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Research Article

Candesartan Suppresses Intestinal Carcinogenesis Partly Through Inhibition of Plasminogen Activator Inhibitor1- Expression

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Abstract

Obesity is a major cause of metabolic syndrome and is a convincing risk factor for colorectal cancer. The renin-angiotensin system (RAS) is activated in obese individuals and has been suggested to play important roles in development of hypertension and several cancers. To cast light on the significance of RAS for obesity-associated colorectal carcinogenesis, we examined the effects of an angiotensin II receptor blocker, candesartan, on colorectal carcinogenesis using obese KK-A^y mice and *Apc*-mutant Min mice. Six-week-old female KK-A^y mice were treated with 200 μg/mouse azoxymethane (AOM) once a week for 3 weeks and given 20 and 50 ppm candesartan in their drinking water, starting 2 days after the last AOM treatment. The numbers of colorectal aberrant crypt foci (ACF) and dysplastic ACF were decreased around 20% and 30%, respectively, in KK-A^y mice treated with 50 ppm candesartan compared with untreated mice. The dose of 50 ppm also reduced the number of PCNA-positive epithelial cells and the expression of c-myc in intestinal mucosa. Furthermore, mRNA expression of plasminogen activator inhibitor-1 (Pai-1) in visceral fat tissue was significantly decreased along with the reduction of visceral adipocyte size. In Min mice, formation of intestinal polyps was suppressed with a decrease of mRNA expression of Pai-1 in visceral fat tissue. These results indicated that candesartan could suppress obesity-associated colorectal carcinogenesis through improvement of Pai-1 expression.

Keywords: Angiotensin II Receptor Blocker; Intestinal Carcinogenesis; Renin-Angiotensin System; Plasminogen Activator Inhibitor-1

Abbreviations

ACF: Aberrant Crypt Foci;
AGT: Angiotensinogen;
Ang: Angiotensin;
AOM: Azoxymethane;
ARB: Angiotensin Receptor Blocker;
H&E: Hematoxylin And Eosin;
IL-6: Interleukin-6;
NF-κB: Nuclear Factor Kappa B;
PAI-1: Plasminogen Activator Inhibitor-1;
RAS: Renin-Angiotensin System;
PCNA: Proliferation Cell Nuclear Antigen;
STAT: Signal Transduction And Activators Of Transcription;
TG: triglycerides;
TNFα: tumor necrosis factor

Introduction

Obesity is a major cause of metabolic syndrome, characterized by hyperglycemia, hyperinsulinemia, dyslipidemia and hypertension, and is a risk factor for cardiovascular disease [1], type 2 diabetes [2] and colorectal cancer [3,4]. However, the mechanisms responsible for these associations, especially with regard to colorectal cancer, have yet to be fully elucidated. Adipocytokines, which are hormones / cytokines produced in adipose tissue, such as angiotensinogen (AGT), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-α (TNF-α) and adiponectin are suggested to play important roles in these disorders [5]. Moreover, dysregulation of adipocytokine production due to the hyperplastic change of adipocytes may enhance such disorders.

It has been reported that AGT, which is the precursor of angiotensin (Ang) II mainly produced in the liver, is also expressed in adipose tissue [6]. In the renin-angiotensin system (RAS), AGT-Ang I-Ang II axis is activated by renin and the angiotensin converting enzyme. Ang II elicits its biofunctions through its receptors AT1R and AT2R. Activation of RAS due to the over-expression of AGT in adipose tissue contributes to obesity-related hypertension [7].

It is now apparent that RAS is involved not only in hypertension but also in development of several cancers. Epidemiologically, long-term use of ACE inhibitors protects against lung and breast cancer [8]. It has been reported that Ang II stimulates the growth of cancer cells, including breast [9], gastric [10] and prostate cancer cells [11] through AT1R and resultant activation of ERK1/2, Nuclear factor kappa B (NF-κB) [10] and MAPK [11]. However, it remains unclear whether activation of RAS due to the hyperplastic change of adipocytes is involved in obesity-related colorectal carcinogenesis.

Previously, we reported that KK-A^y mice, characterized by obesity [12], are highly susceptible to azoxymethane (AOM)-induced colorectal carcinogenesis, with increased levels of adipocytokines, such as Pai-1, except for adiponectin, compared with lean mice [13]. Therefore, KK-A^y mice could be useful to clarify the mechanisms of obesity-related colorectal carcinogenesis and to evaluate candidate chemopreventive agents.

In the present study, to assess the possible role of activation of RAS in intestinal carcinogenesis, we investigated the impact of an angiotensin receptor blocker (ARB), candesartan, on the formation of AOM-induced aberrant crypt foci (ACF) and dysplastic ACF putative preneoplastic lesions, using obese KK-A^y mice. Most of ACF is hyperplastic lesion and dysplastic ACF are reported to be more progressive phenotype. In addition, to examine its influence on more advance stages of intestinal carcinogenesis, formation of intestinal polyps in *Apc*-mutant Min mice, showing activation of Wnt signaling and dyslipidemia status [14,15], was studied. The results showed that candesartan might have potential suppressive effects on obesity-related colorectal carcinogenesis with the improvement of Pai-1 expression in visceral fat tissue. Furthermore, the possible significance of obesity and RAS in colorectal carcinogenesis is discussed.

Materials and Methods

Animals and chemicals

Five-week old female KK-A^y/TaJcl (KK-A^y) and 6-week old female C57BL/6J-*Apc*$^{Min/+}$ (Min) mice, were purchased from CLEA Japan (Tokyo, Japan) and The Jackson Laboratory, respectively. Min mice were genotyped as previously reported [16]. The mice were acclimated to laboratory conditions for 1 week. Three to five mice were housed per plastic cage with sterilized softwood chips as bedding, in a barrier-sustained animal room, air-conditioned at 24 ± 2°C and 55% humidity, on a 12:12 hours light:dark cycle and were allowed free access to commercial diet (AIN-76A; CLEA Japan) and water. The mice were observed daily for clinical signs and body weights were measured weekly. Candesartan was kindly provided by Takeda Chemical Industries, Ltd. (Osaka, Japan).

Cell culture and chemicals

Human colon cancer cell lines, SW480 and HCT116 cells (American Type Culture Collection, Manassas, VA, USA), were cultured in DMEM medium containing 5% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT, USA) and antibiotics at 37°C in a humidified incubator with 5% CO2. PAI-1 was purchased from Merck Millipore (Billerica, MA, USA). AngiotensinII was purchased from Sigma-Aldrich Co. (St. Louis MO, USA).

Animal experimental schedule

KK-A^y mice (n=12 in each group) were treated intraperitoneally with either AOM (Nard Institute, Ltd., Amagasaki, Japan) at a dose of 200 μg/mice or the saline vehicle, once a week for 3 weeks from 6 weeks of age. Starting 2 days from the last AOM administration, the mice were given drinking water containing candesartan at concentrations of 20 and 50 ppm until the end of the experiment.

Min mice (n=10 in each group) underwent the same schedule but with concentrations of 10 and 20 ppm. The water containing candesartan was freshly prepared two times per week. Systolic blood pressure was measured at 1 week before the end of the experiment in an unanesthetized condition using a tail-cuff blood pressure monitor (MK-2000ST; Muromachi Kikai Co.,

Tokyo, Japan). All KK-A^y mice and Min mice were sacrificed at 13 and 20 weeks of age, respectively, and each colorectum of the KK-A^y mice, the intestinal tracts of Min mice, and mesenteric fat tissue of both were excised. Blood samples were collected from the inferior vena cava. The experiments were conducted according to the 'Guidelines for Animal Experiments in the National Cancer Center', and approved by the Institutional Ethics Review Committee for Animal Experimentation of the National Cancer Center.

Analysis of colorectal ACF formation in KK-A^y mice treated with or without candesartan

The excised colorectums of KK-A^y mice were filled with 10% buffered formalin, opened longitudinally, and fixed flat between sheets of filter paper in 10% buffered formalin. After fixation, each was divided into 4 segments, the proximal segment, the rectum (1.5 cm in length), and then the proximal (middle) and distal halves of the remainder. These segments were stained with 0.2% methylene blue (Merck, Darmstadt, Germany) in PBS for 10 minutes. The mucosal surface was assessed for ACF with a stereoscopic microscope, as previously reported [17].

Analysis of dysplastic ACF formation in KK-A^y mice treated with or without candesartan

After the analysis of ACF formation, the same segments were further stained with 0.2% methylene blue in PBS for 30 min and subsequently decolorized with 70% methanol with gentle shaking at room temperature for 4–6 min. The mucosal surface was then assessed for dysplastic ACF with a stereoscopic microscope as detailed earlier [18].

Analysis of intestinal polyp development in Min mice treated with or without candesartan

The intestinal tracts of Min mice were removed and separated into the small intestine, cecum and colon. The small intestine was divided into the proximal segment (4 cm in length), and then the proximal (middle) and distal halves of the remainder. All segments were opened longitudinally and fixed flat between sheets of filter paper in 10% buffered formalin. The numbers and sizes of polyps and their distributions in the intestine were assessed with a stereoscopic microscope [15].

Histological examination

The middle segment of the colorectum after analysis of colorectal ACF formation and fixed mesenteric fat tissues was sliced and routinely processed to sections stained with hematoxylin and eosin (H&E). Serial sections of colorectum were also stained immunohistochemically with antibodies against proliferation cell nuclear antigen (PCNA) (DAKO, Carpinteria, CA, USA). PCNA labeling indices were calculated as the percentages of PCNA-positive epithelial cells within a crypt at three different arbitrarily selected points in the colorectal mucosa [13]. The extent of enlargement of adipocytes was evaluated by quantification of the numbers of adipocyte nuclei observed in fields (x200) of visceral fat tissue in KK-A^y mice as previously reported [13].

Western blotting analysis

Samples from small intestinal mucosa in KK-A^y mice were lysed and sonicated in 100 mL lysis buffer [0.0625 M Tris-HCl (pH 6.8), 20% 2-mercaptoethanol, 10% glycerol, 5% SDS]. Fifty mg aliquots of protein were separated in 10% PAGE-SDS gels and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were preincubated for 1 hour with 5% Nonfat Dry Milk (Cell signaling Technology Inc., Danvers, MA, USA) in PBS. Antibodies against phosohor-p44/42 MAPK (Erk1/2) (Thr 202/Tyr 201), phospho-AMPKα (Thr 172), phospho-Akt (Ser 473) (Cell signaling Technology Inc.), cyclin D1 (Ab-3; Calbiochem, San Diego, CA, USA), c-myc (N-262; Santa Cruz Biotecnology, Inc., Santa Cruz, CA, USA) and β-actin (Sigma-Aldrich) were prepared at a 1:2000 dilution. Peroxidase-conjugated secondary antibodies for anti-rabbit IgG or anti-mouse IgG were obtained from GE Healthcare (Buckingham shire, UK). After exposure, blots were developed with ECL western blotting detection reagents (GE Healthcare).

Analysis of mRNA expression of adipocytokines in the visceral fat tissue

Visceral fat tissues of KK-A^y mice and Min mice were rapidly deep-frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated from tissue using an RNeasy® Lipid Tissue mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, and treated with DNase (Invitrogen, Carlsbad, CA, USA). One-μg total RNA in a final volume of 20 μL was used for synthesis of cDNA using an Omniscript® RT Kit (Qiagen) with an oligo (dT) primer. Real-time PCR was performed using a DNA Engine OpticonTM 2 (MJ Japan Ltd., Tokyo, Japan) with SYBR Green Real-time PCR Master Mix (Toyobo Co., Osaka, Japan). Primers for GAPDH (5'primer, TTGTCTCCTGCGACTTCA; 3'primer, CACCACCCTGTTGCTGTA), IL-6 (5'primer, ACAACCACGGCCTTCCCTACTT; 3'primer, CACGATTTCCCAGAGAACATGTG), leptin (5'primer, CCAAAACCCTCATCAAGACC; 3'primer, GTCCAACTGTTGAAGAATGTCCC), MCP-1 (5'primer, CCACTCACCTGCTGCTACTCAT; 3'primer, TGGTGATCCTCTTGTAGCTCTCC), Pai-1 (5'primer, ACAGCCTTTGTCATCTCAGCC; 3'primer, AGGGTTGCACTAAACATGTCAG), TNF-α (5'primer, TGTGCTCAGAGCTTTCAACAAC; 3'primer, GCCCATTTGAGTCCTTGATG) were employed [13,19-21]. To assess the specificity of each primer set, amplicons generated from the PCR reaction were analyzed for melting curves.

Luciferase assays for evaluation of Wnt/β catenin signaling

To examine activation of Wnt/β catenin signaling, colon cancer cell lines SW480 and HCT116 were seeded in 96-well plates (4×104 cells/well). After 24 hours incubation, cells were transiently transfected with 100 ng/well of TOPFLASH or FOPFLASH (Millipore) reporter plasmid, and 5 ng/well pGL4.73 [*hRluc/*SV40] (Promega, Madison, WI, USA) control plasmid using LIPOFECT AMINE™ 2000 Transfection Reagent (Invitrogen), and pre-cultured for 24 hours. The cells were then treated with test agents, and 24 hours later, Firefly luciferase and Renilla luciferase activity was determined by Luciferase Assay Systems and Renilla Luciferase Assay Systems (Promega), respectively. Basal luciferase activity of untreated cells was set as 1. The relative ratio of luciferase activity with each treatment was calculated from data for triplicate wells. TOPFLASH and FOPFLASH values were normalized to *Renilla*. Fold induction for each cell line was calculated as normalized relative light units of TOPFLASH divided by normalized relative light units of FOPFLASH.

Statistical analysis

The results were expressed as mean ± SD, and statistical analysis was carried out using Dunnett's multiple comparison test. In addition, a linear regression test was used. Differences were considered to be statistically significant with *P*-values less than 0.05.

Results and Discussions

Numbers of ACF in KK-A^y mice treated with AOM and candesartan

Final body weights in the 13-week-old KK-A^y mice in each group with AOM were 44.3 ± 3.0 g (mean ± SD in untreated group), 42.2 ± 3.7 g (20 ppm candesartan group) and 41.2 ± 2.9 g (50 ppm candesartan group). The systolic blood pressure of 12-week-old KK-A^y mice measured at the tail was 104.5 ± 19.2 mmHg (mean ± SD in untreated group). Treatments of 20 and 50 ppm candesartan significantly decreased the systolic blood pressure to 68.4 ± 5.3 mmHg ($p<0.05$) and 62.6 ± 11.0 mmHg ($p<0.01$), respectively.

The observed numbers of ACF and dysplastic ACF in KK-A^y mice are summarized in Figure 1 and 2, respectively. All KK-A^y mice treated with AOM had developed ACF in the colorectum at 13 weeks of age. Administration of 50 ppm candesartan had a tendency to reduce the total number of ACF per colorectum to 81% of the AOM-treated control value. No ACF were found in the colorectums of KK-A^y mice untreated with AOM irrespective of candesartan administration. There were no significant differences in the mean numbers of ACs per focus among the each group.

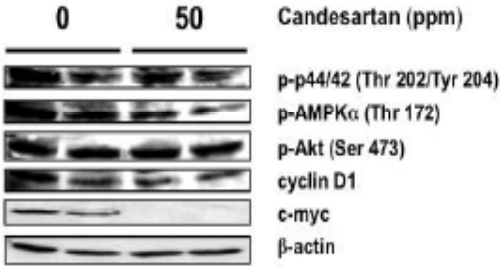

Figure 1. Western blotting for p-p44/42 (Thr 202/Tyr 204), p-AMPK-α (Thr 172), p-Akt (Ser 473), cyclin D1 and c-myc in mucosa of the small intestine of KK-A^y mice treated with or without 50 ppm candesartan. Actin was used as a loading control.

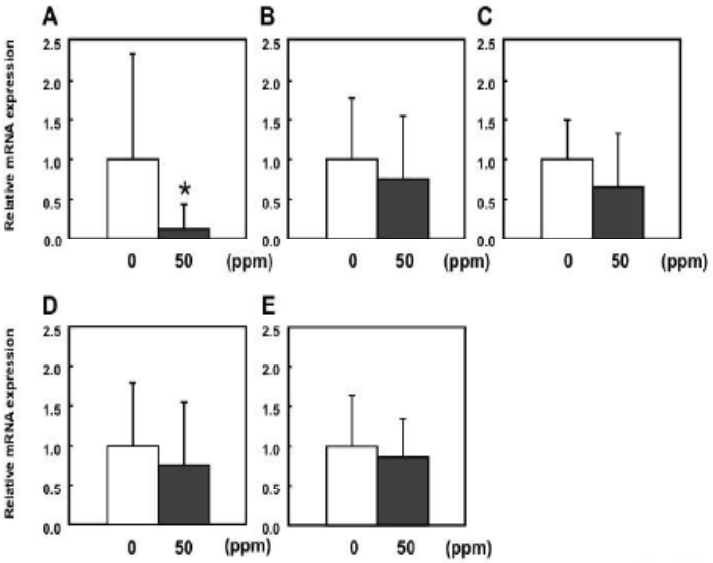

Figure 2. Relative mRNA expression of Pai-1 (A), IL-6 (B), Leptin (C), MCP-1 (D), TNF-α (E) in visceral fat tissue of KK-A^y mice treated with candesartan. Values were set at 1.0 for untreated controls and relative levels expressed as mean ± SD. * $p<0.05$ vs untreated control.

In dysplastic ACF analysis, administration of 50 ppm candesartan also had a tendency to reduce the total number of dysplastic ACF per colorectum to 68% of the AOM-treated control value. In distal segments, the number of dysplastic ACF in the mice with 50 ppm candesartan treatment was significantly decreased compared with those without candesartan treatment.

Evaluation of intestinal epithelial cell growth in KK-A^y mice treated with candesartan

To investigate the effect of candesartan on colon epithelial cell growth, similar portions of distal parts of colon were immunohistochemically stained with anti-PCNA antibody. The rates for PCNA positive cells per crypt (PCNA positive cells/total cells/crypt) in colon mucosa in KK-Ay mice treated with 50 ppm candesartan and untreated controls were 20.5 ± 10.5 % and 30.3 ± 8.8% (mean ± SD), respectively, the difference being sig-

nificant (p<0.05). The total cells per crypt in those mice were 36.8 ± 9.6 and 33.9 ± 9.6, and PCNA positive cells in those mice were 11.0 ± 3.6 and 7.1 ± 4.5, respectively, the difference not being significant.

To assess the cell growth inhibition mechanisms by candesartan, several cell growth-related molecules were analyzed by western blot using AOM-treated intestinal mucosa with or without 50 ppm candesartan. Down-regulation of expression of c-*myc* in the small intestinal mucosa of KK-A^y mice was apparent compared with that of the untreated group (Figure 1). However, there were no significant differences in the expression of phosoho-p44/42, phospho-AMPKα, phospho-Akt and cyclin D1 (Figure 1).

Effects of candesartan on the size of adipocytes and on the expression levels of adipocytokines in KK-A^y mice

The average weight of mesenteric fat in KK-A^y mice treated with 50 ppm candesartan and untreated controls was 1.50 ± 0.30 g and 1.74 ± 0.37 g (mean ± SD), respectively. The number of adipocyte nuclei per one field (x200) in visceral fat tissue of KK-A^y mice treated with 50 ppm candesartan was 107.6 ± 11.8, and that of untreated control mice was 78.3 ± 12.9 (mean ± SD, p<0.05). These results indicated that the size of adipocytes in visceral fat tissue in KK-A^y mice treated with 50 ppm candesartan were smaller than in untreated control mice.

Data for the mRNA expression levels of adipocytokines in visceral fat tissue are shown in Figure 2. The mRNA expression level of Pai-1 in KK-A^y mice treated with 50 ppm candesartan was significantly decreased compared with untreated KK-A^y mice (p<0.05). There were no significant differences in those of IL-6, leptin, MCP-1 and TNF-α between the two groups.

Among the serum adipocytokines, serum Pai-1 level was also significantly decreased in KK-A^y mice treated with 50 ppm candesartan, the concentration being 3.3 ± 0.6 ng/mL as compared with 4.2 ± 0.7 ng/mL in untreated KK-A^y mice (mean ± SD, p<0.05).

Effects of PAI-1 and angiotensin II on Wnt/β catenin signaling in human colon cancer cells

Development of dysplastic ACF is closely related to Wnt/β catenin signaling. Thus, we examined the effect of PAI-1 or AngII on β-catenin/T-cell factor transcription by β-catenin/T-cell factor transcriptional (TOP-FLASH) or FOP-FLASH control assays in SW480 and HCT116 cells. Expression of the TOP-FLASH reporter was slightly induced by PAI-1 and AngII treatment (Figure 3). Of note, expression of the FOP-FLASH reporter was unchanged.

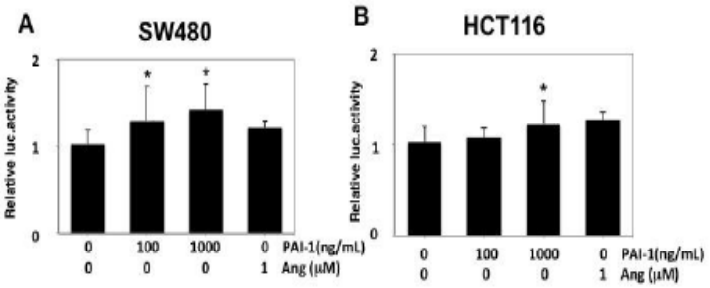

Figure 3. Effects of PAI-1 and angiotensin II on β-catenin/T-cell factor transcription. SW480 (A) and HCT116 (B) cells were seeded in 96-well plates, and transfected with TOPFLASH or FOPLASH plasmids for 48 h and then further incubated with the indicated dose of PAI-1 and 1 μM angiotensin for 24 hours. Relative luciferase activities were normalized to Renilla. Data are means ± SD, n=3. All experiments were repeated at least two times and representative results are shown.

Number of intestinal polyps in Min mice treated with candesartan

As we obtained the data that suggested a correlation between Pai-1/AngII and Wnt signaling, we next investigated the effects of candesartan on the development of intestinal polyps in Min mice, in which activation of Wnt signaling by *Apc* allele deletion plays an important role on the development of intestinal polyps. The observed numbers of intestinal polyps in Min mice are summarized in Figure 4. Administration of candesartan at the concentrations of 10 and 20 ppm significantly decreased the total number of polyps to 68.1% and 56.6%, respectively, of the value for untreated controls. The decrease in the proximal, middle and distal parts of the small intestine was by 42.2%, 51.0% and 80.0% with 10 ppm and by 48.4%, 40.8% and 65.2% with 20 ppm, respectively. Treatment with candesartan did not affect the numbers of colon polyps.

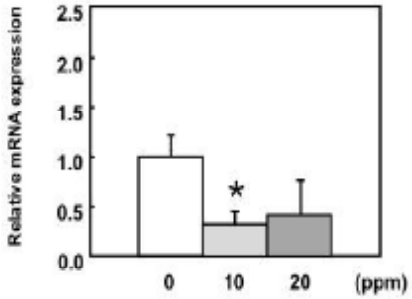

Figure 4. Relative mRNA expression of Pai-1 in visceral fat tissue of Min mice treated with candesartan. Values were set at 1.0 in untreated controls and relative levels expressed as mean ± SD. * p<0.05 vs untreated control.

Effects of candesartan on expression levels of Pai-1 in visceral fat tissue of Min mice

Data for the mRNA expression levels of Pai-1 in visceral fat tissue of Min mice are presented in Figure 4. The mRNA expression level of Pai-1 in Min mice treated with 10 ppm

candesartan was significantly decreased compared with that in untreated Min mice ($p<0.05$). The mRNA expression level of Pai-1 in Min mice treated with 20 ppm candesartan also showed a tendency to decrease compared with untreated Min mice.

In the present study, 50 ppm candesartan treatment suppressed the formation of AOM-induced colorectal ACF and dysplastic ACF in KK-A^y mice. Furthermore, proliferation of colorectal epithelial cells was reduced. Ang II via AT1R activation is known to contribute to not only regulation of systemic and local blood pressure but also cell proliferation. In several prostate cancer cells, LNCaP, DU145 and PrSC cells, activation of AT1R increased MAPK activation, signal transduction and activators of transcription (STAT) activation, and enhanced cell proliferation [11,22]. Additionally, in isolated rat hepatocytes, Ang II induced expression of c-fos, c-mos, c-myc via AT1R activation [23]. Furthermore, the experiments conducted in the present study revealed expression of c-*myc* to be remarkably down-regulated in intestinal mucosa of KK-A^y mice with candesartan treatment. Thus, the observed suppression of cell proliferation and ACF formation might have been partially due to down-regulated expression of c-*myc* in KK-*Ay* mice.

Because candesartan suppressed formation of dysplastic ACF more effectively than total ACF formation in KK-*Ay* mice with AOM treatment, it was expected to suppress neoplastic lesions at the more advanced stages of colon carcinogenesis. In fact, administration of candesartan suppressed the formation of intestinal polyps in Min mice. Considering the finding that candesartan suppressed the expression of c-myc and the formation of intestinal polyps of Min mice, which are known to have lost Apc function and demonstrate activation of β-catenin signaling, it is conceivable that candesartan might have the potential to inhibit the Wnt/β-catenin pathway. Recently, it was reported that Ang II induces GSK3β phosphorylation and promotes β-catenin nuclear translocation and stabilization in an Akt- and GSK3β-dependent manner in neonatal rat cardiomyocytes [24]. We also obtained data showing activation of the Wnt/β-catenin pathway by AngII or PAI-1 treatment in human colon cancer cells.

Moreover, examination of the effects of candesartan on adipogenesis and the expression of adipocytokines demonstrated the size of adipocytes in visceral fat tissue in KK-A^y mice treated with candesartan to be smaller than in untreated control mice with resultant reduction of certain adipocytokines. Further, the serum AGN in KK-A^y and Min mice was significantly elevated as compared with wild type C57BL/6J mice (data not shown). Adipose RAS has been indicated to contribute to regulation of adiposity by its stimulation of preadipocyte differentiation and adipocyte lipogenesis [25,26]. Indeed, loss of Ang II production by targeted deletion of the Agt gene dramatically reduces adipose mass though adipocyte hypotrophy [27]. Furthermore, candesartan has been demonstrated to decrease fat mass due to hypotrophy of epidermal and retroperitoneal fat tissue with decreased size of adipocytes [28] in the rat, consistent with the present findings.

In addition, Ang II regulates the production of adipocytokines such as leptin [29], IL-6 [30], Pai-1 [31] and adiponectin [32]. In this study, candesartan treatment decreased the mRNA expression level of Pai-1 in visceral fat tissue of both KK-A^y (Figure 1) and Min mice (Figure 4). Pharmacological inhibition of Pai-1 by its specific inhibitor, SK-116 and SK-216, suppresses the formation of intestinal polyps in Min mice, in which high serum levels of Pai-1 are observed [21]. Thus, decreased expression of Pai-1 by candesartan treatment might have been involved in the suppression of intestinal carcinogenesis in the present study.

It is well established that PAI-1 is induced by c-*myc*, triglycerides (TG), very low-density lipoprotein (TG-rich lipoprotein), transforming growth factor α, tumor suppressor p53 and NF-κB [33-35]. In addition, it is reported that PAI-1 is a direct downstream target of Wnt/β-catenin signaling in HKC human proximal tubular epithelial cells [36]. In the present study, as the serum levels of TG were not significantly altered by candesartan treatment (data not shown), down-regulation of c-myc or Wnt/β-catenin signaling might have been involved in suppression of Pai-1 expression in visceral fat tissue.

Conclusion

We demonstrated suppressive effects of candesartan on AOM-induced ACF formation in KK-A^y mice and intestinal polyp formation in Min mice with inhibition of Pai-1 expression in visceral fat tissue, suggesting that candesartan may be a candidate chemopreventive agent for obesity-related colorectal cancer.

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