(–)-Epigallocatechin Gallate Suppresses Azoxymethane-Induced Colonic Premalignant Lesions in Male C57BL/KsJ-*db*/*db* Mice

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Abstract Obesity and diabetes mellitus are risk factors for colon cancer. The activation of the insulin-like growth factor (IGF)/IGF-IR axis plays a critical role in this carcinogenesis. (–)-Epigallocatechin gallate (EGCG), the major constituent of green tea, seems to have both antiobesity and antidiabetic effects. This study examined the effects of EGCG on the development of azoxymethane-induced colonic premalignant lesions in C57BL/KsJ-*db*/*db* (*db*/*db*) mice, which are obese and develop diabetes mellitus. Male *db*/*db* mice were given four weekly s.c. injections of azoxymethane (15 mg/kg body weight) and then they received drinking water containing 0.01% or 0.1% EGCG for 7 weeks. At sacrifice, drinking water with EGCG caused a significant decrease in the number of total aberrant crypt foci, large aberrant crypt foci, and β-catenin accumulated crypts in these mice, all of which are premalignant lesions of the colon. The colonic mucosa of *db*/*db* mice expressed high levels of the IGF-IR, phosphorylated form of IGF-IR (p-IGF-IR), p-GSK-3β, β-catenin, cyclooxygenase-2, and cyclin D1 proteins, and EGCG in drinking water caused a marked decrease in the expression of these proteins. Treating these mice with EGCG also caused an increase in the serum level of IGFBP-3 while conversely decreasing the serum levels of IGF-I, insulin, triglyceride, cholesterol, and leptin. EGCG overcomes the activation of the IGF/IGF-IR axis, thereby inhibiting the development of colonic premalignant lesions in an obesity-related colon cancer model, which was also associated with hyperlipidemia, hyperinsulinemia, and hyperleptinemia. EGCG may be, therefore, useful in the chemoprevention or treatment of obesity-related colorectal cancer.

Colorectal cancer is a serious health care problem worldwide. Recent evidence indicates that obesity and related metabolic abnormalities, including hyperglycemia, hyperlipidemia, and hyperleptinemia, are associated with an increased incidence of colorectal cancer (1–5). Obesity is the main determinant of insulin resistance and hyperinsulinemia, which is also a key factor for the development of colorectal cancer (6). Insulin itself stimulates the growth of colon cancer cell lines while also promoting colorectal cancer tumor growth in animal model (7–10). Insulin resistance also causes alterations in the insulin-like growth factor (IGF)/IGF-IR axis, which is involved in the development, progression, and metastatic potential of colorectal cancer (11–13). These reports suggest that hyperinsulinemia may be the essential consequence of obesity that increases the risk of colorectal cancer and, therefore, agents improving insulin resistance and targeting the IGF/IGF-IR axis might be able to inhibit the development of obesity-related colorectal cancer.

Numerous studies indicate that green tea catechins can exert anticancer and/or chemopreventive effects in various organ sites, including the colorectum (14, 15). Recent studies also show that green tea catechins possess antiobesity and antidiabetic properties (16). Experimental studies in rodents have shown that treatment with green tea or its constituents result in a significant reduction in body weight and, therefore, improve hyperlipidemia, hyperinsulinemia, and hyperleptinemia (17–19). These results suggest that long-term consumption of green tea is beneficial for the suppression of obesity and might reduce the risk of obesity-associated diseases, including the development of colorectal cancer. However, detailed studies whether green tea catechins can prevent the development of obesity-associated colorectal cancer have not yet been conducted.

Among the green tea catechins, (–)-epigallocatechin gallate (EGCG), the major biologically active component of green tea, is the most potent polyphenolic compound with respect

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to inhibiting proliferation and inducing apoptosis in cancer cells (14, 15). For instance, EGCG inhibits cell growth, induces apoptosis, and inhibits the expression of cyclooxygenase (COX)-2 by inhibiting the activation of epidermal growth factor receptor family of receptor tyrosine kinases (RTK) and their downstream signaling molecules, such as extracellular signal-regulated kinase and Akt proteins, in human colorectal cancer cells (20, 21). In addition, EGCG also inhibits the activation of IGF-IR, which belongs to a separate family of RTKs, in human colorectal cancer and liver cancer cells (22, 23).

The development of azoxymethane-induced aberrant crypt foci (ACF) and β-catenin accumulated crypts (BCAC), both of which are putative precursor lesions for colonic adenocarcinoma (24, 25), have been previously reported to be enhanced in C57BL/KsJ-*db/db* (*db/db*) mice with hyperinsulinemia and hyperleptinemia, when compared with *db*/+ or +/+ mice (26). This is a useful preclinical animal model to examine the possible underlying mechanisms on how chemopreventive agents inhibit the development of obesity-related colorectal cancer because *db/db* mice are more susceptible to azoxymethane as described above, and the chemopreventive effect by specific agent is more apparent in these mice than in *db*/+ or +/+ mice (27). In view of the evidence that insulin resistance plays an important role in the development of colorectal cancer via activation of the IGF/IGF-IR axis (1, 2, 11–13), and the previous studies indicating that EGCG inhibits activation of this axis in human cancer cells (22, 23), in the present study we examined in detail the effects of EGCG on the development of colonic premalignant lesions, ACF and BCAC, in *db/db* mice initiated with azoxymethane.

Materials and Methods

Animals, chemicals, and diets

Four-week-old male homozygous *db/db* mice were obtained from Japan SLC, Inc. All mice were maintained at Gifu University Life Science Research Center, according to the Institutional Animal Care Guidelines, and were housed in plastic cages with free access to drinking water (tap water with or without EGCG) and a basal diet, CRF-1 (Oriental Yeast Co., Ltd.). A colonic carcinogen azoxymethane was purchased from the Sigma Chemical Co. EGCG was obtained from Mitsui Norin Co., Ltd.

Experimental procedure

A total of 45 male *db/db* mice were divided into five groups (Fig. 1). At 5 wk of age, mice in groups 3 through 5 were s.c. injected with azoxymethane (15 mg/kg body weight) once a week for 4 wk. Group 3 was given tap water throughout the experiment. Groups 4 and 5 were given tap water containing 0.01% EGCG and 0.1% EGCG, respectively, with free access to drinking for 7 wk, starting 1 wk after the last injection of azoxymethane. Group 2 was given 0.1% EGCG alone for 8 wk. Group 1 served as an untreated control. At the termination of the study (16 wk of age), mice were sacrificed by CO_2 asphyxiation to analyze the number of colonic ACF and BCACs.

Identification of ACF and BCACs

The presence of ACF and BCACs was determined according to the standard procedures that are routinely used in our laboratory (27, 28). After the colons were fixed flat in 10% buffered formalin, the mucosal surface of the colons were stained with methylene blue and then the number of ACF were counted under a light microscope. After counting the ACF, the distal parts (5 cm from anus) of the colon were cut to count the number of BCACs. To identify intramucosal lesions BCACs, the distal part of the colon was embedded in paraffin, and then a total of 20 serial sections (4-μm thick each) per colon were made by an *en face* preparation (27, 28).

Histopathology and immunohistochemistry

Immunohistochemical analyses for β-catenin or IGF-IR were done using the labeled streptavidin-biotin method (LSAB kit; DAKO) or stain system kit (Zymed), respectively (26–28). Three serial sections were made from the paraffin-embedded tissue blocks. Two sections were subjected to H&E staining for histopathology and β-catenin immunohistochemistry to count the number of BCACs. The other section was for IGF-IR immunohistochemistry. Immunoreactivity was regarded as positive if apparent staining was detected in the cytoplasm and/or nuclei to determine the BCACs (27, 28).

Protein extraction and Western blot analysis

Total proteins were extracted from the scraped mucosa from the remaining colon of the azoxymethane-treated mice (groups 3 through 5) and equivalent amounts of proteins (40 μg/lane) were examined by a Western blot analysis (21–23, 29). The primary antibodies for IGF-IR, p-IGF-IR, p-GSK-3β, COX-2, and glyceraldehyde-3-phosphate dehydrogenase were described previously (23, 29). Anti–β-catenin antibody was obtained from Transduction Laboratories. Anti–cyclin D1 antibody was from Santa Cruz Biotechnology, Inc. An antibody to glyceraldehyde-3-phosphate dehydrogenase served as a loading control.

Clinical chemistry

At sacrifice, blood samples were collected to measure the serum concentrations of insulin, leptin, triglyceride, total cholesterol, IGF-I, and IGFBP-3. The levels of insulin, IGF-I, and IGFBP-3 were measured in the mice from groups 3, 4, and 5, and other measurements were determined in all the groups. The serum triglyceride and cholesterol were assayed as described previously (27). The serum insulin, leptin, IGF-I, and IGFBP-3 were determined by an enzyme immunoassay according to the manufacturer's protocol (R&D Systems).

Statistical analysis

The results were given as mean ± SD and were analyzed using the GraphPad Instat software program version 3.05 (GraphPad Software) for Macintosh. Differences between groups were analyzed by one-way ANOVA or, as required, by two-way ANOVA. When ANOVA showed a statistically significant effect ($P < 0.05$), comparisons of each experimental group with the control group was made using Dunnett's test, which corrects for multiple comparisons. The differences were considered significant when two-sided P was <0.05.

Results

General observations

Figure 1 shows the growth curves of all groups during this experiment. The body weight gain of the mice that received 0.1% EGCG alone (group 2) was slightly larger than that of group 1 (untreated), but the difference was not significant. The body weight gains of groups 3 (azoxymethane alone), 4 (azoxymethane plus 0.01% EGCG), and 5 (azoxymethane plus 0.1% EGCG) were smaller than those of groups 1 and 2. However, the values were comparable among the groups 3 through 5. The body weights of the mice in all groups at the end of the study are listed in Table 1. The mean body weights in the azoxymethane-treated groups (groups 3 through 5) were significantly lower than those of group 1 ($P < 0.05$). This finding is consistent with our previous report and might be associated with the toxicity of azoxymethane because db/db mice are more susceptible to azoxymethane toxicity due to lower maximum tolerated dose of this carcinogen when compared with +/+ mice (26). However, the values were comparable among

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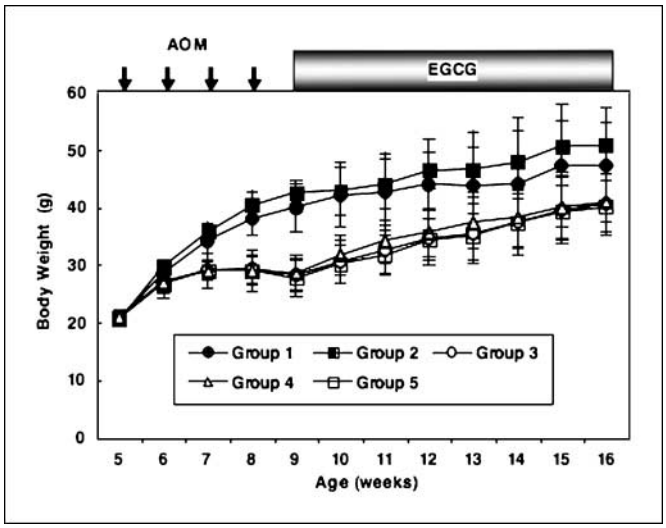

Fig. 1. Growth curves of the experimental mice. *Points*, mean; *bars*, SE. For experimental protocol in each group, see Table 1.

the groups 3 through 5 (azoxymethane alone versus azoxymethane plus EGCG–treated groups). There was no significant difference in the weights of the liver and kidney (per body weight) among these groups. A histopathologic examination also revealed no alterations, thus suggesting the absence of toxicity of EGCG in the liver and kidney of the mice in groups 2, 4, and 5 (data not shown).

Effects of EGCG on azoxymethane-induced ACF and BCAC formations in db/db mice

Table 1 summarizes the number of total ACF, large ACF with four or more crypts, and BCACs (Fig. 2A and B) in the mice of all groups. ACF and BCACs developed in the colons of all the mice that received azoxymethane (groups 3-5), but not in the colons of the mice in groups 1 and 2 that did not receive azoxymethane. In comparison with the azoxymethane alone group (group 3), treatment with a high dose (0.1%) of EGCG (group 5) significantly inhibited the development of total ACF (33% reduction, $P < 0.05$), large ACF (60% reduction, $P < 0.001$), and BCACs (57% reduction, $P < 0.01$). Similarly, administration of a low dose (0.01%) of EGCG (group 4) also reduced the numbers of total ACF (31% reduction, $P < 0.05$), large ACF (40% reduction, $P < 0.001$), and BCACs (50% reduction, $P < 0.01$).

Effects of EGCG on serum levels of triglyceride, total cholesterol, and leptin in db/db mice

The serum concentrations of triglyceride, total cholesterol, and leptin are listed in Table 2. The EGCG administration in the drinking water caused a significant decrease in the serum levels of triglyceride in groups 2 ($P < 0.01$), 4 ($P < 0.01$), and 5 ($P < 0.01$), irrespective of azoxymethane exposure. A significant decrease of triglyceride was observed in group 3 ($P < 0.01$) compared with group 1, and this finding might be explained by the body weight loss caused by azoxymethane toxicity (Table 1) because obesity is the metabolic stressor most frequently associated with hypertriglyceridemia (30). Among the azoxymethane-treated groups, the serum levels of total cholesterol also significantly decreased when the mice were given EGCG at doses of 0.01% ($P < 0.01$) and 0.1% ($P < 0.01$). The mean concentration of triglyceride and total cholesterol of group 3 were significantly lower than that of group 1 ($P < 0.01$). The serum leptin levels of groups 4 and 5 were also significantly smaller than those of group 3 ($P < 0.01$ for each comparison).

Immunohistochemical analysis of IGF-IR in BCACs

Recent studies indicate that the activation of the IGF/IGF-IR axis stabilizes and activates the Wnt/β-catenin pathway, which is involved in colorectal carcinogenesis (31, 32). Therefore, the expression of IGF-IR was examined in BCACs by immunohistochemical analysis (Fig. 2). Immunohistochemistry of IGF-IR revealed a strong reactivity in the cytoplasm and, in part, the nuclei of atypical cells in all of BCACs, when compared with their surrounding cryptal cells (Fig. 2C). This finding suggests that up-regulation of the IGF/IGF-IR axis and the accumulation of β-catenin might be associated with obesity-related colorectal carcinogenesis.

Table 1. Effects of EGCG on azoxymethane-induced ACF and BCAC formations in *db/db* mice

Group no.	Treatment	No. mice	Body weight (g)	Length of colon (cm)	Total no. of ACFs/colon	Total no. of large ACFs*/colon	Total no. of BCACs/cm^2
1	None	6	48.5 ± 9.9[†]	12.5 ± 0.9	0	0	0
2	0.1% EGCG	6	50.9 ± 3.8	12.0 ± 0.6	0	0	0
3	AOM alone	11	40.6 ± 5.4[‡]	12.3 ± 0.7	83.2 ± 7.2	33.5 ± 5.3	13.2 ± 8.5
4	AOM + 0.01% EGCG	11	41.0 ± 5.7[‡]	12.0 ± 0.6	57.1 ± 8.2[§]	20.1 ± 4.5[‖]	6.6 ± 3.5[¶]
5	AOM + 0.1% EGCG	11	40.2 ± 4.4[‡]	12.4 ± 0.5	55.9 ± 10.3[§]	13.5 ± 4.6[‖]	5.7 ± 2.6[¶]

Abbreviation: AOM, azoxymethane.
*"Large ACFs" are ACFs with four or more aberrant crypts.
[†]Mean ± SD.
[‡]Significantly different from group 1 ($P < 0.05$).
[§]Significantly different from group 3 ($P < 0.05$).
[‖]Significantly different from group 3 ($P < 0.001$).
[¶]Significantly different from group 3 ($P < 0.01$).

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Effects of EGCG on the serum levels of insulin, IGF-I, and IGFBP-3 and on the expression levels of IGF-IR, p-IGF-IR, p-GSK-3β, β-catenin, COX-2, and cyclin D1 proteins in azoxymethane-treated db/db mice

The effects of EGCG on the serum levels of insulin, IGF-I, and IGFBP-3 and on the activation of IGF-IR protein were next examined because hyperinsulinemia and up-regulation of IGF/IGF-IR axis play a role in obesity-related colorectal cancer development (Fig. 2; refs. 6, 11–13, 26). We found that drinking water containing EGCG caused a significant decrease in the serum levels of both insulin and IGF-I (Fig. 3A and B). On the other hand, drinking with 0.1% EGCG caused a significant increase in the level of IGFBP-3 in azoxymethane-treated mice (Fig. 3C).

As shown in Fig. 3D, a Western blot analyses showed that drinking water with EGCG markedly decreased the levels of IGF-IR and the phosphorylated (i.e., activated) form of IGF-IR (p-IGF-IR) proteins in the colonic mucosa of azoxymethane-treated mice. In addition, there was also a marked decrease in the expression levels of the phosphorylated (i.e., inactivated) form of GSK-3β (p-GSK-3β), β-catenin, COX-2, and cyclin D1, a downstream molecule in the Wnt/β-catenin signaling pathway (33), after drinking water containing EGCG (Fig. 3D). The finding that EGCG caused a decrease in the expression of COX-2 protein seems to be of interest because an increase in this protein expression has been reported to play a significant role in colorectal cancer development and therefore might be one of the targets for chemoprevention of colorectal cancer (34).

Discussion

The present study clearly indicated that EGCG administration in drinking water effectively suppressed the development of putative precursor lesions (ACF and BCACs) of colonic adenocarcinoma, thus suggesting the inhibitory effects of EGCG on the early phase of obesity-related mouse colon carcinogenesis initiated with azoxymethane (Table 1). In addition, this suppressing effect of EGCG was associated with the improvement of hyperlipidemia (Table 2) and hyperinsulinemia (Fig. 3A). Although green tea consumption is not associated with decreased risk of colorectal cancer in general epidemiologic studies (35), our findings may suggest that EGCG might effectively prevent colorectal cancer development, at least, in humans with high risk for developing colorectal cancer, such as obese people.

What is the target of EGCG for its chemopreventive effects on obesity-related cancers? A previous study indicated that EGCG suppressed biosyntheses of lipids and cell proliferation in human breast cancer MCF-7 cells by inhibiting the epidermal growth factor receptor/phosphatidylinositol 3-kinase/Akt signaling pathway (36). EGCG seems to down-regulate adipocyte differentiation by inhibiting activation of extracellular signal-regulated kinase protein (37). With respect to targets at RTKs and its downstream signaling pathways, EGCG also inhibits activation of the epidermal growth factor receptor family of RTKs and its downstream signaling molecules, including extracellular signal-regulated kinase and Akt proteins, thereby inhibiting the growth of colon cancer cells (15, 20, 21). These findings suggest that EGCG exerts both anticancer and antiobesity effects, at least in part, by inhibiting the activation of some types of RTKs and their downstream molecules. We are now trying to reveal evidence that EGCG prevents obesity-related colorectal carcinogenesis by improving such metabolic abnormalities as hyperlipidemia and hyperinsulinemia, while focusing on the effects of EGCG with regard to specific RTKs in an ongoing study.

IGF-IR is also a membrane-associated RTK. It is widely appreciated that the IGF/IGF-IR system is involved in colorectal carcinogenesis and might play a critical role as a molecular target with respect to the prevention and treatment of colorectal cancer (11–13). Ealey et al. (11) showed that both IGF-I and insulin are important promoter of colon cancer development using liver-specific IGF-I–deficient mice. Blockade of the IGF/IGF-IR axis by soluble IGF-IR inhibits IGF-I–induced activation of Akt and growth of colon cancer xenografts *in vivo* (38). The results of the current study indicate that EGCG inhibits the activation of IGF-IR by decreasing the serum levels of the ligand for this receptor, IGF-I, but also increases the levels of IGFBP-3, which can sequester IGF-I and thereby inhibits its activity as an agonist (Fig. 3B and C; refs. 12, 13). Similar effects by green tea catechins are also reported in a prostate cancer chemoprevention study using transgenic mice (39). The finding that a low concentration (0.01%) of EGCG is sufficient to decrease the serum levels of IGF-I (Fig. 3B) might be explained by the direct effect of EGCG on the transcriptional activity of this molecule. Namely, EGCG directly inhibits the expression of IGF-I/2 mRNAs by inhibiting the Ras/mitogen-activated protein kinase and phosphatidylinositol 3-kinase/

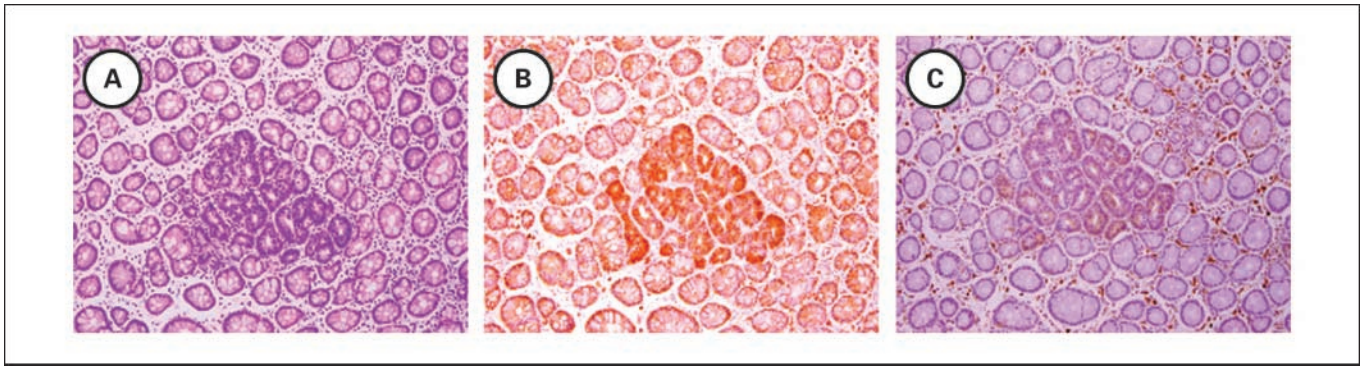

Fig. 2. Histopathology and immunohistochemical expression of β-catenin and IGF-IR in BCACs. *A*, a representative photograph of BCACs induced by azoxymethane in *db*/*db* mice (H&E staining). The epithelium had basophilic cytoplasm and hyperchromatic nuclei. *B*, immunohistochemistry of β-catenin protein in BCACs. The localization of the accumulated β-catenin protein is apparent in the cytoplasm and nucleus of atypical cryptal cells. *C*, immunohistochemical pattern of IGF-IR protein in BCACs. Strong cytoplasmic expression of the IGF-IR protein is observed in BCACs.

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Table 2. Serum triglyceride, total cholesterol, and leptin levels of the experimental mice

Group no.	Treatment	No. mice	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	Leptin (ng/mL)
1	None	6	556.2 ± 27.7*	207.2 ± 22.5	270.0 ± 13.9
2	0.1% EGCG	6	353.4 ± 49.9†	204.2 ± 4.9	248.8 ± 21.0
3	AOM alone	11	244.8 ± 33.8†	176.4 ± 18.8†	273.8 ± 7.2
4	AOM + 0.01% EGCG	11	146.2 ± 39.4‡	131.8 ± 13.6‡	228.0 ± 8.8‡
5	AOM + 0.1% EGCG	11	158.6 ± 39.8‡	127.4 ± 16.1‡	224.3 ± 12.3‡

*Mean ± SD.
†Significantly different from group 1 ($P < 0.01$).
‡Significantly different from group 3 ($P < 0.01$).

Akt signaling pathways in human colorectal cancer and liver cancer cells (22, 23). EGCG also increased the expression of IGFBP-3 protein in these cancer cells. However, the production of IGFBP-3 might be a secondary effect because EGCG indirectly induces this protein by controlling the expression of other molecules, including transforming growth factor-β and matrix metalloproteinase-7 and matrix metalloproteinase-9 (22). Therefore, a high concentration (0.1%) of EGCG might be required to increase the serum levels of IGFBP-3 in the current study (Fig. 3C).

Among the IGF-IR–related downstream molecules, GSK-3β, which is phosphorylated by phosphatidylinositol 3-kinase/Akt, is considered as a key kinase for colorectal cancer development because inactivation of GSK-3β leads to dissociate the adenomatous polyposis coli/axin/β-catenin complex and cytosolic β-catenin accumulation (40). The stability of β-catenin protein caused by IGF-I stimulation results in an enhanced transcriptional activity of target genes in combination with the inhibitor of GSK-3β (31). Free accumulated β-catenin translocates into nucleus and forms a complex with transcription factor "T-cell factor," thereby activating the transcription of target genes, including cyclin D1, and thus contributes to colorectal cancer progression (33). These reports might explain the current finding that the expression of the IGF-IR protein was relatively strong in the cytoplasm of atypical cells in BCACs compared with the surrounding cryptal cells (Fig. 2C), and that the p-IGF-IR, p-GSK-3β, β-catenin, and cyclin D1 proteins were constitutively overexpressed in the colonic mucosa of azoxymethane-treated mice (Fig. 3D).

In addition, recent studies have revealed that COX-2, one of the main mediators in the inflammatory signaling pathway, and its product prostaglandin E_2 (PGE_2), both of which are involved in colorectal cancer development, also stimulate the β-catenin/T-cell factor–mediated pathway in colorectal cancer development (41, 42). PGE_2 increases the p-GSK-3β and consequently accumulates β-catenin, thereby activating the β-catenin/T-cell factor–dependent transcription and increasing the expression of cyclin D1 in colon cancer cells (43). In the present study, blockade of IGF/IGF-IR axis by EGCG caused a decrease in the p-GSK-3β, β-catenin, cyclin D1, and COX-2 proteins in the colonic mucosa (Fig. 3D). EGCG also inhibits the expression of COX-2 and production of PGE_2 both in colorectal cancer cells (22) and in an inflammation-related mice colon carcinogenesis (29). Such findings seem to be significant because obesity-related insulin resistance is associated with a state of chronic inflammation, thus promoting carcinogenesis in certain tissues, including the colon (44, 45).

Leptin is considered to be involved in intestinal carcinogenesis. However, the data showing whether leptin inhibits or promotes the carcinogenesis remain contradictory. Leptin inhibited azoxymethane-induced precancerous lesions in the rat colon (46). Leptin also did not promote the growth of colon cancer xenografts in nude mice or intestinal tumorigenesis in Apc(Min/+) mice (47). On the other hand, in the present study, EGCG decreased the serum leptin level (Table 2) and this is regarded as one of the anticarcinogenic mechanisms of EGCG. In addition, we recently reported that the serum leptin level in mice of colitis-related colorectal cancer model induced by azoxymethane plus dextran sulfate sodium was six times higher than that in untreated mice. In this study, Nobiletin, a citrus flavonoid, abolished colonic malignancy and notably decreased the serum leptin level by 75% (48). These findings, therefore, suggest that a higher serum leptin level exerts tumor promotion effect, at least in part, in obesity- and inflammation-related colorectal cancer.

We should emphasize that not only a high (0.1%) but also a low (0.01%) concentration of EGCG similarly decreased the development of colonic premalignant lesions (Table 1) by improving the obesity-related metabolic abnormalities to the same extent (Table 2; Fig. 3). Similar results which show that drinking low as well as high doses of EGCG could inhibit the development of adenocarcinoma to the same extents are also observed in inflammation-related mouse colon carcinogenesis (29). These reports indicate that a low dose of EGCG (0.01%) is sufficient to prevent the development of colon tumor and suggest the possibility that EGCG dose thus can be further decreased, although the feeding protocol of EGCG at a high dose (0.1%) mimics an approximate consumption of six cups of green tea per day by an average adult human and has been used in mice in many prior chemopreventive studies (14, 39, 49). These findings, therefore, might be more significant when considering the clinical use of this agent because a lower dose is more acceptable for administration to patients.

Finally, when checking the potency of ACF reduction by many chemopreventive agents in the Chemoprevention Database,[5] that of EGCG was below the average (average database potency around 2.0, compared with EGCG 1.48 = 83.2/57.1 or

[5] http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html

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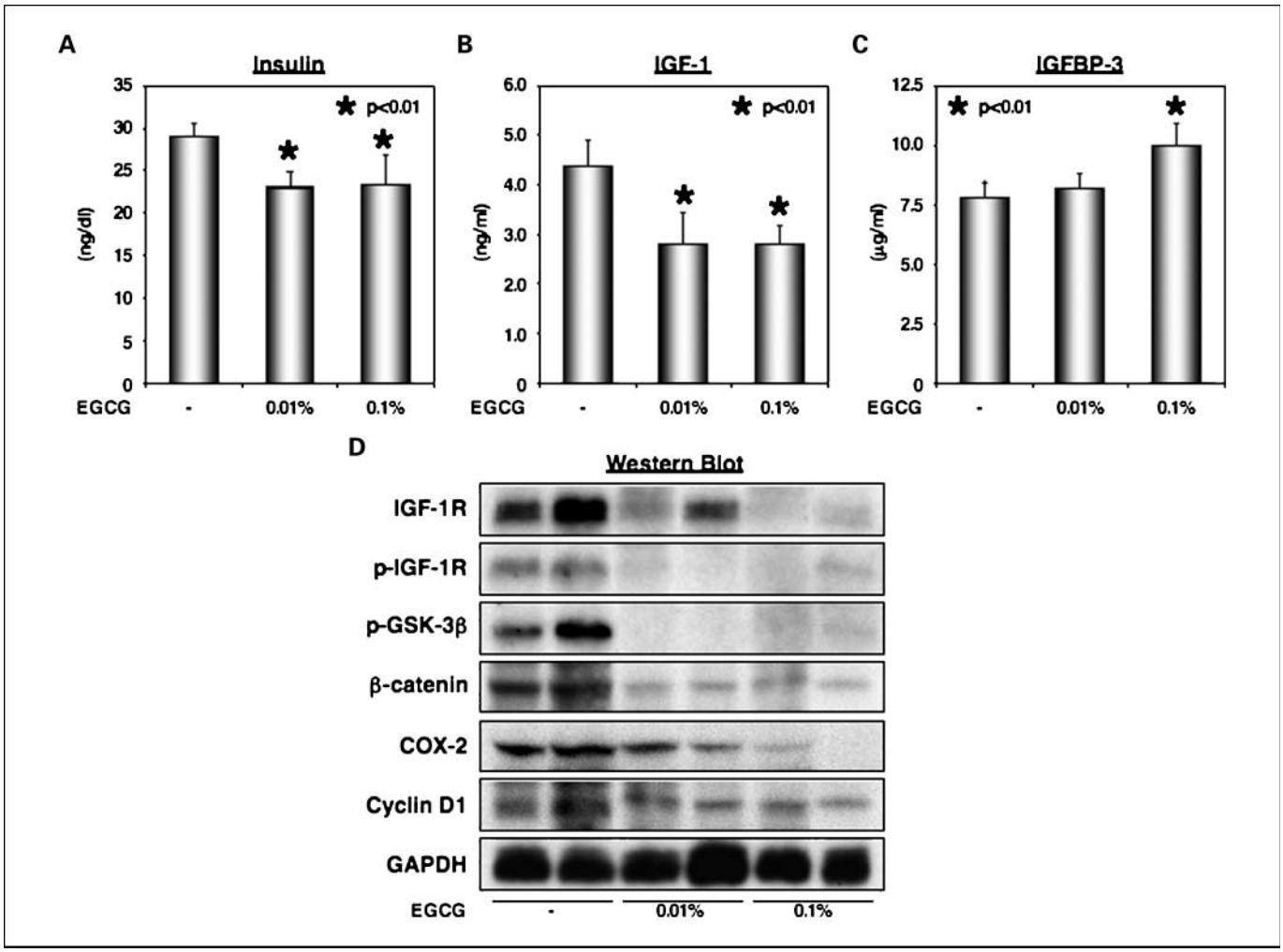

Fig. 3. The effect of EGCG on serum levels of insulin, IGF-I, and IGFBP-3 and on activation of the IGF-IR and its downstream signaling molecules in azoxymethane-treated *db/db* mice. The serum concentrations of insulin (*A*), IGF-I (*B*), and IGFBP-3 (*C*) were measured by an enzyme immunoassay. The asterisks indicate a significant difference ($P < 0.01$) between the control azoxymethane-treated group and the EGCG-treated groups. *Bars,* SD of triplicate assays. *D,* total proteins were extracted from the scraped colonic mucosa of azoxymethane-treated mice and equivalent amounts of proteins were examined by a Western blot analysis, as described in Materials and Methods. Two lanes represent protein samples from two different mice in each group (groups 3–5). Repeat Western blots gave similar results.

83.2/55.9; see Table 1). However, we should evaluate the inhibitory effects of EGCG with regard to the actual development of colonic tumors. In conclusion, the prevention of colorectal cancer by targeting the dysregulation of energy homeostasis might be one of the promising strategies for obese patients who are at increased risks of colorectal cancer. EGCG seems to be a potentially effective and critical candidate for this purpose because this agent can exert a depressant effect on the IGF/IGF-IR and COX-2/PGE_2 axes, and both of these axes are critical targets for colorectal cancer chemoprevention (12, 13, 34).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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