline (pH 7.8) for 1 h. Samples were then incubated with von Willebrand factor (1:200) overnight at 4°C. The endothelial cells in blood vessels were labeled by the addition of peroxidase-conjugated streptavidin followed by 3,3'-diaminobenzidine. The number of blood vessels was expressed as the number of blood vessel per square millimeter (mm^2) in five to eight randomly selected fields (×200).

Statistical analysis. Results were expressed as the mean ± SE of at least triplicate determinations, and statistical comparisons were based on Student's *t*-test or ANOVA followed by the Turkey's *t*-test. $p < 0.05$ was considered to be significant.

RESULTS

Oral Administration of Nicotine Increased Cotinine and Adrenaline Levels in Plasma of Nude Mice

To investigate the effects of nicotine on tumorigenesis in animals, nude mice were injected s.c. with HT-29 cells and

TABLE 2

Effects of β_1- or β_2-Selective Antagonists on Final Tumor Volume (cm^3) in HT-29 Human Colon Cancer Xenograft. *$p < 0.05$; **$p < 0.005$, Significantly Different from the Tap Water–Treated Group. +$p < 0.05$; ++$p < 0.005$, Significantly Different from the Respective Control Group. ##$p < 0.005$, Significantly Different from the Nicotine-Treated Group

Treatment group	Final tumor volume (cm^3)
Tap water	0.77 ± 0.19
Tap water + 5 mg/kg Atenolol	0.77 ± 0.17
Tap water + 10 mg/kg Atenolol	0.74 ± 0.15
Tap water + 5 mg/kg ICI 118,551	0.68 ± 0.13
Tap water + 10 mg/kg ICI 118,551	0.66 ± 0.11
200 μg/ml Nicotine	1.82 ± 0.22**,++
200 μg/ml Nicotine + 5 mg/kg Atenolol	1.77 ± 0.18**,++
200 μg/ml Nicotine + 10 mg/kg Atenolol	1.63 ± 0.15*,+
200 μg/ml Nicotine + 5 mg/kg ICI 118,551	1.21 ± 0.18
200 μg/ml Nicotine + 10 mg/kg ICI 118,551	1.06 ± 0.19##

treated with nicotine (0, 100, or 200 μg/ml) in drinking water. The effects of oral nicotine administration on various parameters were examined (Table 1). The average body weight and the total drinking solution consumed by animals were not significantly different among treatment groups. Cotinine levels in plasma were significantly increased in the nicotine-treated mice (50 μg/ml, 43.2 ± 7.10 ng/ml; 200 μg/ml, 169.4 ± 12.21 ng/ml) than in the tap water–treated animals (8.7 ± 0.66 ng/ml). Adrenaline levels in plasma were significantly elevated ($p < 0.05$) in mice treated with the maximal concentration of nicotine (200 μg/ml, 19.5 ± 1.68 ng/ml) than in the tap water–treated group (7.1 ± 0.83 ng/ml).

Nicotine Promoted Tumor Growth in HT-29 Human Colon Cancer Xenograft in Nude Mice

Seven days after implantation of HT-29 colon cancer cells into nude mice, tumor size became palpable, and there was no significant difference between different treatment groups (Fig. 1). On day 25, tumor volumes in the nicotine-treated animals (50 μg/ml, 1.10 ± 0.11 cm^3; 200 μg/ml, 1.78 ± 0.17 cm^3) were significantly larger than those in the tap water–treated group (0.77 ± 0.13 cm^3).

Nicotine Increased β-Adrenoceptors, COX-2 Expression, and PGE_2 Levels in Tumor Tissues

Nicotine treatment dose dependently increased mRNA expression of β_1-, β_2-adrenoceptors, and COX-2 in tumor tissues (Figs. 2A and 2B). The levels of β_1-, β_2-adrenoceptors, and COX-2 were enhanced by 35%, 65%, and 70%, respectively, in tumor tissues of nude mice treated with 200 μg/ml nicotine in drinking water when compared to the tap water–treated group. The same dose of nicotine treatment also significantly elevated PGE_2 concentrations in tumor tissues (Fig. 2C).

Nicotine Stimulated Neovascularization in Association with Increased VEGF Expression

To further examine the effects of nicotine in promoting colon tumor growth in colon cancer xenograft, microvessel densities were determined. The number of blood vessels was significantly increased in the 200 μg/ml nicotine–treated group when compared to the tap water–treated mice (Fig. 3A). The expression of VEGF in tumor tissues corresponded well with the microvessel densities, and it was significantly increased by 1.5-fold in the 200 μg/ml nicotine–treated group (Fig. 3B). Herein, we used 200 μg/ml nicotine in drinking water for the subsequent experiments in this study.

β-Adrenoceptors Antagonists Attenuated Nicotine-Promoted Tumor Growth

To study the involvement of β-adrenoceptors in the promoting actions of nicotine on colon tumor growth and to delineate which receptor isoform mediates the process, β_1- or β_2-selective antagonist, atenolol, or ICI 118,551 (5 or 10 mg/kg) was injected i.p. thrice per week into nude mice bearing HT-29 tumor xenograft. Nicotine treatment similarly and significantly elevated cotinine and adrenaline plasma levels as shown in the experiment without β-adrenoceptor antagonist treatment. Both β_1- or β_2-selective antagonists dose dependently attenuated the stimulatory effect of nicotine on tumor growth in which β_2-selective antagonist produced a more prominent effect (Table 2).

β-Adrenoceptor Antagonists Reversed Nicotine-Stimulated COX-2 Expression and PGE_2 Levels

The results above showed that COX-2 expression was increased in the nicotine-treated group. Nevertheless, whether COX-2 expression was coupled to β-adrenoceptors was unknown. Both β_1- or β_2-selective antagonists suppressed the stimulatory action of nicotine on COX-2 expression as well as PGE_2 levels in tumor tissues in which β_2-selective antagonist produced a significant effect (Figs. 4A and 4B).

β-Adrenoceptor Antagonists Abolished Nicotine-Increased Microvessel Densities and VEGF Expression

The stimulatory effect of nicotine on microvessel densities was abolished by β_1- or β_2-selective antagonists dose dependently, in which the high dose of ICI 118,551 (10 mg/ml) produced a significant effect (Fig. 5A). VEGF is a major angiogenic factor involved in solid tumor neovascularization, and β_1- or β_2-selective antagonist treatment also reduced the

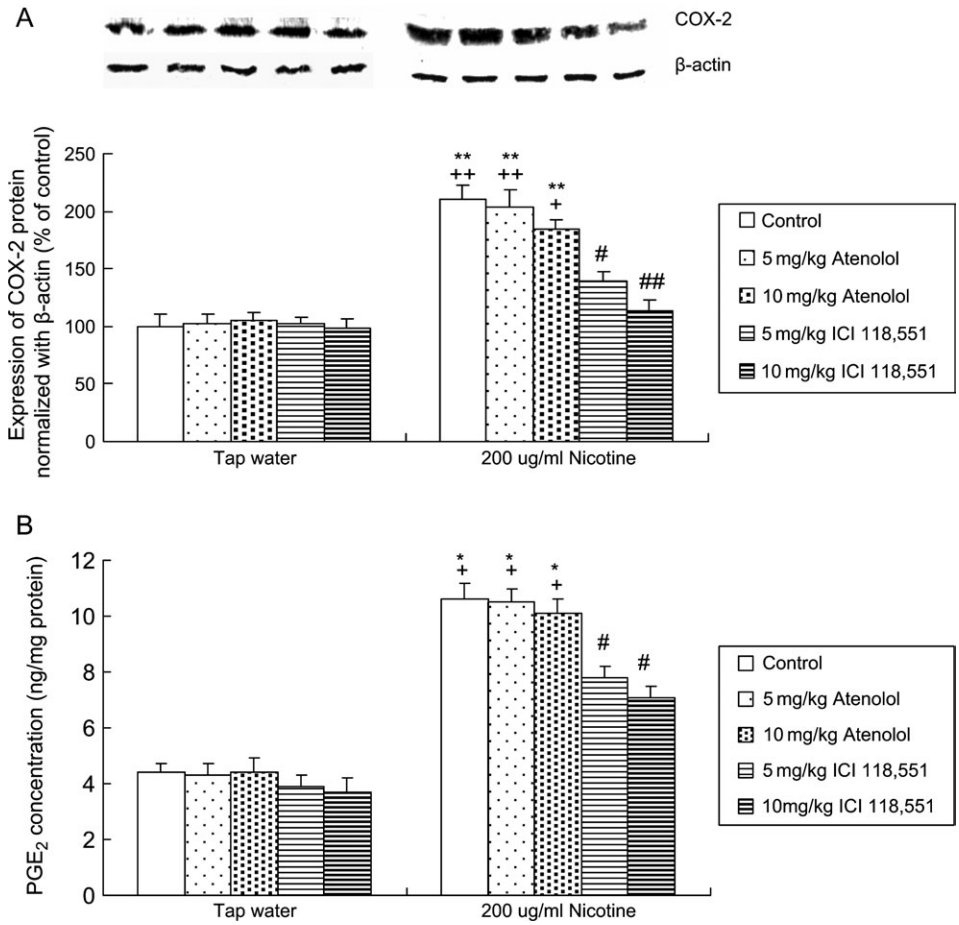

FIG. 4. Nicotine exposure increased COX-2 expression (A) and PGE_2 concentrations (B) in colon tumor tissues, and the effects were reversed by i.p. injections of β_1- or β_2-selective antagonists. $*p < 0.05$; $**p < 0.005$, significantly different from the tap water–treated group. $+p < 0.05$; $++p < 0.005$, significantly different from the respective control group. $\#p < 0.05$; $\#\#p < 0.005$, significantly different from the nicotine-treated group.

stimulatory effect of nicotine on VEGF expression in tumors (Fig. 5B).

DISCUSSION

Nicotine is the main component in tobacco smoke. In the present study, we demonstrated that oral nicotine administration promoted colon tumor growth and angiogenesis using human colon cancer xenograft model. Athymic nude mice bearing HT-29 tumor xenografts were treated with 0, 50, or 200 μg/ml nicotine for 25 days. Mice exposed to oral nicotine had a concentration-dependent elevation in plasma cotinine and adrenaline levels. This treatment also significantly increased tumor growth but did not alter the average body weight gain and the total drinking solution consumed when compared to the tap water–treated group. These findings indicate that oral administration of nicotine did not affect the normal growth of animals. In addition, oral nicotine exposure also dose dependently elevated β_1-, β_2-adrenoceptors, COX-2, and PGE_2 levels as well as microvessel densities in association with VEGF expression in tumor tissues. Cotinine is a primary metabolite of nicotine, and plasma level of cotinine is used as an indicator of nicotine exposure, which has also been used as a predictor of risk for lung cancer in smokers (Boffetta *et al*., 2006). Nicotine and cotinine have been shown to increase VEGF protein expression in endothelial cells (Conklin *et al*., 2002). In the present study, we demonstrated that nicotine *in vivo* elevated VEGF expression, which was associated with an increase in microvessel densities in colon tumor tissues. VEGF is considered as a major contributor in tumor angiogenesis, a process which is important for tumor growth and metastasis (Battegay, 1995). Work done by Natori *et al*. (2003) also supports this observation. This study showed that nicotine promoted colon tumor growth by promoting neovascularization. In other studies, nicotine treatment was found to stimulate angiogenesis and promote tumor growth of Lewis lung cancer and gastric cancer (Heeschen *et al*., 2001; Shin *et al*., 2004). Other carcinogenic actions of nicotine have also been reported. In this connection, nicotine was shown to promote other types

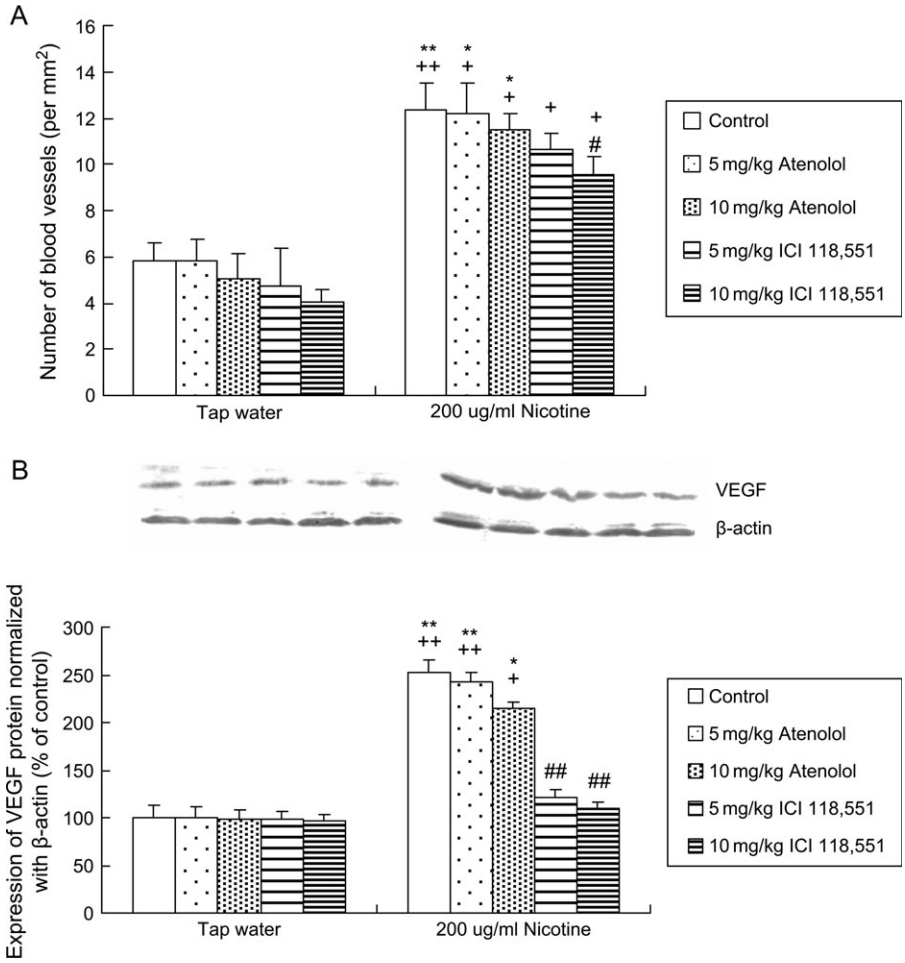

FIG. 5. Nicotine exposure increased microvessel densities (A) in association with VEGF expression (B) in colon tumor tissues and the effects were reversed by i.p. injections of β_1- or β_2-selective antagonists. $*p < 0.05$; $**p < 0.005$, significantly different from the tap water–treated group. $+p < 0.05$; $++p < 0.005$, significantly different from the respective control group. $\#p < 0.05$; $\#\#p < 0.005$, significantly different from the nicotine-treated group.

of cancer growth through the increase of proliferation (Waggoner and Wang, 1994) or inhibition of apoptosis (Heusch and Maneckjee, 1998).

Nicotine administration also elevated plasma levels of adrenaline in nude mice. Adrenaline is the most potent naturally occurring β_2-adrenoceptor agonists (Hardman *et al*., 2001). Adrenaline has been shown to stimulate VEGF expression in ovarian tumor, and the effects were reversed by β-blockers (Lutgendorf *et al*., 2003). Therefore, nicotine together with adrenaline may play a coregulatory role in the tumor growth and angiogenesis in colon cancer xenograft. In addition to that, nicotine treatment upregulated β_1-, β_2-adrenoceptors, and COX-2 mRNA expression and PGE_2 concentrations in tumor tissues in a dose-dependent manner. COX-2 is aberrantly overexpressed in colon cancer and plays an essential role in cancer progression (Eberhart *et al*., 1994). The stimulation of β-adrenoceptors has been linked to the arachidonic acid cascade, especially COX-2 expression, in various types of cancer (Cakir *et al*., 2002; Schuller and Cole, 1989; Weddle *et al*., 2001).

We further examined the involvement of β-adrenoceptors activation and its down-stream signal pathway in mediating the stimulatory actions of nicotine on colon tumor growth. Athymic mice bearing HT-29 tumor xenografts were treated with 0 or 200 μg/ml nicotine for 25 days with i.p. injection of atenolol or ICI 118,551 (5 or 10 mg/kg) thrice per week. Both β_1- or β_2-selective antagonists reversed the nicotine-induced tumor growth indicating that β-adrenoceptors could involve in this biological process. The blockade is more prominent in the ICI 118,551–treated group, suggesting that β_2-adrenoceptor plays a dominant role in mediating the tumor-promoting effect of nicotine on colon cancer. The stimulatory actions of nicotine on COX-2 expression and PGE_2 concentrations were also abolished by β_1- or β_2-selective antagonists, in which β_2-selective antagonists produced a more significant effect. Similar action was observed on tumor microvessel densities

and this corresponds well with the downregulation of VEGF expression. These data suggest that β-adrenoceptor is the upstream receptor for the actions of nicotine to stimulate COX-2, PGE_2, and VEGF expression in colon cancer xenograft. These findings signify the involvement of β-adrenoceptor and its associated genes in tumor-associated growth and angiogenesis in colon cancer. Accumulating evidence from *in vitro* and *in vivo* experimental studies also showed that nicotine could activate β-adrenoceptors, and its actions were reversed by β-blockers (Gadek-Michalska *et al*., 2002; Jin *et al*., 2004; Uchida *et al*., 2002). The direct action of nicotine on β-adrenoceptors and its stimulatory action on systemic adrenaline level would partially explain the potential tumorigenic action of nicotine on colon cancer.

There have been reports showing that intake of β-blockers can reduce cancer risk (Algazi *et al*., 2004; Perron *et al*., 2004), while others reported that β-blockers inhibit breast and prostate cancer migration (Drell *et al*., 2003; Palm *et al*., 2006). These findings along with the present experimental data strongly suggest that adrenergic modulation plays an important role in the carcinogenesis of solid tumors. The present study demonstrates for the first time that β-adrenergic activation can stimulate colon cancer cell growth *in vivo* through the upregulation of COX-2, PGE_2, and VEGF expression. All these effects can be potentiated by nicotine administration. These data suggest that blockade of β-adrenoceptor–stimulated signal pathways could have therapeutic implications for the prevention and treatment of colon cancer, especially in cigarette smokers.

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