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Prospective Targets for Colon Cancer Prevention: from Basic Research, Epidemiology and Clinical Trial

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The step-wise process of colorectal carcinogenesis from aberrant crypt foci, adenoma to adenocarcinoma, is relatively suitable for chemopreventive intervention. Accumulated evidences have revealed that maintaining an undifferentiated state (stemness), inflammation, and oxidative stress play important roles in this colon carcinogenesis process. However, appropriate molecular targets that are applicable to chemopreventive intervention regarding those three factors are still unclear. In this review, we summarized appropriate molecular targets by identification and validation of the prospective targets from a comprehensive overview of data that showed colon cancer preventive effects in clinical trials, epidemiological studies and basic research. We first selected a study that used aspirin, statins and metformin from FDA approved drugs, and epigallocatechin-gallate and curcumin from natural compounds as potential chemopreventive agents against colon cancer because these agents are considered to be promising chemopreventive agents. Experimental and observational data revealed that there are common target molecules in these potential chemopreventive agents: T-cell factor/lymphoid enhancer factor (TCF/LEF), nuclear factor-&B (NF-κB) and nuclear factor-erythroid 2-related factor 2(NRF2). Moreover, these targets, TCF/LEF, NF-κB and NRF2, have been also indicated to suppress maintenance of the undifferentiated state, inflammation and oxidative stress, respectively. In the near future, novel promising candidate agents for colon cancer chemoprevention could be identified by integral evaluation of their effects on these three transcriptional activities.

Key Words: Colon cancer; Chemoprevention; TCF/LEF, NF-κB and NRF2

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world. Nearly[1.4] million new cases occurred in 2012, and worldwide it is expected to increase to 2.4 million cases annually by 2035.1 Despite the extensive efforts to develop many anti-cancer drugs, the mortality rate of CRC is still high. Therefore, a new strategy to controlling development of this cancer is in great demand.[2] In this context, primary prevention, including chemoprevention, is one of the important strategies for fighting this malignancy.

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The colon and rectum are ideal target organs in which to develop a chemopreventive strategy because of the accumulation of a lot of knowledge of the relative sequence of genetic events required for tumor formation. In 1988, Vogelstein et al. reported a multistep genetic model of colorectal carcinogenesis.[3] Now, it is evident that colorectal carcinogenesis is a multistage process consisting of initiation, promotion and progression phases, and occurs through the accumulation of gene mutations in oncogenes, tumor suppressor genes, and genomic stability genes. Mutations in four or five genes may be necessary to develop a malignant neoplasm over a period of 10-20 years. Therefore, there are various aspects for interventions to inhibit, slow down and/or reverse each process of carcinogenesis.

Mutations in the *adenomatous polyposis coli (APC)* gene are assumed to be the first step in the carcinogenesis process.[4] The *APC* gene was isolated as a responsible gene of familial ade nomatous polyposis (FAP), and is involved in the regulation of cytoskeleton organization, apoptosis, cell cycle cont-

rol and cell adhesion.[5] *APC* mutations occur in up to 80% of adenomas and adenocarcinomas, and 4.3% of sporadic aberrant crypt foci (ACF).[6,7] *APC* protein translated from the *APC* gene is a main player of the Wnt signal pathway, and *APC* regulates cell proliferation by binding with phosphorylated and degraded β-catenin protein that promotes cell proliferation.[8] However, mutant *APC* protein can not bind and degrade β-catenin protein, and as a result, β-catenin protein translocates to the nucleus and binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factor, which targets c-*myc*, *Lgr*[5], and *c-jun genes*.[9,10] As Wnt activity is crucial for maintaining intestinal stem cells and crypt homeostasis under physiological conditions, that aberrant activation of Wnt signaling has a central function in the carcinogenic process via promoting proliferation and maintaining the undifferentiated status of the cells.[11] In fact, APC restoration showed rapid tumor regression by promoting cellular differentiation and reestablishing crypt homeostasis in established tumors *in vivo*.[12]

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD) of unknown etiology. It is well-known that the relative risk of CRC in patients with IBD has been estimated to be between 4- to 20-fold.[13] In this context, chronic inflammation has been considered to play a major role in the initiation, promotion and progression of CRC. Nuclear factor-κB (NF-κB)[14] is a common transcription factor that regulates several inflammation related genes, such as cyclooxygenase-2 (COX-2)[15], inducible nitric oxide synthase (iNOS)[16], interferon-α (IFN-ɣ), tumor necrosis factor-α (TNF-β) and interleukin-1β (IL-1β).[17] These genes are overexpressed in inflamed mucosa and colonic neoplasms. The aberrant activation of NF-κB is reported in over 50% of CRCs.[18] In addition, NF-κB is a central regulator of the transcriptional activation of a number of genes involved in cell adhesion, immune and pro-inflammatory responses, apoptosis, differentiation and growth. Induction of these genes in intestinal epithelial cells through activation of NF-κB profoundly influences mucosal repair as well. On the other hands, chronic activation of NF-κB induces promotion of epithelial cell turnover and generation of reactive oxygen species (ROS).

The inductions of DNA mutations by ROS attack appears to be principally involved in the early stage of colon carcinogenesis linked to inflammatory processes. ROS could thereby overwhelm the tissue's antioxidant defenses and induce DNA damage. One of the major players in the antioxidant defense system of various tissues is the nuclear factor-erythroid 2-related factor 2 (NRF2). NRF2 is a basic leucine zipper redox-sensitive transcriptional factor that plays a central role in the regulation of antioxidant and/or detoxifying genes. Interestingly, NRF2 knockout mice showed increased susceptibility to dextran sulfate sodium (DSS)-induced inflammation in the colorectum (colitis)[19] and carcinogenesis[20] compared with wild-type mice.

Thus, maintaining the stemness by Wnt signaling activation, inflammation and oxidative stress play important roles in the initiation and promotion stages of colon carcinogenesis. However, appropriate molecular targets in those three factors to prevent CRC are still unclear. In this review, we aim to identify, validate and summarize prospective targets for colon cancer prevention by a comprehensive overview of promising agents against colon cancer prevention, supported by epidemiological studies, clinical trials and chemopreventive researches *in vitro* and *in vivo*.

1. Aspirin

1) Background and Association with CRC Risk

Aspirin (acetylsalicylic acid) is the most traditional common type of nonsteroidal anti-inflammatory drugs (NSAID) and is widely used against inflammation and pain, mainly due to its inhibition of prostaglandins (PGs) biosynthesis. Moreover, long-term use of aspirin has been one of the most performed CRC chemoprevention trials. Meta-analysis data of aspirin randomized trials demonstrated that about 5 years use of aspirin reduces CRC incidence and mortality by 30-40% 20 years later.[21] We recently performed a double-blind, randomized study using aspirin and/or placebo for 2 years, in which included Asian subjects (n=311) that resected all the colorectal tumors (adenomas or early-stage adenocarcinomas) endoscopically at the entry of the trial. After 2 years, we performed colon endoscopy and obtained the odds ratio (OR) for the presence or absence of tumor recurrence as 0.60 (CI, 0.36-0.98).[22] We concluded that this trial is totally in line with the observations of other aspirin adenoma trials, and aspirin might be a promising cancer chemopreventive agent, regardless of ethnic group.

2) Effects on Inflammation-related Factors

NF-κB protein in the cytoplasm is maintained in an inactive state by the inhibitory subunit I-κBα Iκ-B is phosphorylated by IKK and degraded by the ubiquitin-proteasome system. Phosphorylation of residues Ser32 and Ser36 of IkB-α results in its subsequent degradation and allows NF-κB protein to be an inactive state. Translocation of NF-κB to the nucleus leads to activation of transcription of downstream targets genes[23], such as COX-2, IL-6 and TNF-α.[24] For example, induc tion of COX-2 produces a lot of PGs, which causes an induction of cell proliferation and inhibits apoptosis, to some extent

mediated by PGE2 receptor subtypes. It has been reported that aspirin inhibits IKK-β activity at millimolar concentrations and inactivates NF-κB transcriptional activity in an *in vitro* setting.[25]

3) Effects on Differentiation-related Factors

A number of protein kinases have been shown to impact Wnt/β-catenin signaling, that is GSK3β, protein kinase C, casein kinase I/II and the serine/threonine protein phosphatase 2A (PP2A).[26] TCF-driven luciferase activity revealed that aspirin at millimolar concentrations causes a dose-dependent inhibition of the Wnt/β-catenin pathway. This suppression is explained by an increased phosphorylation of PP2A and the resultant break down of β-catenin in colorectal cancer cells.[27]

4) Effects on Oxidative Stress Related Factors

Aspirin itself has a free radical scavenging property and is able to protect cells from the deleterious effects of oxidative stress, in human and experimental models.[28] Moreover, aspirin can inhibit transcriptional factor NRF2. It is demonstrated that H_2O_2 activate the NRF2/HO-1 signaling pathway and aspirin directly inhibits NRF2 activation in primary melanocytes.[29] The NRF2-ARE pathway plays an important role in the protective function against oxidative stress by induction of phase II detoxification and antioxidant enzymes.[30] Under normal conditions, NRF2 exists in an inactive state in the cytoplasm due to the effect of the cytosolic repressor Kelch-like ECH-asso ciated protein 1 (Keap1). When aspirin disrupts its complex with Keap1, NRF2 translocates to the nucleus, and initiates the transcription of genes coding for phase II detoxification and antioxidant enzymes, including NAD(P)H: quinone oxidoreductase-1 (NQO1) and glutamate-cysteine ligase (GCL). In addition, NRF2 is related to the modification of NADPH oxidase. It has been reported that NRF2-deficient mice showed an increase in NADPH oxidase 2.[31] NADPH oxidase is a well-known ROS-producing enzyme that acts against bacterial infection and inflammation. Recently, NADPH oxidase has been suggested to be involved in colorectal carcinogenesis. Apocynin, an NADPH oxidase inhibitor, has been shown to reduce the numbers of intestinal polyps in Apc-mutant Min mice.[32]

5) Physiological Effects on Carcinogenesis Related Factors in Humans

Aspirin is in widespread use as an analgesic, antipyretic and cardioprotective agent, mainly due to its anti-inflammatory properties. It has been reported that aspirin at a dose of 300 mg/day suppressed the blood levels of inflammatory markers, high-sensitivity C-reactive protein (hs-CRP), TNF-α, IL-6 and the platelet aggregation mediator, thromboxane B2, after 2 weeks of treatment in Chinese patients with metabolic syndrome.[33] Of note, another suggested cancer preventive mechanism of aspirin is platelet-mediated mechanism. In addition to the above listed chemopreventive functions, induction of apoptosis is another ability of aspirin. Aspirin at 100 micromolar dosage could acetylate the p53 protein, one of the transcriptional factors. p53 targets p21, involved in cell cycle arrest, and Bax, involved in apoptosis[34] and this may also explain the chemopreventive function of aspirin.

2. Statins

1) Background and Association with CRC Risk

Statins are among the most widely prescribed medications in the world. The term "statin" is the general name for the lipid-lowering drugs, inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in the mevalonate pathway. Thus, statins are commonly used in patients with high levels of blood low-density lipoprotein (LDL) cholesterol. High cholesterol can contribute to plaque formation in the arteries, which narrows the blood vessels and restricts blood flow. There is strong evidence that statins have the potential to reduce cardiovascular diseases (CVD), such as strokes, heart attacks and other circulation problems. Also, statins could be useful for the prevention of some malignant neoplasms, which was summarized in previously published excellent reviews.[35,36]

Broadly speaking, in addition to CVD, statins were considered as they functioned as good preventive reagents against cancer, including CRC. At first, a nested case-control study using the large Quebec Administrative Health Database found a reduction of CRC risks associated with statin use (OR; 0.83).[37] This OR value resembles other consecutive reports of the Dutch Database of 8 Cities (OR; 0.87)[38], the Population-Based Danish Cohort Study (OR; 0.85)[39], and so on. These cohort studies indicated the possibility of statins to reduce CRC risk, nevertheless the data were not significant. Thenceforth, the Molecular Epidemiology of Colorectal Cancer (MECC) study in Israel showed that at least 5 years intake of statins was associated with a significant reduction in the risk for developing CRC (OR; 0.53), solidifying the potential importance of statins for preventing CRC.[40] Some recent reports showed a similar or stronger usefulness of statins for CRC prevention.[41-44]

Nowadays, the association between prescription of statins and reduction of CRC might be thought as a significant one.

After an adjustment for NSAID use, the association was strengthened. Meanwhile, a meta-analysis of three adenoma chemoprevention trials[45] did not clarify the effectiveness of the short-term statin exposure to the colorectal adenoma risk.

2) Effects on Inflammation-related Factors

Statins have often been used in some other diseases due to their pleiotropic effects, such as anti-inflammatory function.[35,36,46] For instance, statins could restrain NF-κ B-dependent transcription, along with the reduction of inflammation-related proteins, such as CRP and various inflammation-related cytokines, at the transcriptional level. As described in a previous section, statins predominantly act as HMG-CoA reductase inhibitors that might not act as the direct transcriptional modulator, however they could cause transcriptional changes on downstreams of signal pathways, neither dependent nor independent on HMG-CoA reductase activity. The assumed molecular mechanisms are as follows (Fig. 1); (1) Statins could reduce mevalonate by their HMG-CoA reductase activity, (2) Decrease of mevalonate could induce the reduction of geranylgeranyl pyrophosphate (GGPP), which is a metabolic-downstream product of mevalonate, (3) GGPP is essential for small-G protein Rho activation, so the Rho could not transduce their signals, (4) Rho inactivation evokes the attenuation of inflammation signals, (5) Finally, the inflammation related dominant transcriptional pathway, NF-κB -dependent transcription, is suppressed.

The above down-regulation pathway might attenuate the production of certain inflammation-related cytokines, chemokines and adhesion molecules, and it might provoke positive-feedback in many types of organs and cell lines. Indeed, there are clear anti-inflammatory effects of some statins on intestinal epithelial cells and on an experimental murine colitis model.[47] Fifty micromolar simvastatin significantly inhibited TNF-α-induced IL-8 gene expression in COLO-205 colon cancer cells through blocking IκB phosphorylation/degradation to inhibit DNA binding activity of NF-κB to their cis-elements and resultant NF-κB transcriptional activity.

It should also be noted that the serum levels of CRP and modulation of these inflammation-related proteins by statin-treatment does not correlate with the serum levels of LDL cholesterol.[48,49]

Besides this, other mechanisms for the suppression of NF-κB-dependent transcription by statins could be existed.[36,50,51] Of note, only the natural statins (simvastatin, mevastatin, lovastatin, and pravastatin), but not the synthetic statins (fluvastatin and atorvastatin) inhibited TNFα-induced NF-κB activation.[52] These results suggest that different statins behave differently from one another.

3) Effects on Differentiation-related Factors

Almost no reports have been published about statin treatment and TCF/LEF transcription, which dominate the colon differentiation and stem-cell maintaining destiny. However,

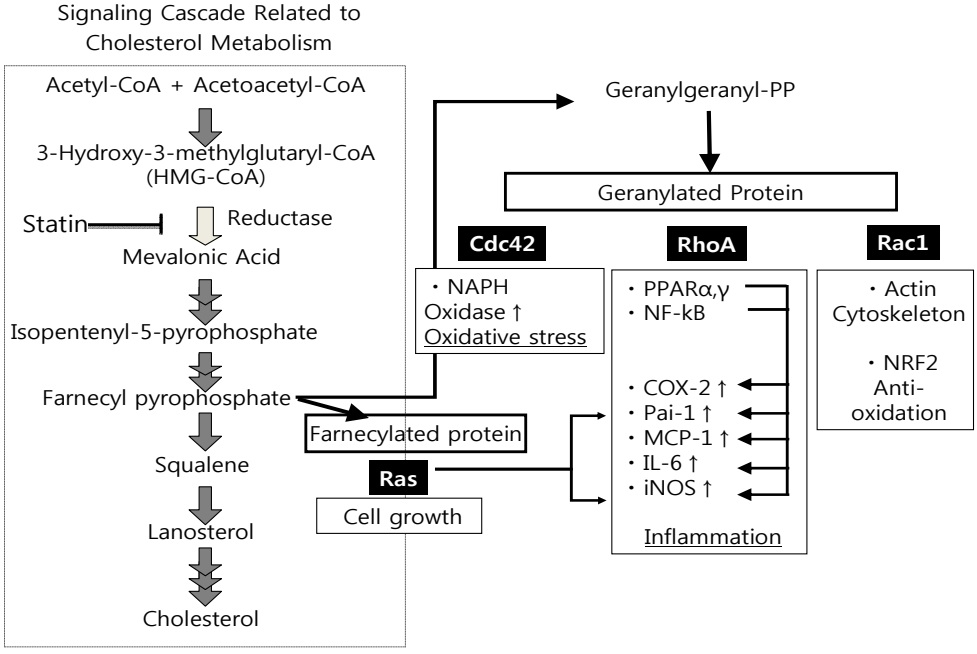

Fig. 1. Proposed molecular mechanisms of statin.

in our preliminary screening data for all the FDA-approved drugs for the effectiveness of colorectal cancer prevention, various statins suppressed TCF/LEF dependent transcriptional activity. By contrast, Salins et al. described lovastatin might inactivate GSK-3β activity, increase nuclear translocation of β-catenin, TCF-3 and LEF-1, resulting in induction of TCF/LEF-dependent gene reporter activity.[53]

4) Effects on Oxidative Stress Related Factors

Oxidative stress is also a key factor for the onset of CVD.[35] Statins have been reported to show a cardio-protective activity, and previous reports indicated that the production and the nuclear translocation of NRF2 were up-regulated by statin treatment, in a mevalonate-dependent and cholesterol-independent fashion.[54]

This NRF2 up-regulation was examined in many cells, including colorectal cancer cell lines, HT-29 and HCT116.[55] Simvastatin, a typical statin, induced dose-dependent up-regulation of NRF2 expression and stimulated NRF2 nuclear translocation. Simultaneously, the transcriptions of antioxidant enzymes (heme oxygenase-1 (HO-1), NQO1 and γ-glutamate-cysteine ligase catalytic subunit (GCLC)) were increased.

Molecular mechanisms of this NRF2 up-regulation are being explored now. Ihoriya et al. showed that NRF2 activity and NRF2-mediated antioxidant gene expression were up-regulated by rosuvastatin in human umbilical vein endothelial cells by reducing NRF2 degradation and accelerating the interaction between NRF2 and p21Cip1.[56] Jang et al. demonstrated the activation and nuclear translocation of NRF2 and the expression of various anti-oxidant enzymes via the ERK and PI3K/Akt pathways in colon cancer cells.[55] On the other hand, some experiments suggested the induction of Kruppel-like factor 2 (KLF2) by statins might be the primary cause of these pleiotropic actions attributed to antioxidant activity.[57,58] Yet another explanation is the formerly denoted deficit of GGPP, bringing about small G protein Rac-inactivation and anti-oxidant responses.[36]

5) Physiological Effects on Carcinogenesis-related Factors in Humans

Pleiotropic functions of statin were mainly focused on the inflammation-related phenomena and anti-oxidant matters. This could be confirmed by the sufficient number of the reports of down-stream gene expression of these two pathways in clinical trials. Regarding inflammation-related protein expression through NF-κB dependent transcription, there is a great deal of papers demonstrating the lowered production of CRP and inflammation-related cytokines, by statin administration.[59-61] From the aspect of anti-oxidant functions of statins, some theses have reported the up-regulation of the HO-1 and superoxide dismutase (SOD) genes that controlled by NRF2.[62,63]

3. Metformin

1) Background and Association with CRC Risk

Metformin, a FDA approved biguanide drug, is a widely used for management of type 2 diabetes mellitus. The results of case-control and prospective studies revealed that obesity is a strong risk factor for CRC, especially in men.[64-67] Obesity is also associated with insulin resistance and hyperinsulinemia that may be involved in CRC pathogenesis.[68] In addition to its function as a gluconeogenesis suppressor (anti-diabetes mellitus), metformin has recently been shown to possess strong anti-cancer properties.

The relation between metformin administration and reduction of CRC risk were confirmed by a meta-analysis of 37 studies with over 1.5 million total subjects published in 2013.[69] This report showed that metformin users compared with non-users, demonstrated its relative risk for colon cancer-incidence as 0.77 (95% CI, 0.64-0.91) and the mortality was 0.66 (95% CI, 0.45-0.97). Hosono et al. conducted clinical trials with non-diabetic subjects, and reported that oral administration of low-dose metformin (250 mg/d) for a month suppressed the formation of colorectal ACF70 without any adverse effects. Recently, the same group assessed the effects of long-term administration of low-dose metformin on adenoma and polyp recurrence. Low-dose metformin significantly reduced the prevalence and number of metachronous adenomas or polyps after polypectomy in an Asian population.[71] This evidence indicates that metformin has a suppressive effect on tumorigenesis and cancer cell growth in the human colon.

2) Effects on Inflammation-related Factors

At the cellular level, metformin activates AMP-activated protein kinase (AMPK), an energy sensor involved in the regulation of cellular metabolism. AMPK is activated by an increase of the intracellular AMP levels. Many studies have shown that activation of AMPK attenuates inflammatory reactions in different animal models, such as autoimmune encephalomyelitis,[72] LPS-induced lung inflammation,[73] cystic fibrosis[74] and colitis.[75] In these contexts, metformin is thought to suppress inflammatory reactions mediated by the transcription factor NF-κB. In fact, metformin pre-treatment reduced expression levels of IL-8 induction in COLO205 colon cancer cells stimulated with TNF-α attenuation of IκB α and NF-κB DNA-binding activity.[76] Moreover, Koh et al. demonstrated that metfor-

min administration significantly reduced IKK activation in a DSS-induced colitis model, and inhibited the development of colitic cancer in IL10$^{-/-}$ mice. Thus, metformin exerts an anti-inflammatory effect through the inhibition of NF-κB activation followed by inhibiting IκB degradation.

3) Effects on Differentiation-related Factors

The effect of AMPK activator or metformin treatment on Wnt activation or cell differentiation in colon cancer cells remains unclear. However, a recent study showed that metformin and another AMPK activator suppressed Wnt3a-induced TCF/LEF transcriptional activity in human osteoblast-like Saos-2 cells.[77] Lithium chloride (LiCl)-induced transactivation of TCF/LEF through inhibiting β-catenin degradation was suppressed by metformin treatment. Takatani et al. concluded that metformin attenuates TCF/LEF activation by reducing β-catenin protein levels.

4) Effects on Oxidative Stress-related Factors

Metformin enters into the cell through transporters, and inhibits mitochondrial complex I. By inhibiting mitochondrial complex I, metformin reduces production of ROS, oxidative stress and DNA damage[78], resulting in reduced risk of mutagenesis. Bordini et al. investigated the anti-oxidative effects of metformin with a 1,2-dinethylhydrazine induced colon carcinogenesis model in Balb/c mice.[79] Metformin (50 mg/kg) treatment significantly suppressed ACF formation at the distal colon section. Moreover, oxidative stress and nitric oxide related parameters were reduced in the metformin treated group compared with the control group. This may partly be explained the increased labeling index of NRF2 in metformin treated colonic epithelial cells.

5) Physiological Effects on Carcinogenesis-related Factors in Humans

Metformin decreases angiogenesis via NF-κB by increasing the antiangiogenic thrombospondin-1 in polycystic ovary syndrome patients.[80] Recent double-blind placebo-controlled study also showed that administration of metformin (500 mg/twice a day for 12 weeks) significantly decreased plasma concentrations and mRNA levels of IL-6 and TNF-β compared to the control group.[81] Interestingly, metformin treatment attenuated NF-κB DNA binding activity, not affecting the expression of the NF-κB p65 subunit, but inhibited its acetylation in mononuclear cells. Chakraborty et al. conducted a double blind randomized study with 208 type 2 diabetes patients for 24 weeks to examine the effect of metformin on the oxidative stress and inflammation parameters.82 ROS generation and advanced oxidation protein products were reduced by metformin treatment compare to a placebo. Lowered inflammation and oxidative stress status by metformin administration could be beneficial for CRC prevention.

4. Epigallocatechin-gallate (EGCG)

1) Background and Association with CRC risk

Green tea is a very popular drink all over the world. In the green tea, epigallocatechin-gallate (EGCG) is the major constituent among green tea catechins.[83] A large population-based case control study was examined in 1997.[84] In this study, 931 newly diagnosed colon cancer cases were compared to controls (n=1,552). An inverse association with colon cancer was observed in men with increasing amounts of green tea consumption, with OR in the highest tea consumption category (300 g/month) of 0.82. In women, the OR for the highest consumption category (200 g/month) was 0.67. There is a large prospective cohort study, in which 69,710 Chinese women were interviewed to determine green tea consumption habits.[85] During 6 years of follow-up, the risk of CRC was significantly decreased along with an increase of the amounts of green tea consumed. Moreover, there is a double-blind randomized clinical trial to determine the preventive effect of green tea extract (GTE) on recurrence of colorectal adenoma.[86] Patients who had no polyps after a year of endoscopic resection of one or more colorectal adenomas were recruited. Administration of GTE tablets for 12 months significantly reduced the incidence of metachronous colon adenomas. The size of relapsed adenomas was statistically smaller in the GTE group than in the control placebo group. These studies suggested that EGCG may have the potential to lower the risk of CRC.

2) Effects on Inflammation-related Factors

It has been shown that the galloyl and hydroxyl groups at the 3' position on EGCG are responsible for its strong anti-inflammatory properties.[87] On the other hands, COX-2 plays an important role in inflammation, and its overexpression was observed during colon tumorigenesis.[88] EGCG significantly suppresses COX-2 *mRNA* and protein overex- pression in human colon cancer cell lines, HT-29 and HCA-7 via modulation of NF-κB activation.[89] In animal experiments, EGCG markedly improved the disease activity index, colon mucosa damage index and histological scores in colitis of rats compared with the placebo groups.[90] In the same study, the production of NF-κB, TNF-α and IFN-γ in EGCG-treated colon tissue was significantly lower than that in rats treated with a placebo. Also in the mouse experiment, EGCG effec-

tively inhibited inflammation-related colon carcinogenesis.91 Administration of EGCG suppressed the protein and *mRNA* expression levels of *COX-2* and the *mRNA* expression of inflammatory cytokines (*TNF-α, IFN-γ, IL-6, IL-12 and IL-18*), target genes of NF-κB. Since NF-κB is considered to play a key role in the development and progression of human can cer, anti-inflammatory effects through the suppression of the NF-κB signaling pathway by EGCG could contribute to its chemopreventive efficacy.

3) Effects on Differentiation-related Factors

Treatment of the HT-29 colon cancer cell line with EGCG caused a significant inhibition of GSK-3α and GSK-3β activity.[92] A concentration-dependent decrease in phosphorylated β-catenin levels and overall reduction in β-catenin and its mRNA levels were also observed after EGCG treatment.[92] In the colon cancer cells, treatment of EGCG inhibited the Wnt/β-catenin pathway through the phosphorylation and degradation of β-catenin.[93] EGCG represses the expression of cyclin D1 and c-myc, which are identified as targets of the β-catenin/TCF/LEF nuclear complex, and inhibited the proliferation of colon cancer cells.[93,94] Animal studies using *Apc*-mutant Min mice also showed that EGCG treatment reduced tumor multiplicity by suppressing nuclear β-catenin levels, suggesting an inhibition of the Wnt mediator β-catenin translocation to the nucleus.[94,95]

4) Effects on Oxidative Stress-related Factors

Varying doses of EGCG significantly decreased 2-amino-3-methylimidazo [4,5-f]quinoline (IQ)-induced colonic formation of the total number of ACF and total AC in nude mice in a dose-dependent manner.[96] Furthermore, the expression of NRF2 and its downstream gene, UGT1A10, had a positive correlation with doses of EGCG administration. Similar results have been reported in the nude mice implanted orthotopically with colon cancer.[97] EGCG inhibited the tumor growth and liver and pulmonary metastases of orthotopic colon cancer compared with the control group.[97] The protein level of NRF2 and the mRNA levels of NRF2, UGT1A, UGT1A8 and UGT1A10 statistically elevated in EGCG-treated mice in comparison with those in the control group.[97] These reports showed that activating the NRF2-UGT1A signaling pathway by administration of EGCG could contribute to colon cancer prevention.

5) Physiological Effects on Carcinogenesis-related Factors in Humans

There are several clinical studies using green tea. One clinical study suggested that the oral ingestion of green tea causes a rapid decrease in the level of PGE2 in the rectal mucosa.[98] This study also showed that EGCG levels in intestinal mucosa are higher than other polyphenols such as epigallocatechin and epicatechin. A green tea containing EGCG is known to inhibit COX-dependent arachidonic acid metabolism in human colon mucosa and colon tumor tissues.[99] These results are speculated to be due to the inhibition of COX-2 activity by EGCG in the colorectum. In healthy male smokers, green tea catechin supplementation for two weeks resulted in the reduction of the plasma concentration of 8-OHdG, IL-6 and TNF-α.[100] Consumption of green tea with high-density catechins for 12 weeks in non-alcoholic fatty liver disease patients significantly decreased serum ALT levels and reduced urinary 8-isoprostane excretion compared with a placebo group.[101] In a double-blind, placebo-controlled trial study, obese/hypertensive patients received green tea extract for 3 months, resulting in considerable reduction in serum TNF-α and increasing the total antioxidant status compared with a placebo group.[102] These studies demonstrated that EGCG might have a beneficial effect on several human diseases through its properties of anti-inflammation and anti-oxidation.

6. Curcumin

1) Background and Association with CRC Risk

Turmeric (rhizomes of Curcuma longa) is prevalent in Asian countries as a dietary spice and colorant. Turmeric contains around 4% curcumin (diferuloymethane) by weight[103] and this is the major polyphenol in turmeric. Curcumin has proven beneficial properties, including anti-inflammation, differentiation and anti-oxidant effects in CRC as described below, and in addition helps the improvement of other conditions. The effects of curcumin are limited by uptake in clinical or animal activities because of its low solubility, rapid breakdown by alkali- or ultraviolet-light, and poor bioavailability that is considered to be approximately 1%.[104,105] In order to overcome these difficulties, several approaches are being tested, such as treating with a high dose, making analogs of curcumin, or include ing it as nanoparticles and so on, for clinical trials and animal experiments.

Curcumin has been proved to be safe with no side effects by oral administration in CRC patients.[106] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) defined an acceptable daily intake (ADI) value of 0-3 mg/kg BW per day for curcumin in 2004. Curcumin supplementation (360 mg/d) for 10-30 d decreased serum TNF-α levels and p53 expression in CRC patients.[107] It is also interesting to mention that intake of 4g/d curcumin resulted in a significant (40%) reduction in the total number

of colonic ACF in CRC patients but was almost as same as that of 2 g/d curcumin.[108] Here, we would like to focus on recent findings for anti-cancer, anti-inflammatory and anti-oxidative effects of curcumin against CRC.

2) Effects on Inflammation-related Factors

Molecular targets regarding inflammatory mediators of curcumin could be NF-κB. CRC patients provided 3.6 g curcumin/d for 4 months resulted in a significant decrease of plasma PGE2 production.[106] Curcumin at 0.002-16 g/kg in diet suppressed the incidence of ACF or adenoma along with attenuation of arachidonic acid metabolism in rats.[109,110] In addition, curcumin at 1 g/kg BW orally for 30 days suppressed the development of tumors with attenuation of cell cycle and activation of caspase in mice.[111] Curcumin inhibited COX-2 expression and NF-κB activation in human CRC cells via attenuation of the NIK-IKK pathway.[112,113]

3) Effects on Differentiation-related Factors

Curcumin at a dose of 20 μM treatment in human CRC cells brought about a reduction of binding activity of β-catenin and TCF/LEF to DNA.[114]

4) Effects on Oxidative Stress-related Factors

Curcumin not only quenches ROS directly but also translocates NRF2 into the nucleus, following transcriptional activation to induce antioxidant enzymes such as HO-1, GST, NQO1 and a relevant metabolite, GSH, with a decrease of some blood oxidative markers. Administration of curcumin 0.44 g/d for 29 d or 3.6 g/d for 7 d decreased a blood oxidative marker of 1-methylguanosine (M1G) in human CRC patients.[115,116] Curcumin (12.5 mg/mouse, i.p.) enhanced EpRE activity related to transactivation of NRF2 in the intestine after 24 h.[117] In addition, the potentiation of GST and NQO1 activities via NRF2 transactivation by curcumin were reported in human CRC cells.[118]

5) Physiological Effects on Carcinogenesis-related Factors in Humans

Patients with CD, depression and pancreatic cancer who were provided curcumin at a range of 0.55-8.0 g/d for several weeks showed significant anti-inflammatory response.[119,120] Particularly, the NF-κB expression levels in peripheral blood mononuclear cells were remarkably down-regulated in the patients with pancreatic cancer.[119] Administration of curcumin at about 0.5 g for 1-12 months brought about anti-oxidative effects for patients with tropical pancreatitis, type II diabetes mellitus and β-thalassemia/Hb E.[121-123] Little information has been found regarding cell differentiation associated with TCF/LEF activity activated by curcumin in clinical trials.

CONCLUSION

This review has focused on identifying and validating molecular targets by an overview of potential chemopreventive agents for colon carcinogenesis that might alter its activity. TCF/LEF, NF-κB and NRF2 could be prospective targets to prevent CRC in light of the numerous studies conducted with aspirin, statins, metformin, EGCG and curcumin.

Many molecules are considered to be up- and down-regulated depending on the stages of colon carcinogenesis. Pesson et al. tried to characterize the change of gene expression by microarray in the progression from colorectal normal mucosa to adenoma, and then adenocarcinoma.[124] Interestingly, 2,393 probes were abnormally expressed in adenoma, even more than in adenocarcinoma (1,805 probes), as compared to normal mucosa. In both adenoma and adenocarcinoma samples, 954 probes were commonly deregulated. A number of TCF/LEF-, NF-κB- and NRF2-related and target genes are included in these commonly deregulated genes.

Effects on NF-κB (anti-inflammation) and NRF2 (anti-oxidative stress) transcriptional activities, but not the effects on TCF (cell differentiation or stemness), are relatively well studied with many drugs and natural compounds in humans. Recently, the mechanisms underlying the maintenance of stemness in intestinal stem cells were revealed by Clevers's group.[125] Since maintaining the stemness by Wnt signaling activation is plays an important role in initiation and promotion stages of colon carcinogenesis, a new screening system is demanded that assess the effects on transcriptional activities of TCF/LEF, NF-κB and NRF2 to identify novel and promising chemopreventive agents.

Authors' contributions

SM, MT, RI, GF, MM wrote the manuscript.

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