Table 1: Summary of animal models with dyslipidemia and cancer high susceptibility.

Animal	Strain	Age (week-old)	Serum TG (mg/dL)	Treatment	Tumor	Reference
Mouse	Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$)	12	~600	—	Intestinal adenoma	[59]
	Min (C57BL/6-$Apc^{Min/+}$)	15	~400	—	Intestinal adenoma	[59, 60]
	KK-A^y	19	481	AOM	Colon cancer	[61]
	ICR	20	159	AOM + DSS	Colon cancer	[62]
Syrian golden hamster	—	6	300	BOP	Pancreatic cancer	[63]

Table 2: Summary of tumor suppressive effects of LPL inducers in animal models.

Agent	Dose	Animal model	Value to the untreated control group	Reference
Pioglitazone	200 ppm	Apc^{1309}	67%	[59]
	1600 ppm	Min	9%	[60]
	800 ppm	BOP-treated hamster	40%	[63]
NO-1886	800 ppm	Min	42%	[65]
Indomethacin	10 ppm	Min	25%	[66]

desaturase-1, β-oxidation genes like *acyl-CoA oxidase* and *carnitine palmitoyl transferase 1*, and gluconeogenesis genes, exemplified by *phosphoenolpyruvate carboxykinase*, demonstrated no variation from wild-type mouse expression.

Obese KK-A^y mice were found to be highly susceptible to azoxymethane- (AOM-) induced colorectal aberrant crypt foci (ACF) and colorectal carcinoma development compared to lean C57BL/6J mice [61]. Surprisingly, colorectal carcinomas developed within a very short-term period, 19 weeks, after AOM injection. The number of total ACF in KK-A^y mice was around 70/mouse and almost 8 times higher than that in lean C57BL/6J mice. The incidences of adenomas and adenocarcinoma were 84% and 88%, respectively, in KK-A^y mice, far higher than the 8% and 4% in C57BL/6J values. KK-A^y mice exhibit abdominal obesity, hypertriglyceridemia, and hyperinsulinemia at the time of ACF and tumor development. At 13 weeks of age, the average serum levels of TG, total cholesterol, and free fatty acids of KK-A^y mice undergoing AOM treatment were 484.1 mg/dL, 101.6 mg/dL, and 1,796 mEq/L, respectively (Table 1). It is interesting that hepatic *LPL* mRNA levels were also suppressed in KK-A^y mice compared with C57BL/6J mice. Moreover, serum proinflammatory adipocytokines, such as IL-6, leptin, and plasminogen activator inhibitor-1 (Pai-1), were elevated. Importantly, expression of proinflammatory adipocytokine mRNAs such as for IL-6, leptin, monocyte chemotactic protein (MCP)-1, Pai-1 and TNF-α was significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Tanaka et al. have developed a novel colitis-related colorectal carcinogenesis model, using AOM plus dextran sodium sulfate (DSS), a colitis-inducing agent [64]. In this model (AOM + 2% DSS in ICR mice), numerous colorectal adenocarcinomas occur within a short-term period and the serum TG levels demonstrate increase to about 134, 175 and 159 mg/dL at 5, 10, and 20 weeks, respectively [62] (Table 1).

Injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) into Syrian golden hamsters is known to induce pancreatic ductal adenocarcinomas, with a histology very similar to typical human pancreatic ductal adenocarcinomas. Moreover, associated genetic mutations, that is, K-*ras* point mutations and *p16* aberrant methylation/homozygous deletions, are found in common in both hamster and human lesions. Interestingly, Syrian golden hamsters exhibit a hypertriglyceridemic state, almost 300 mg/dL at 6 weeks of age, even when not fed a high-fat diet [63] (Table 1). Also, in the case of this animal model, a low activity of LPL could be one of the causes of hypertriglyceridemia, activity of this enzyme in the liver being only 20% and 30%, respectively, of the values in C57BL mice and F344 rats.

5. Tumor Suppressive Effects of LPL Inducers

Pioglitazone, {(±)-5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride}, is a potent peroxisome proliferator-activated receptor (PPAR)γ ligand with a weak binding affinity for PPARα. In the promoter region of the *LPL* gene, there exists a PPRE, and pioglitazone treatment successfully induced LPL expression in the liver and intestinal epithelial cells in *Apc*-deficient mice. The total numbers of polyps in the groups treated with 100 and 200 ppm pioglitazone in the Apc^{1309} were reduced to 67% of the value in the untreated control group [59] (Table 2). With another *Apc*-deficient model, Min mice given 100–1600 ppm pioglitazone for 14 weeks showed decrease of intestinal polyps to 63–9% of the control number [60] (Table 2 and Figure 1).

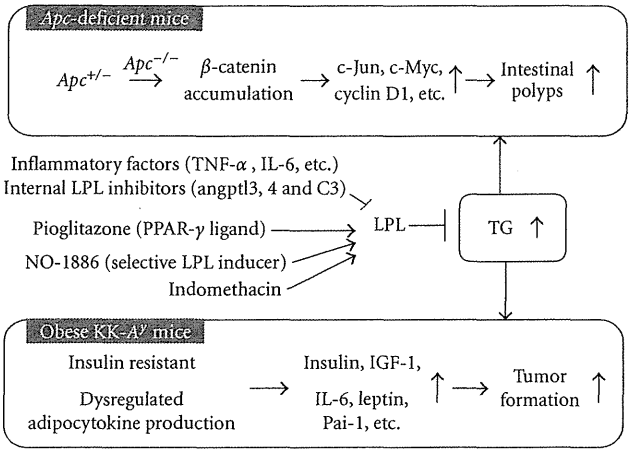

FIGURE 1: Involvement of triglycerides in animal intestinal carcinogenesis models. Angptl-3,4: angiopoietin-like protein-3,4; IGF-1: insulin like growth factor-1; IL-6: interleukine-6; LPL: lipoprotein lipase; Pai-1: plasminogen activator inhibitor-1; PPAR: peroxisome proliferator-activated receptor; TG: triglyceride; TNF-α: tumor necrosis factor-α.

Pioglitazone possesses other functions rather than just simply inducing LPL, such as causing cell growth arrest and apoptosis. Thus, data regarding LPL selective inducers are necessary for determining the relationship between hypertriglyceridemia and intestinal carcinogenesis. NO-1886, 4-[(4-bromo-2-cyanophenyl)carbamoyl] benzylphosphonate, chemically synthesized at Otsuka Pharmaceutical Factory [67] is one useful tool for clarifying this issue. Using a reporter gene assay, NO-1886 demonstrated no PPAR agonistic activity, unlike bezafibrate and pioglitazone [68].

Administration of 400 and 800 ppm NO-1886 also significantly decreased the total number of intestinal polyps to 48% and 42% of the untreated control value, respectively, in Min mice, along with causing marked increase in *LPL* mRNA levels in the liver and the small intestine. Moreover, treatment with NO-1886 also significantly decreased the numbers of colon polyps [65] (Table 2, Figure 1).

In the case of BOP-treated hamsters, pioglitazone has been demonstrated to improve hyperlipidemia and suppress ductal adenocarcinoma development. The incidences of ductal adenocarcinoma in the BOP plus 800 ppm pioglitazone and BOP alone groups were 38% and 80%, and the multiplicities were 0.55 and 1.37, respectively [63] (Table 2). Expression levels of hepatic LPL mRNA were elevated by treatment with 800 ppm pioglitazone. Moreover, quantitative real-time RT-PCR assays demonstrated almost 1.7-fold higher mRNA levels of LPL than that of pioglitazone-nontreated hamsters.

Indomethacin is a conventional nonsteroidal anti-inflammatory drug which has long been clinically employed to improve inflammation. It has demonstrated potent chemopreventive activity against intestinal tumor development in animal models, and a clinical trial in FAP patients also showed reduction in intestinal polyp development [69, 70]. We earlier reported that indomethacin suppresses intestinal polyp formation in Min mice together with ameliorating the hyperlipidemic state by regulating LPL, other lipid metabolic factors and inflammatory pathways [66]. Reduction of serum TG levels was 90% in Min mice with 10 ppm indomethacin treatment and higher than that with 400 ppm pioglitazone (83%) observed in our other previous study [59, 60]. The PPARγ agonistic activity of indomethacin is reported to be 50 times weaker than that of troglitazone, a well-established PPARγ agonist [71]. These results indicate that functions other than agonistic activity of indomethacin are responsible for its strong lipid-lowering effects (Figure 1).

6. Involvement of LPL in Inflammation, Obesity, and Others

6.1. LPL and Inflammation and Apoptosis. In addition to the lipid modifying function of LPL, two different mechanisms might be involved in *LPL* influence on carcinogenesis. The first involves anti-inflammatory action of LPL. It has been reported that LPL suppresses TNF-α- and interferon (IFN)-γ-evoked inflammation-related gene expression in endothelial cells through inactivation of transcription factor nuclear factor kappa B (NF-κB) [72]. Conversely, TNF-α, IFN-γ, IL-1β, IL-6, and leukemia inhibitory factor (LIF) decrease LPL activity.

It is well known that cyclooxygenase-2 (COX-2) is markedly elevated in human colon cancers, in AOM-treated rats, and in intestinal polyps of *Apc*-deficient mice. COX-2 is in fact thought to play important roles in both cancer cell proliferation and angiogenesis. Experiments conducted to clarify the mechanisms of NO-1886 effects on colon carcinogenesis revealed that the expression levels of mRNA for COX-2, in DLD-1 human colon cancer cells, were reduced under conditions of TGFα stimulation. On the other hand, there was no obvious change in the mRNA levels for COX-1 and inducible nitric oxide synthase (iNOS). The results obtained by RT-PCR analysis were also confirmed by

β-gal reporter gene assay in DLD-1 cells [65]. Consistent with the *in vitro* data, administration of 400 and 800 ppm NO-1886 reduced COX-2 mRNA levels in normal parts of small intestine of Min mice at 20 weeks of age [65]. In addition, NO-1886 ameliorates and induces regression of experimental steatohepatitis through increasing LPL activation and suppression of proinflammatory agents, such as TNF-α, IL-6, and COX-2 [73]. Recently, mice lacking *angiopoietin-like protein family 4* (*Angptl4*), which is the inhibitor of LPL, showed a severe and lethal phenotype characterized by fibrinopurulent peritonitis, ascites, intestinal fibrosis, and cachexia in response to a saturated fat diet [74].

The second mechanism is modification of the apoptosis pathway by LPL activation. Phosphatase type 2Cβ activation by unsaturated fatty acids has been demonstrated to induce apoptosis [75]. Unlike ester bodies of fatty acids, free fatty acids have cytotoxic effects *in vitro* and the products produced by hydrolysis of plasma TG may be implicated in such an apoptotic effect.

6.2. LPL and Obesity. Given the importance of LPL for lipid metabolism, its activity would be expected to be intimately involved in obesity effects and development of the metabolic syndrome. A large number of studies in rodents and humans have revealed that obesity results in increased LPL activity in adipose tissue [15, 35, 76–78]. Interestingly, LPL is regulated in opposite directions in adipose tissue and muscle. Feeding increases adipose LPL activity with a corresponding decrease in muscle LPL activity [35, 79]. Exercise stimulates LPL activity in the muscle and leads to increase fatty acid oxidation [80]. In an animal study, NO-1886 suppressed high-fat diet-induced fat accumulation in rats due to the increase of muscle LPL activity [81].

7. Conclusion

Targeting LPL activity or expression levels for development of reagents against cancer seems particularly challenging, because LPL is expressed ubiquitously and plays essential roles in maintaining homeostasis in the body. Data from LPL homozygous knockout mice, which die within one day of birth, underline its importance. However, appropriate suppression of serum TG levels could be achieved by using drugs, even if the number of selective inducers of LPL is limited. Thus, it might be important to develop selective LPL inducers or search for agents focusing on the aspect of "drug repositioning" to obtain the tools for investigating correlation between LPL and cancer. It should be borne in mind that LPL is inhibited by intrinsic factors, such as angptl3, angptl4, and C3 (Figure 1). These could clearly be candidate target molecules for development of LPL inducers. Considering that LPL activity has impact on obesity and metabolic syndrome, its targeting may also affect the regulation of adipocytokines, which may also be involved in carcinogenesis. Further investigations are warranted to clarify the importance of LPL and to accumulate evidence as to the worthiness as a target for cancer chemopreventive and chemotherapeutic agents.

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Role of adipocytokines in colorectal carcinogenesis

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Abstract

The metabolic syndrome and obesity-associated cancers are increasing in Asian countries in accordance with Westernization of lifestyle. Excessive-accumulation of visceral adipose tissue causes insulin resistance, dyslipidemia and adipocytokine dysbalance, and these factors are suggested to be involved in cancer promotion. In this review, we focus on involvement of dysbalance of three adipocytokines, adiponectin, plasminogen activator-1 and leptin, in colorectal carcinogenesis, and of the potential for cancer prevention by targeting these adipocytokines.

Introduction

The metabolic syndrome is caused by metabolic abnormalities due to excessive accumulation of visceral adipose tissue and is characterized by obesity, hyperlipidemia, type 2 diabetes and hypertension. Recently, this syndrome has attracted much interest as a risk factor for several cancers, including colorectal cancer, which constitute so-called "obesity-associated cancers". The metabolic syndrome and such obesity-associated cancers are extremely common in Western countries, and they are currently increasing in Eastern countries as well. Although the mechanisms underlying how the metabolic syndrome is linked with carcinogenesis are not fully understood yet, it is clear that factors such as insulin resistance, dyslipidemia and subsequent adipocytokine dysbalance may be involved in the promotion of carcinogenesis. Elucidation of the biological significance of changes in adipocytokine

levels is particularly important given their potential as important targets for chemoprevention of cancer. In this review, we focus on our recent findings for three adipocytokines, adiponectin, plasminogen activator inhibitor-1 (PAI-1) and leptin, and their relevance to carcinogenesis and cancer prevention.

Involvement of adiponectin in colorectal carcinogenesis

Adiponectin was first discovered as an adipocyte complement-related protein of 30 kDa, secreted from adipose tissue (1) and present at high levels in plasma (range, 3-30 μg/mL) as a multimer. Levels of both adiponectin in plasma and messenger RNA (mRNA) in adipose tissue are inversely correlated with body mass index and whole-body adipose mass (2, 3). Furthermore, a decrease in plasma adiponectin levels is associated with insulin resistance, type 2 diabetes, and coronary artery disease (4-6).

There are two different adiponectin receptors, designated as AdipoR1 and AdipoR2 (7). Full-length adiponectin binds with highest affinity to AdipoR2 (8) and cleaved adiponectin, known as the globular form (gAcrp30) (9), binds with highest affinity to AdipoR1 (7). Physiological functions of adiponectin are elicited through these receptors, stimulating AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPARα), respectively (10,11).

Decrease in the level of plasma adiponectin is significantly associated with increased risk of various cancers, including colorectal cancer (12, 13). Polymorphism of two adiponectin-related genes, ADIPOQ and ADIPOR1, which code adiponectin and AdipoR1 respectively, have been associated with risk for insulin resistance. However, how low adiponectin be involved in cancer risk/progression is not well understood but animal studies using *Apc*-deficient *Min* mice ($Apc^{Min/+}$), a model of familial adenomatous polyposis (FAP), have provided pointers regarding colorectal carcinogenesis. *Min* mice show a 2- or 3-fold increase in the total number of intestinal polyps compared to those of adiponectin-wild *Min* mice in both males and females at the ages of 9 and 12 weeks (14). Adiponectin-deficient C57BL/6J mice treated with azoxymethane (AOM) demonstrated increased incidences and multiplicities of colorectal tumors, again with gene dosage-dependence. Moreover, administration of a high-fat diet enhanced aberrant crypt foci (ACF) formation and tumor development in the colon of adiponectin deficient C57BL/6J mice compared with normal diet (15). It is conceivable that abolished signaling from AdipoR1 could enhance cell growth and it is known that activation of AMPK is

of fibrosis (pathological formation of connective tissue). Presumably, lower PAI-1 levels would slow down fibrinolysis and conversely accelerate fibrin degradation.

PAI-1 is mainly produced by the endothelium and hepatocytes, but is also secreted by other cell types, such as in adipose tissue so that it is recognized as one of the adipocytokines. Present at increased levels with obesity and the metabolic syndrome, it has been linked to the occurrence of thrombosis in affected patients. PAI-1 is known to be induced by triglyceride (TG), very low-density lipoprotein (TG-rich lipoprotein), transforming growth factor β (TGFβ), various growth factors, tumor suppressor p53 and nuclear factor kappa B (NFκB) (25-28), all of which are plausibly involved in carcinogenesis. In this context, it is of interest that changes in PAI-1 expression have been shown in a number of cancers. For example, PAI-1 levels and a polymorphism have been prospectively validated as markers of poor prognosis for breast, ovarian, renal and bladder, and testicular cancers (29-36).

On the surface, it seems counterintuitive that increased levels of a proteolytic inhibitor like PAI-1 might be associated with a worse prognosis in patients with cancer. However, despite its anti-proteolytic activity, it has been reported that PAI-1 promotes cancer invasion and metastasis (37). The answer to the question of 'How?' is most likely is not related to the direct inhibitory action of PAI-1 on uPA. Molecular mechanisms are not fully established, but PAI-1 could modulate cell proliferation and stimulate angiogenesis (38-43). Importantly, cancer invasion and angiogenic activity were found to be abolished in Pai-1-deficient mice (44).

There is evidence that serum PAI-1 concentration may be a reliable indicator of a poor prognosis in colorectal cancer. The mean level of PAI-1 increases during the transformation of normal colonic epithelium (42-43, 45-49) and one study demonstrated a 10-fold increase in human colonic adenocarcinoma compared to normal colonic epithelium. The same authors also demonstrated an intermediate level of PAI-1 for adenomas (50). Several studies have demonstrated that higher levels of PAI-1, but not PAI-2, are associated with large tumors and a worse prognosis with colorectal cancer. Patients with a PAI-1 level in their colon tumors of < 3.10 ng/mg protein had a 75% 3-years survival, in sharp contrast to the rate of only 12% seen with a PAI-1 level > 3.10 ng/mg ($p = 0.006$)

It should be mentioned here that PAI-1 may also be upregulated in neoplastic lesions of the human colon due to a polymorphism (51). The PAI-1 promoter 4G/5G polymorphism, in which the 4G allele is associated with high PAI-1 expression, may

decreased with adiponectin deficiency in colon epithelial cells. AMPKα activation through AdipoR1 inhibits Akt followed mammalian target of rapamycin (mTOR) inactivation (11, 15). In immortalized colon epithelial cell culture, it was shown that addition of adiponectin blocked leptin-induced cell proliferation (16), presumably through actions on the leptin-activated phosphatidylinositol-3-kinase (PI3K)/Akt signal pathway known to contribute to cell survival, cell growth, and the cell cycle leading to carcinogenesis (17). Thus, the Akt pathway is possibly a major pathway involved in the protective effect of adiponectin against colon carcinogenesis. In primary cell culture, fibroblasts from adiponectin$^{-/-}$ C57BL/6J mice are resistant to apoptosis through expression of Bcl-2 (14, 18).

Adiponectin is known to act as a regulator of other adipocytokines and under starvation conditions stimulates AMPK in the hypothalamus to promote food intake and inhibit leptin activation (19). In peripheral tissues, such as skeletal muscle, adiponectin activates AMPK, insulin receptor substrates-1 and fatty acid transport protein-1, to stimulate fatty acid combustion and glucose intake, these being inhibited by tumor necrosis factor α (TNFα) activation. Thus, it is assumed that adiponectin deficiency affects other adipocytokine production *in vivo*, with consequent affects on intestinal tumorigenesis. Adiponectin-deficient *Min* mice exhibit increase in serum Pai-1 levels with the adiponectin gene dosage (14), in agreement with the tendency for elevation observed with adiponectin-deficiency at the age of 55 weeks in C57BL/6J mice (14). Treatment with an AMPK activator also reduced hepatic Pai-1 mRNA levels in *Min* mice, in line with earlier reports (20, 21). Thus, it is conceivable that PAI-1 expression levels are usually depressed by adiponectin through AdipoR1 receptor activity. Adiponectin and PAI-1 are thus considered to be key molecules involved in obesity-associated cancers.

Involvement of PAI-1 in colorectal carcinogenesis

PAI-1 is the principal inhibitor of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), exerting its influence by direct binding. In fibrinolysis, the physiological breakdown of blood clots, tPA and uPA activate plasminogen to produce plasmin through serine protease activity, so PAI-1 is also termed a serine protease inhibitor (serpin) protein (gene name; SERPINE1) (22-24). The relevant substrate for plasmin is fibrin, but plasmin can also activate cytokines and matrix metalloproteinases, these further influencing the fibrotic process.

Under inflammatory conditions in which fibrin is deposited in tissues, PAI-1 appears to play a significant role in the progression

influence the development of aggressive fibromatosis in FAP patients.

In colorectal cancer, obesity and hyperlipidemia are known to increase tumor risk. In our experiments, *Min* mice develop intestinal polyps along with high serum TG levels up to 10-fold those observed in wild-type mice (52). We further found that serum Pai-1 levels in the 15-week-old male *Min* mice were eight times higher than in wild-type mice and hepatic Pai-1 mRNA levels were 11-fold increased. Immunostaining for Pai-1 was also strong in small intestinal epithelial cells of *Min* mice. Thus, it is conceivable that PAI-1 is one of the factors that might explain linkage between hyperlipidemia and intestinal tumorigenesis.

Administration of a PAI-1 inhibitor, SK-216, at 25, 50 and 100 ppm doses in the diet for 9 weeks reduced serum Pai-1 levels and hepatic Pai-1 mRNA levels of *Min* mice compared to the wild-type levels. Moreover, *Min* mice receiving SK-216 at 50 and 100 ppm exhibited significantly reduced total numbers of intestinal polyps, to 64 and 56% of the untreated group value, respectively. Serum TG levels were also decreased by 43% at the dose of 100 ppm. In addition, administration of 50 ppm SK-116, another PAI-1 inhibitor, for 9 weeks similarly reduced serum Pai-1 levels and total numbers of intestinal polyps to 70% of the untreated group value (53). These results indicate that Pai-1 induction associated with hypertriglyceridemia may contribute to intestinal polyp formation with *Apc* deficiency, and PAI-1 could thus be a novel target for colorectal chemopreventive agents (54).

Involvement of leptin in colorectal carcinogenesis

Leptin, a 16-kDa protein produced by the *ob* gene, plays an important role in regulation of food intake and energy balance. Thus, a good correlation of serum leptin levels is observed with the percentage of body fat, values being markedly elevated in obese individuals (55-57). The effects of leptin on peripheral tissue are mediated through binding to its receptor (Ob-R) leading to activation of NFκB, Erk1/2, PI3K/Akt and JAK/STAT signaling (58). All the pathways exert roles in cell survival and proliferation.

The published effects of leptin on the development of colorectal cancer have been somewhat contradictory. In human clinical data, several case–control studies provided evidence that high serum leptin correlated with an elevated risk of colorectal cancer (59, 60), but this was not confirmed by others (61, 62). In the rodent models, leptin exerted no influence on HT-29 colon cancer growth grafted in nude mice or on the intestinal polyp development in *Min* mice, even though plasma leptin levels were 2.4- to 4.3-fold increased by delivery of exogenous leptin (63). In the case of

carcinogen induced colon carcinogenesis models, obese *ob/ob* mice (leptin-deficient mice) and *db/db* mice (Ob-R-deficient mice), it has been reported that intraperitoneal injection of the carcinogen AOM resulted in the development of around 15 colorectal ACF in both strains (64). Another report demonstrated AOM-induced colorectal tumor development, i.e. multiplicity and tumor size, in *ob/ob* and *db/db* mice to be reduced as compared to wild-type mice (65). These findings indicate that leptin acts as a growth factor at some stages in colorectal carcinogenesis. KK-A^y mice are also obese but possess intact leptin and leptin receptors (66). They were established by cross-mating KK mice, Type 2 diabetes model mice, with C57BL/6J-A^y mice (67, 68), which carry the *Agouti* gene (*Ay*), to induce severe hyperphagia, hyperinsulinemia and hyperlipidemia as compared with C57BL/6J mice. C57BL/6J mice are generally used as non-obese, non-diabetic controls compared with KK-A^y mice (69, 70). In this obese KK-A^y mice model, the mice were found to be highly susceptible to induction of ACF, and developed colorectal carcinomas. Furthermore, some of the tumors exhibited cancer cell invasion under the muscular layer of mucosa and remarkable tumor angiogenesis (66). The number of ACF/mouse and tumor/mouse developing in KK-A^y mice (≈70 ACF/mouse and ≈8 tumors/mouse) in response to AOM also

Table 1. Development of colorectal ACF/tumors in obese mice treated with AOM

Mice	AOM treatment (dose, weeks after the last AOM)	No. of ACF / colorectum	No. of tumors / colorectum	Ref.
ob/ob	2-weekly ip (10 mg/kg, 4 weeks)	~35		65
	6-weekly ip (10 mg/kg, 14 weeks)		~2	
db/db	6-weekly ip (10 mg/kg, 14 weeks)		~2	
C57BL	2-weekly ip (10 mg/kg, 4 weeks)	~5		
	6-weekly ip (10 mg/kg, 14 weeks)		~3.5	
KK-A^y	2-weekly ip (200 mg/mouse, 5 weeks)	~70		66
	6-weekly ip (200 mg/mouse, 7 weeks)		~8	
C57BL	2-weekly ip (200 mg/mouse, 5 weeks)	~9		
	6-weekly ip (200 mg/mouse, 7 weeks)		~0.1	
ob/ob	4-weekly ip (5 mg/kg, 100 days)	~15		64
db/db	4-weekly ip (5 mg/kg, 100 days)	~16		
C57BL	4-weekly ip (5 mg/kg, 100 days)	~6		

AOM: azoxymethane; ACF: aberrant crypt foci; ip: intraperitoneal

appeared higher than in other obese mice, *ob/ob* or *db/db* mice (Table 1). The KK-A^y mouse exhibited abdominal obesity, hypertriglyceridemia and hyperinsulinemia at the time-points of colorectal ACF and cancer development. Moreover, serum adipocytokines such as interleukin-6 (IL-6), leptin and Pai-1 were also elevated compared with values for lean C57BL/6J mice. Expression of pro-inflammatory adipocytokine mRNA such as for IL-6, leptin, monocyte chemoattractant protein-1 (MCP-1), Pai-1 and TNF-α were significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Taking together, it would appear that further investigations are required to clarify the role of leptin in colorectal carcinogenesis. Interactions between leptin and insulin may be one of the factors making the story complex. The concept of leptin resistance, INDEX= ln (serum leptin levels/%body fat) (71), might help to clarify the importance of leptin for colorectal and other organ carcinogenesis in the future.

Future aspects

For the present purpose, we have focused on the involvement of three major adipocytokines, adiponectin, PAI-1 and leptin, in colorectal carcinogenesis. Very recently, it has become clear that some of these hormones may play important roles not only in the stage of progression of colorectal cancer, but also in very early stages of colorectal cancer development. However, cancer prevention/therapies targeting these adipocytokines have yet to be established.

Although improvement of adipocytokine imbalance must be considered as an important goal for prevention of cancer, targeting adipocytokine expression levels for development of cancer chemopreventive and chemotherapeutic agents seems particularly challenging. This is because adipocytokines are expressed in many tissues and play essential roles for biologically indispensable metabolism regulation. Thus, it might be important to develop selective adipocytokine regulating drugs or search for agents from drugs with little side effects, i.e. from the aspect of "drug repositioning".

As an alternative to administration of adipocytokine inducers/activators and suppressors/inhibitors, it is highly desirous that public health measures be taken to increase sportive physical activity and to encourage temperance in daily life so that a meritorious balance of the adipocytokine production is naturally maintained.

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REVIEW ARTICLE

Potential Cancer Chemopreventive Activity of Protocatechuic Acid

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A natural phenolic compound, protocatechuic acid (3,4-dihydroxybenzoic acid), is present in many edible and medicinal plants. Recent studies, including our animal experiments, indicate that this simple phenolic acid could be protective against the development of epithelial malignancy in different tissues and cardiovascular diseases as well. The mechanism of the action is mostly associated with antioxidant activity, including inhibition of generation as well as scavenging of free radicals and upregulating antioxidant enzymes. The influence on Phases I and II of the metabolism of certain carcinogens and, perhaps, direct blocking of specific binding sites of ultimate carcinogens with DNA molecule, thus preventing adduct formation that may result in mutations and neoplastic transformation, also account for its cancer protective action. However, other biological aspects of the chemopreventive activity of protocatechuic acid are not fully studied. They include influence on the activity of inducible isoenzyme of cyclooxygenase and nitric oxide synthase, cell cycle–regulating proteins, or inflammatory cytokines, which are involved in oncogenesis. In view of its reported biological properties and relative safety, protocatechuic acid is a potential cancer chemopreventive product.

1. Introduction

Prevention of chronic diseases, including cancer, is an old but important concept regarding human health. Dietary factors are known to influence cancer development.[1,2] An important consideration in cancer research today is that exposure to pharmacologically active chemicals (natural and synthetic) may play an important role in reducing the risk of cancer development.[1,3,4] Cancer chemoprevention could be possible by the use of exogenous factors to enhance endogenous mechanisms that reduce the risk of cancer development because of exposure to different environmental factors. Some of these exogenous factors are dietary constituents, drugs, immunizations, and supplements. Edible plants and plants used for folk medicine are rich sources of such cancer chemopreventive agents.[3–6]

Protocatechuic acid (3,4-dihydroxybenzoic acid; Figure 1) is a simple phenolic compound widely distributed in nature. Like many other simple phenolic acids, protocatechuic acid is detected in almost all plants and is, therefore, a very common component of human diet,[7] such as the bran and grain brown rice (*Oryza sativa* L.)[8] and onion (*Allium cepa* L.),[9] especially in the scales. Protocatechuic acid is detected in many fruits, such as plums (*Prunus domestica* L.)[10]; gooseberries (*Ribes uva-crispa* L.)[9]; grapes (*Vitis vinifera*)[11]; and nuts, such as almonds ordinary (*Prunus amygdalus*).[12] It is present in products of plant origin, such as olive oil or white wine.[13–15] Protocatechuic acid is also found in many plants and spices, such as star anise (*Illicium verum*), melisie (*Melissa officinalis* L.), a medical rosemary (*Rosmarinus officinalis* L.), and cynamonowcu (*Cinnamomum aromaticum*).[9] This compound is one of the biologically active components of some medicinal plants, including those used in natural medicine, such as sudan Mallow (*Hibiscus sabdariffa* L.),[16,17] Japanese ginkgo (*Ginkgo biloba* L.),[18] and St. John's wort (*Hypericum perforatum* L.).[19]

We demonstrated the chemopreventive ability of protocatechuic acid in chemically induced carcinogenesis in mainly the digestive organs of experimental animals.[20,21] In this review, we have highlighted the protective mechanisms of protocatechuic acid against carcinogenesis.

2. Effects of Protocatechuic Acid on Chemical Carcinogenesis in Rodents

The chemopreventive action of protocatechuic acid was evaluated in several models of chemically induced carcinogenesis in laboratory animals (Table 1).[16,22–33] The results indicate that the protocatechuic acid at doses of 200–2000 ppm in diet effectively inhibited the development of most of the cancers, especially of the digestive

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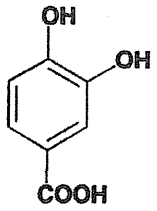

Figure 1 Chemical structure of protocatechuic acid (3,4-dihydroxybenzoic acid). Chemical abstracts service (CAS) No.: 99-50-3; Molecular weight (MW): 154.12.

system,[21] when administered both in the initiation phase and in the promotion/progression of carcinogenesis.

Efficacy of protocatechuic acid was demonstrated in the prevention of cancers of the oral cavity in several experimental models using rats and hamsters. Dietary feeding with protocatechuic acid during the initiation phase or the promotion/progression of tongue carcinogenesis of rats reduced the incidence and the number of preneoplastic lesions (hyperplasia and dysplasia) and epithelial neoplasms (squamous cell carcinomas and papillomas) induced by 4-nitroquinoline oxide.[22] A recent study with modified experimental protocol using the same carcinogen, which was able to cause a greater percentage of carcinomas in the advanced stage, has shown that treatment with protocatechuic acid in the progression phase reduced the incidence of precancerous lesions and cancers invading adjacent organs or metastatizing to the lungs.[23] Protocatechuic acid given during the promotion/progression of carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene in the cheek pouch of hamsters significantly reduced the size of tumors and the area occupied by a precancerous lesion, squamous cell dysplasia.[24]

In the model of rat colon carcinogenesis induced by azoxymethane, protocatechuic acid in diet during the initiation or promotion/progression lowered the number of aberrant crypt foci, which are considered to be putative precancerous lesions,[34–36] and the incidence and number of colorectal adenocarcinoma.[5,25,34]

Protocatechuic acid administered during promotion/progression of pancreatic carcinogenesis induced by *N*-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters caused a significant reduction in the incidence of large pancreatic cancer (>3 cm) invading the adjacent organs.[26]

Protocatechuic acid also affected the development of neoplasia in the rat liver induced by diethylnitrosamine.[27] When protocatechuic acid was administered in diet during the initiation or promotion/progression phases of carcinogenesis, the incidence of altered hepatocellular foci, characterized by lack of iron accumulation and being positive for the reactivity against placental isoform of glutathione *S*-transferase (GST), was decreased. The treatment also reduced the multiplicities of both liver cell adenomas and carcinomas. Chemoprevention effects of protocatechuic acid in urinary bladder carcinogenesis was also revealed in rats initiated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine by the reduction of the incidence of precancerous lesions (hyperplasia and dysplasia) and cancers when rats were fed the diet containing the chemical during the initiation or promotion/progression stages.[28]

Our data on cancer chemopreventive ability of protocatechuic acid indicate that dietary administration with protocatechuic acid at 500 or 1000 ppm during the initiation and postinitiation stages suppresses chemically induced carcinogenesis in the tongue, glandular stomach, colon, liver, and urinary baldder of rats,[21,28] suggesting that 500 ppm is enough to inhibit the carcinogenesis in these tissues. In addition, we have found the protective effect of 2000 ppm of protocatechuic acid in diet against progression of tongue carcinogenesis.[23] Therefore, it is possible that less than 2000 ppm of this compound might inhibit all phases (initiation, promotion, and progression) of tongue carcinogenesis.

There are also reports showing negative chemopreventive activity of protocatechuic acid. In the experimental lung tumorigenesis of

Table 1 Evaluation of the activities of protocatechuic acid in preventing different chemical carcinogenesis in rodents

Species/strain/gender of animals	Carcinogen/promoter	Protocatechuic acid		Target tissue/cancer	Response	Authors/yr (ref. no.)
		Dose/rout	Experimental protocol			
F344 rats/males	4-NQO	500, 1000, 2000/in diet	4-NQO + PCA and 4-NQO → PCA	Oral cavity (tongue)/SCC	Inhibition	Tanaka T et al/1994[22]
F344 rats/males	4-NQO	2000/in diet	4-NQO → 4-NQO + PCA	Oral cavity (tongue)/SCC	Inhibition	Suzuki R et al/2003[23]
Syrian golden hamsters/males	DMBA	200 ppm/in diet	DMBA → PCA	Bucccal pouch/SCC	Inhibition	Ohnishi M et al/1997[24]
F344 rats/males	MNNG	1500 ppm/in diet	MNNG → PCA	Forestomach/SCC	No effects	Hirose M et al/1992[30]
F344 rats/males	AOM	1000, 2000 ppm/in diet	AOM + PCA → PCA	Colon/ADC	Inhibition	Kawamori T et al/1994[33]
F344 rats/males	AOM	250, 500, 1000 ppm/in diet	AOM + PCA and AOM → PCA	Colon/ADC	Inhibition	Tanaka T et al/1993[25]
Syrian golden hamsters/females	BOP	500, 1000 ppm/in diet	BOP → PCA	Pancreas/ADC	Inhibition	Nakamura H et al/2000[26]
F344 rats/males	DEN	500, 1000 ppm/p.o.	DEN + PCA and DEN → PCA	Liver/AD	Inhibition	Tanaka T et al/1993[27]
A/J mice/females	NNK	1000 ppm/in diet	NNK + PCA and NNK → PCA	Lung/AD	No effects	Mori H et al/1999[29]
F344 rats/males	BBN	500; 1000; 2000 ppm/in diet	BBN + PCA and BBN → PCA	Urinary bladder/TCC	Inhibition	Hirose Y et al/1995[28]
CD-1 mice/females	B[a]P/TPA	5, 10, 20 μM/locally on the skin 5 min before TPA	DMBA → TPA + PCA	Skin/PAP	Inhibition	Tseng TH et al/1998[16]
ICR mice/females	DMBA /TPA	16, 160, 1600 nM/topically to the skin 0; 40 min or 3 hr before TPA	DMBA → TPA + PCA	Skin/PAP	Inhibition (16nM); no effects (160nM and 1600nM) Enhancement of skin papilloma by 1600nM PCA	Nakamura Y et al/2000[32]
F344 rats	PhIP	2000 ppm /in diet	PhIP + PCA → PCA	Breast/ADC	No effects	Mori H et al/1999[31]

AD = adenoma; ADC = adenocarcinoma; AOM = azoxymethane; B[a]P, benzo[a]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine; BOP = *N*-nitrosobis(2-oxopropyl) amine; DEN = *N*-diethylnitrosamine; DMBA = 7,12 dimethylbenz[*a*]anthracene; MMMG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NNK = 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; 4-NQO = 4-nitroquinoline oxide; PAP = squamous cell papilloma; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SCC = squamous cell cancer; TCC = transitional cell carcinoma; TPA = 12-*O*-tetradecanoylphorbol 13-acetate.

mice, induced by 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, protocatechuic acid in diet at a dose of 1000 ppm during the initiation or promotion phase did not exert any protective effects on the development of lung tumors.[29] Protocatechuic acid at a dose of 2000 ppm was also ineffective in the prevention of cancers in the forestomach[30] and mammary gland[31] of rats, induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and 2-amino-1-methyl-6-fenylimidazo-[4,5-*b*] pyridine, respectively.

Protocatechuic acid influences the development of squamous cell papillomas in skin cancers in the classic two-stage carcinogenesis model using 7,12-dimethylbenz[*a*]anthracene as an initiator and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) as a promoter. Topical application of protocatechuic acid at small doses (<20nM) for 5 minutes before painting of TPA inhibited the incidence and number of papillomas in mice.[16] In another experiment carried out in the same model, the effect of protocatechuic acid depended on the dose used and the timing between administration of the acid and TPA application. Protocatechuic acid at a dose of 16nM, which was applied 40 minutes before TPA treatment, caused a reduction in the number of tumors, whereas a dose of 1600nM increased the number of papillomas. Application of protocatechuic acid at a high dose (20,000nM) 5 minutes before TPA decreased the number of tumors, whereas administration 3 hours before TPA significantly increased the number of skin tumors in mice.[32] Therefore, much attention should be paid to the administration dose or time of protocatechuic acid in human chemoprevention studies. These data indicate an organotropic activity of protocatechuic acid and suggest that possible cancer chemopreventive compounds need to be carefully examined for effectiveness in multiple organs by different models.

3. Mechanisms of Chemopreventive Action of Protocatechuic Acid

3.1. Effects of protocatechuic acid on the redox balance in cells

Chemopreventive action of protocatechuic acid is largely because of its antioxidant properties. Reactive oxygen species (ROS) and reactive nitrogen species, occurring as a result of exposure to environmental chemicals and radiation in the course of physiological or metabolic processes, may damage or modify macromolecules, that is, nucleic acids, structural proteins and enzymes, and membrane lipids. This may lead to mutation and disruption in the signaling pathways in the cell and, consequently, to the development of cancer. It has been shown that ROS and reactive nitrogen species can affect every stage of carcinogenesis, from initiation phase to the phase of promotion/progression.[35]

The antioxidant activity of protocatechuic acid is well documented. Studies using *in vitro* cellular system and ROS generation have shown that protocatechuic acid inhibits both the formation of free radicals, including the highly reactive hydroxyl radical, and the scavenging of free radicals.[37,38] Inhibition of the formation of free radicals is associated with the ability of protocatechuic acid to form complexes with transition metal ions, Cu (II) and Fe (II), or lowering the activity of enzymes catalyzing reactions in the course of which such radicals are formed, such as xanthine oxidase.[39,40] The neutralization of free radicals is the result of their reaction with hydroxyl groups of protocatechuic acid. *In vitro* models showed that protocatechuic acid prevents oxidative DNA damage and lipid peroxidation.[41,42]

The antioxidant activity of protocatechuic acid was also observed *in vitro* on cell cultures under conditions of oxidative stress.[13] Protocatechuic acid was able to completely prevent the low-density lipoprotein (LDL) oxidation mediated by inhibited rat macrophage-like cells J774A.1.[14] Protocatechuic acid inhibits the concentration of reduced glutathione content and restores glutathione reductase and glutathione peroxidase activities. Protocatechuic acid also restores the mRNA levels of γ-glutamylcysteine synthetase, glutathione reductase, and glutathione peroxidase to control levels.[14]

Protocatechuic acid at concentrations 0.02–0.1 mg/mL and 50–100 mg/kg prevented the undesirable consequences of oxidative stress in the primary culture of rat hepatocytes and in liver of rats exposed to *tert*-butyl hydroperoxide (*t*-BHP).[43,44] The findings demonstrated that protocatechuic acid reduced the cytotoxicity of *t*-BHP and raised the level of glutathione (GSH). Furthermore, protocatechuic acid inhibited lipid peroxidation, DNA repair processes, and oxidative damage induced by nucleic acid and prevented mitochondrial membrane depolarization. In addition, protocatechuic acid caused a reduction in phosphorylation of tyrosine residues of proteins of hepatocytes, suggesting an impact on signal transduction mechanisms in the cell.[43] It is known that one of the ROS and lipid peroxidation products—4-hydroxy-2-nonenal—can regulate the phosphorylation of signaling proteins by stimulating the tyrosine kinase activity.[45]

Protocatechuic acid affects the oxidative stress in the skin of CD-1 mice that received local application of another inducer, TPA.[16] Protocatechuic acid at doses of 5–20mM reduced the inflammation caused by the administration of TPA, inhibited the production of hydrogen peroxide (H_2O_2), and decreased the activity of myeloperoxidase in the skin. Myeloperoxidase present in neutrophils is responsible for the oxidation of certain carcinogens with H_2O_2, which plays an important role in their biotransformation.

However, protocatechuic acid, like many other well-known antioxidants, such as ascorbic acid and a-tocopherol, may exhibit pro-oxidant action under certain conditions.[46] Protocatechuic acid works as an antioxidant at low concentrations, whereas at high concentrations, it works as a pro-oxidant.[32,47–49] Protocatechuic acid at high concentrations (>10mM) was demonstrated to induce oxidative stress in immortalized human gingival S-G epithelial cells and salivary gland cancer cells HSG1, as evidenced by the intensity of lipid peroxidation in the presence of Fe (II) and lower levels of GSH. Protocatechuic acid at a concentration of 2.5nM potentiated the toxicity of *t*-BHP.[47] Similar observations were reported in some *in vivo* studies. Intraperitoneal administration of protocatechuic acid at a toxic dose (500 mg/kg) reduced the concentration of GSH in the liver and the kidney of ICR mice.[49] Dose-dependent effect of protocatechuic acid was confirmed by alterations in the skin of mice after exposure to TPA.[32,48] Several independent *in vivo* experiments showed that when given protocatechuic acid at doses of 1600 and 20,000nM for 0.5 or 3 hours before application of TPA, the oxidative stress and inflammation were potentiated in the skin. Increased levels of lipid peroxidation, H_2O_2 generation, and myeloperoxidase activity, and decreased levels of GSH were also observed. Use of 16nM protocatechuic acid was protective against oxidative stress and papilloma formation in the skin. Results of these *in vivo* studies and *in vitro* works using human promyelocytic leukemia cells HL-60 suggested that the effects of higher doses of protocatechuic acid may be associated with the oxidation of the chemical by tyrosinase, which leads to a more reactive derivative, probably quinone that can react with glutathione and proteins. The consequence is the loss of GSH and disruption of detoxification systems, and modifications—sometimes necessary to proteins—which can induce a local immune response increasing the effects of oxidative stress.

3.2. Effect of protocatechuic acid on the metabolism of carcinogens

The chemopreventive action of protocatechuic acid is also linked to its effects on the metabolism of carcinogens. The process involves two groups of enzymes, Phase I and Phase II. Phase I biotransformation enzymes are mainly from the superfamily of cytochrome

P-450 and catalyze hydroxylation reactions. In the course of this transformation, metabolic activation can occur, and the resulting metabolites may react with the DNA and form adducts. In contrast, Phase II enzymes that detoxify carcinogens catalyze the conjugation of glucuronic acid, sulfuric acid, or glutathione. This increases the solubility of these compounds in water and facilitates excretion. However, these reactions can also lead to the activation of some carcinogens. This group includes GST, uridine 5′-diphosphate (UDP)-glucuronosyltransferase, and reduce nicotinamide adenine dinucleotide phosphate (NAD(P)H):quinone (NQO1).[50] It has been shown that protocatechuic acid has an effect on enzymes involved in both Phase I and II biotransformation of carcinogens.[51–54]

Protocatechuic acid inhibited the catalytic activity of certain cytochrome p-450, especially CYP1A2 and, to a lesser extent, CYP1A1 and CYP2B, induced by sodium phenobarbital or 5,6-benzophenone in microsomes from mouse hepatocytes of *in vitro* stduy.[51] *In vivo* studies have shown that a single administration of protocatechuic acid to rats have stronger effects on the activities of Phase I and II enzymes than subchronic administration of protocatechuic acid to rats at a dose of 250- or 500-mg/kg body weight. Exposure of protocatechuic acid reduced the activities of CYP1A1, CYP1A2, and CYP2B in the liver but only CYP2B in microsomes obtained from kidney homogenates. Giving protocatechuic acid 1 hour before the application of *o*-toluidine, which is an aromatic amine metabolizable by CYP1A1, caused increases in the activities of CYP1A1 and CYP1A2 in the liver and an increase in CYP1A1 activity only in the kidney. Protocatechuic acid also increased the activity of GST, which was reduced after the administration of *o*-toluidine.[54] However, protocatechuic acid administered at a dose of 50 mg/kg every 3 days for 2 weeks to rats that were exposed to 3-methylcholantren at the 12th day of protocatechuic acid treatment resulted in decreases in activities and expression of CYP1A1, CYP1A2, and CYP2E1, and an increase in GST and NQO1 activities in the livers of animals.[55] Protocatechuic acid also reduced the constitutive activity induced by 3-methylcholanthrene and isoenzyme CYP2E1 in the kidneys of rats. Administration of protocatechuic acid had no effect on cytochrome P-450 activity, whereas it decreased the activity of detoxification enzymes studied, that is, GST, UDP-glucuronosyltransferase, and NQO1, in the livers of animals.

These results indicate that protocatechuic acid not only affects the activities of enzymes involved in the metabolism of carcinogens, but also neutralizes reactive intermediate metabolites, thereby preventing their binding to DNA. Blocking the DNA-binding site with carcinogens by protocatechuic acid is likely to prevent DNA mutations and tumor initiation.[52] Different effects of protocatechuic acid on the activity of enzymes involved in the biotransformation of the studied organs may be partly explained by differences in the efficacy of phenol in the prevention of chemically induced carcinogenesis in experimental animals.

3.3. Other mechanisms of anticarcinogenesis action of protocatechuic acid

Protocatechuic acid possesses antiproliferative action on several human cell lines, including immortalized breast cells HBL 100, breast cancer cells T47D, gastric adenocarcinoma cells MKN45, lung cancer cells PC14, and promyelocytic leukemia cells HL-60.[8,56–58] Zheng et al[59] reported that protocatechuic acid exerts tumor-preventive action through apoptosis- and cell proliferation–independent mechanisms in human colon cancer cell lines. Inhibition of the proliferation of nonlesional epithelial cells, including those of oral cavity,[22,24] colon,[25] liver,[27] and bladder,[28] was also observed in chemical carcinogenesis models.

Antiproliferative action of protocatechuic acid can be derived from its antioxidant properties. Some ROS, such as H_2O_2, act as a second messenger in cell signal. They can activate transcription factors, such as nuclear factor kappa B (NF-κB)[60–63] or activator protein-1, and thus, affect the expression of genes involved in cell cycle regulation and apoptosis.[35] Interestingly, protocatechuic acid suppresses the expression of inducible nitric oxide synthase (iNOS)[61], cyclooxygenase-2,[61] and tumor necrosis factor (TNF)-α,[63] which are involved in carcinogenesis and/or inflammation.

Polyamines, including putrescine, spermine, and spermidine, play an important role in the growth and differentiation of cells by interfering with the mechanisms of signal transduction.[64] Polyamines induce a cascade of kinases, such as tyrosine kinase and mitogen-activated protein kinase, and stimulate the transcription of some oncogenes, such as c-*myc*, c-*jun*, and c-*fos*. Protein products of these oncogenes function as transcription factors and may stimulate the proliferation of cells. The main enzyme in polyamine biosynthesis is ornithine decarboxylase, which catalyzes the conversion of ornithine to putrescine. Increased enzyme activity of ornithine decarboxylase was observed in many cancers and in precancerous changes as well. Protocatechuic acid reduces the enzymatic activity of ornithine decarboxylase in target organs and levels of polyamines in serum in some chemically induced carcinogenesis models in rodents.[22,23,25,27,33]

A recent study has shown that protocatechuic acid has a direct effect on the process of DNA replication. Stagos et al[15] demonstrated that protocatechuic acid is a potent inhibitor of topoisomerase I, the enzyme responsible for the catalytic reaction of cutting and joining DNA polynucleotide chains.

Protocatechuic acid also affects apoptosis to eliminate damaged and neoplastic cells.[65,66] In human promyelocytic leukemia cell line HL-60, protocatechuic acid increased the proportion of cells in the G1 phase of the cycle and induced apoptosis; treatment with protocatechuic acid increased the level of hypophosphorylated RB protein and the expression of proapoptotic protein, BAX, whereas the treatment caused a decline in hyperphosphorylated RB and a decrease in the expression of antiapoptotic protein Bcl-2.[58] These data suggest that protocatechuic acid is an apoptosis inducer in human leukemia cells, and that RB phosphorylation and Bcl-2 protein may play a crucial role in the early stage. It should be pointed out that RB protein plays an important role in the regulation of cell cycle and apoptosis. At the end of G1 phase, after phosphorylation by protein complexes of cyclin D-dependent kinase 4/6 and cyclin E-dependent kinase 2, transcription factors are released from the E2F family, which after joining the heterodimeric DNA binding protein partners called DP are associated with promoter areas of target genes, stimulating the transcription of cells and the transition into a new phase of the cycle. However, poorly phosphorylated RB protein binds E2F factors, which prevents the gene transcription and stops the cell cycle in G1 phase.[67] Regardless of stopping the cycle, RB protein directly inhibits the transmission of signals in cell death.[37] Reduction of the levels of both forms of proteins 9 hours after administration of protocatechuic acid may be associated with the inhibition of polyamine biosynthesis by caspases and degradation, which in turn, triggers apoptosis.[58] Recent studies indicate, however, that some of the reduced RB protein molecule can inhibit apoptosis.[68]

There have been reports showing that protocatechuic acid prevents apoptosis initiation by affecting death receptors. In human umbilical vein endothelial cell line and T-cell lymphoma cell line Jurkat, protocatechuic acid inhibited apoptosis induced by TNF-α.[63] Protocatechuic acid also increased the activity of the transcription factor NF-κB as a consequence of worsening degradation of the inhibitory protein IκBα, which creates cytosolic complex with NF-κB and prevents its translocation to the nucleus. After