I. Abstract

Colorectal cancer (CRC) is one of the most common human malignancies and a leading cause of cancer-related deaths in developed countries. Identifying effective preventive strategies aimed at inhibiting the development and progression of CRC is critical for reducing the incidence and mortality of this malignancy. The prevention of carcinogenesis by anti-inflammatory agents including nonsteroidal anti-inflammatory drugs (NSAIDs), selective cyclooxygenase-2 (COX-2) inhibitors, and natural products is an area of considerable interest and research. Numerous anti-inflammatory agents have been identified as potential CRC chemopreventive agents but vary in their mechanism of action. This review will discuss the molecular mechanisms being studied for the CRC chemopreventive activity of NSAIDs (i.e. aspirin, sulindac, and ibuprofen), COX-2 inhibitors (i.e. celecoxib), natural products (i.e. ...
curcumin, resveratrol, EGCG, genistein, and baicalein), and metformin. A deeper understanding of how these anti-inflammatory agents inhibit CRC will provide insight into the development of potentially safer and more effective chemopreventive drugs.

II. Introduction

Chemoprevention strategies utilizing natural products or synthetic drugs provide opportunities to prevent or delay the development and reverse the progression of cancer. Carcinogenesis is a multistage process often occurring over decades that involves cumulative molecular and cellular aberrations. The stages consist of initiation, promotion and progression in which, each stage involves a disruption of a multitude of signaling cascades (126). This multistage sequence of biological processes provides opportunities to target specific biochemical targets by small molecules for cancer intervention (99). Cancer chemoprevention strategies can be divided into three types: 1) primary prevention, a strategy aimed to prevent cancer in all individuals; 2) secondary prevention, a strategy to prevent cancer in individuals with premalignant conditions; and 3) tertiary prevention, a strategy to prevent further disease progression (i.e. metastasis) after an initial diagnosis of malignant disease (47, 56). The use of non-steroidal anti-inflammatory drugs (NSAIDs) and certain natural products has been shown to prevent CRC during each stage of carcinogenesis. The preventive activities of both NSAIDs and natural products have been a major focus of research involving in vitro and in vivo experimental models, epidemiologic studies, and clinical trials (38, 47, 99). Chronic inflammation is associated with the development of cancer and promoting progression into a malignant phenotype (25). Because of this association, the use of anti-inflammatory agents to disrupt and retard carcinogenesis is considered to provide a highly effective strategy for cancer chemoprevention.

III. Colorectal Cancer

Colorectal cancer is the third leading cause of cancer related-deaths in men and women in the United States (U.S.) (6). CRC is the second most commonly diagnosed cancer in women and the third in males world-wide (52). Colorectal cancer rate trends are higher in developed countries in comparison to developing countries, which is supported by evidence from ecological, migrant, and secular trend studies suggesting that environmental, dietary, and lifestyle factors may be important causes of CRC (9, 52). This trend may be due to the variations in risk factors such as diet, red meat consumption, cigarette, and alcohol consumption (52). In Western countries, especially the United States, there is a recent decline in CRC incidence rates that may be due to increased screening (i.e. colonoscopy and fecal occult blood test). Increased screening allows for the early detection and removal of precancerous polyps (52, 107). Indeed, the 5-year survival rate of CRC if diagnosed at an early and localized stage is 90% (6, 99). Unfortunately, greater than 50% of patients are diagnosed with advanced disease when distant metastasis has already occurred in which the 5-year survival rate precipitously drops to 10% (6, 99, 128). The use of anti-inflammatory agents could substantially reduce the incidence and mortality of CRC (50), although safety and efficacy are important considerations for developing such approaches.
Colorectal cancer can be divided into two classes: 1) sporadic where there is no (or very little) prior family history of CRC, and 2) familial or hereditary in which inherited gene mutations predispose an individual to develop CRC. Only about 5-10% of CRCs are due to inherited gene defects, while the majority of CRC cases are sporadic (2). Although age, sex and ethnicity are risk factors for CRC, there are key lifestyle risk factors that can be moderated including smoking, physical inactivity, obesity, heavy alcohol consumption and diets high in saturated fats (52, 101, 125). Diets high in red and processed meat intake are also associated with increased risk of CRC development (101, 125). Red and processed meats have been shown to be high in DNA-damaging N-nitroso compounds as well as meats cooked at high temperatures (i.e. grilled, charbroiled or blackened) that contain high levels of mutagenic heterocyclic amines (125).

In 1990, Fearon and Vogelstein described colon tumorigenesis as a multistep process in which the accumulation of multiple genetic mutations is required for cancer initiation, promotion and progression (31). The colorectal mucosa consists of a large number of invaginations called the crypts of Lieberkühn. Epithelial cell renewal occurs in these crypts through a series of events involving proliferation, differentiation, and migration (124). The progression of CRC is though stages that range from single crypt lesions (e.g. aberrant crypt foci) to adenomatous polyps to malignant carcinomas (77). The progression of these stages is believed to require mutations in multiple oncogenes and tumor suppressor genes (124). The progression of precancerous cells into an invasive phenotype can span over a decade (42). A key molecular step in colorectal tumorigenesis is the loss of genomic stability. Genomic instability is the hallmark of CRC and creates an environment suitable for alterations in tumor suppressor genes and oncogenes (35, 50). Identification of anti-inflammatory agents that suppress and/or prevent the consequences of genetic mutations that give rise to colorectal cancer is therefore of critical importance.

Hereditary CRC

There are multiple inherited conditions that predispose an individual to CRC. The two major hereditary CRC syndromes are hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). HNPCC, or Lynch syndrome, is the most commonly inherited CRC syndrome (15). Patients with HNPCC have an 80% chance of developing colorectal cancer (128). HNPCC is a distinct autosomal dominant inheritable condition caused by germline mutations in DNA mismatch repair (MMR) genes. The loss of MMR function leads to mutations in microsatellites (repetitive sequences) and microsatellite instability (MSI) thus causing a mutable phenotype (15, 66). The primary MMR enzymes associated with this MSI are MLH1 and MSH2 (69, 128). It is estimated that HNPCC accounts for 1-5% of CRC cases. FAP is rare autosomal dominant disorder due to germline mutations in the adenomatous polyposis coli (APC) gene, resulting in loss of function of the APC protein, which has tumor suppressor function (128). FAP is characterized by the occurrence of hundreds to thousands of colorectal polyps, which if not removed, can develop into adenocarcinoma (15, 66).

The APC protein plays a key regulatory role in suppressing the oncogenic Wnt/β-catenin pathway (104). In FAP, mutations in the APC gene by insertions, deletions, or nonsense mutations result in a truncated APC protein. The truncated APC protein is associated with loss of β-catenin binding sites, thus allowing for the constitutive activation of β-catenin signaling (73,
In colonocytes in the absence of Wnt signaling, cytoplasmic β-catenin is normally degraded by the Axin complex (73). This complex consists of APC, the scaffold protein, Axin, casein kinase 1 (CK1), and glycogen synthase kinase 3β (GSK3β). CK1 and GSK3β phosphorylate β-catenin resulting in the ubiquitination and proteasomal degradation of β-catenin (71, 73). The degradation of β-catenin prevents its availability to act as a nuclear co-activator of the transcription factor family, T cell factor/lymphoid enhancer factor (TCF/LEF), thus inhibiting Wnt target gene expression such as c-Myc, cyclin D1, survivin and PPAR (44, 72, 73, 95).

In unstimulated cells free β-catenin levels are regulated by ubiquitination and proteasomal degradation through the promotion of phosphorylation by the APC/Axin/GSK3β complex (72). The Wnt/β-catenin pathway is activated when Wnt ligands (e.g. WNT3A), cysteine-rich proteins consisting of 350-400 amino acids and containing a N-terminal secretion peptide, bind the Frizzled receptor and its co-receptor low-density lipoprotein receptor-related protein 6 (LRP6) or LRP5 (73). This complex along with the scaffold protein Dishevelled (Dvl) results in the recruitment of the APC/Axin/GSK3β complex, thus preventing β-catenin degradation, leading to its translocation into the nucleus and binding of TCF/LEF to stimulate transcription of proliferative and oncogenic genes (i.e. c-myc and cyclin D1) (71, 73, 127). In both inherited and sporadic CRC, mutations in the APC gene results in the deregulation of β-catenin degradation and thus leads to increased stability and activation of β-catenin transcriptional activity (Figure 4) (8). Disruption of the APC pathway is one of the most prevalent genetic events identified in CRC that is altered in 95% of colorectal tumors (77). Therefore, identifying inhibitors that target this pathway either directly or indirectly can delay or prevent the development and/or progression of CRC.

IV. NSAIDs and COX-2 inhibitors

Non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors (Coxibs) have been proven to be promising agents for chemoprevention of CRC. The suppressive effect of these agents on tumorigenesis has been supported by a number of epidemiological studies, clinical trials and animal studies. NSAIDs and Coxibs inhibition of tumorigenesis is believed to be mediated through multiple cellular processes including apoptosis, cell-cycle arrest, and inhibition of angiogenesis (40, 50). For the purposes of this review, the NSAIDs and Coxibs discussed are aspirin, sulindac, ibuprofen and celecoxib (Figure 1). The non-COX inhibitory sulfone metabolite of sulindac, which is not a NSAID is also discussed. A commonality of both NSAIDs and Coxibs is the inhibition of COX enzyme activity, although they vary in their potency and selectivity to inhibit COX-1 and/or COX-2.

There are three isoforms of cyclooxygenase (COX) enzymes. COX-1 is widely distributed, constitutively expressed, regulates renal blood flow and protects intestinal mucosal integrity (115). COX-2 is highly inducible and has been shown to play a role in tumor progression, while COX-3 is a splice variant form of COX-1 (42, 116). COX-1 and COX-2 catalyze the rate-limiting step in metabolic conversion arachidonic acid (AA) to prostaglandins (PGs) and thromboxanes (131). The COX-2 gene is an early response gene transcriptionally regulated by neoplastic and inflammatory stimuli (i.e. cytokines, growth factors and mitogens), and plays an important role in tumorigenesis (42, 116). COX-2 levels have been demonstrated to be elevated in premalignant and malignant tissues (116). In 85-90% of CRC and 40-50% of...
adenomas, COX-2 expression is over-expressed and associated with lower survival of patients
with CRC (116, 128). Both COX enzymes catalyze the conversion of AA into prostaglandin H2
(PGH$_2$) which is the PGs precursor (Figure 5) (40). PGs, specifically PGE$_2$ has been shown to be
an important mediator of the pro-inflammatory and tumor-promoting effects of COX-2 (66).
PGE$_2$ induces cell growth and numerous processes associated with inflammation (51). The
degradation of PGE$_2$ is catalyzed by the tumor suppressor, 15-prostaglandin dehydrogenase (15-
PGDH) which has been shown to be down-regulated in FAP patients and is a target of β-catenin
gene repression (110, 116). The increased production of PGE$_2$ is correlated with lipid
peroxidation and the formation of DNA adducts to accelerate colon carcinogenesis (85).

The inhibition of COX and PG synthesis may not be the only mechanisms of action of NSAIDs. Although the initial mechanism thought to be responsible for the chemopreventive activity of NSAIDs was attributed to the inhibition of COX, it is now recognized that the chemopreventive activity may involve COX-independent mechanisms (3, 39, 90, 93, 106).

Aspirin

NSAIDs are a chemically diverse group of compounds that inhibit COX-1 and COX-2 and share similar side effects (32). The oldest and most common NSAID is aspirin (acetylsalicylic acid), which irreversibly inhibits both COX-1 and COX-2, by acetylation of serine residues Ser529 and Ser516 in the catalytic domain (40, 66). However, the inhibition of COX-1 and COX-2 is concentration dependent. At low concentrations aspirin preferentially inhibits COX-1, while higher concentrations will inhibit both COX-1 and COX-2 (123). The irreversible inhibition of COX due to acetylation is a unique property of aspirin that is not shared by other NSAIDs. Aspirin inhibits PG synthesis in tumors, but the effect is not complete, which may explain its modest efficacy or suggest that other mechanisms are involved (104).

Because of its widespread use for preventing heart attacks and strokes, aspirin is the most investigated NSAID for CRC prevention in epidemiological studies (Figure 1A) (19). An inverse association between aspirin use and CRC risk has been observed in numerous cohort and case-control studies (19, 119). In the Cancer Prevention Study II men and women showed that aspirin use at least sixteen times per month over 6-year period was associated with a 40% reduced risk of colon cancer mortality. The U.S. Health Professionals and U.S. Nurses’ Health studies showed similar findings that regular aspirin use (>2X/week) was associated with a reduced risk of CRC with 18-20 years follow up (19). A consistent observation of these studies is that the benefits of aspirin use increase with longer duration of use (19). Although aspirin has been shown to be an effective chemopreventive agent in sporadic CRC, it has shown little efficacy in clinical trials of hereditary CRC. In the Colorectal Adenoma/Carcinoma Prevention Programme 1 (CAPP1) and CAPP2 studies, aspirin use did not significantly inhibit polyp formation in patients with FAP or Lynch syndrome (19).

A COX-independent mechanism for the chemopreventive activity of aspirin may involve the inhibition of the transcription factors, specificity proteins (Sp) Sp1-4. Sp proteins regulate the expression of several critical genes involved in cell proliferation, cell survival, angiogenesis and inflammation pathways (21). Aspirin and its major metabolite, sodium salicylate, inhibit the expression of Sp1, Sp3 and Sp4 through caspase-dependent proteolysis in colon cancer cells (88). The COX-2 inhibitor, celecoxib, can downregulate Sp1 and Sp4 proteins in several colon cancer cells (1). Aspirin has been reported decrease colon tumor growth and Sp1, Sp3 and Sp4 expression in a xenograft model (88). The inhibition of expression and activity of Sp1, Sp3 and
Sp4 by aspirin may be a potential explanation for the anti-tumorigenic activity of aspirin that involves a COX-independent mechanism.

**Sulindac**

The NSAID, sulindac is a prodrug with a sulfoxide moiety that requires in vivo reduction to the pharmacologically active sulindac sulfide (SS) by colonic bacteria (18). Sulindac and SS are irreversibly oxidized to a sulfone metabolite following oral administration (Figure 2). SS is known to be a potent inhibitor of COX-1 and COX-2 enzyme activity in comparison to the sulindac sulfone metabolite which does not inhibit COX-1 or COX-2 (91, 92). In clinical trials, FAP patients had reduced size and number of polyps after 6-9 months of treatment with sulindac (33). Sulindac was also shown to reduce and/or regress adenomatous polyps in sporadic CRC patients (76).

Sulindac sulfone (exisulind) demonstrates an anti-tumor effect that is independent of COX inhibition in multiple rodent models of cancer including colon, mammary, lung, bladder, and prostate (75, 81, 90, 94, 102, 117). In colon cancer cells, sulindac sulfone inhibits tumor cell growth by inducing apoptosis and causing cell cycle arrest (93). Sulindac sulfone was later demonstrated to reduce the number of colonic neoplasms in AOM-treated rats in comparison to controls (90) and with modest activity in patients with sporadic polyposis (7), but never received FDA approval because of hepatotoxicity.

Mechanistic studies showed that sulindac sulfone can suppress oncogenic β-catenin signaling by inhibiting cyclic guanosine monophosphate phosphodiesterase (cGMP PDE) (118). Cyclic nucleotide PDEs are an important enzyme superfamily responsible for regulating second messenger signaling by hydrolyzing the 3’,5’-phosphodiester bond in cGMP or cAMP to suppress cyclic nucleotide signaling. The inhibition of cGMP PDEs lead to the increased levels of cGMP and activation of protein kinase G (PKG) (17). Activation of PKG has been shown to induce apoptosis in colon cancer cells (28). While sulindac sulfone inhibits cGMP PDE activity at concentrations equivalent to those required for apoptosis, its inhibitory activity did not appear to be selective for any specific PDE isozyme. Sulindac sulfide, however, showed greater potency and selectivity to inhibit specific cGMP PDEs (i.e. PDE2, 3, 5 and 10), consistent with its improved potency to inhibit colon tumor cell growth (40). The selective inhibition of the cGMP-specific PDE5 isozyme by SS was reported to be critical for the induction of apoptosis and inhibition of the Wnt/β-catenin signaling pathway in human colon cancer cells, although the involvement of additional or alternative cGMP PDE isozymes could not be ruled out (120).

SS and other NSAIDs also induce the expression of NSAID-activated gene 1 (NAG-1). Studies investigating the proapoptotic and anti-tumorigenic activity of NAG-1 suggest that it acts as a tumor suppressor gene (11, 12). Increased NAG-1 expression inhibited tumor growth and development in mouse models of CRC (12). Colon cancer cells treated with SS showed an increase at both the mRNA and protein levels of NAG-1 (10). The mechanism of SS regulation of NAG-1 appears to be associated with the transcription factor, early growth response-1 (EGR-1) (10). The inhibition of the Wnt/β-catenin pathway and induction of NAG-1 provide a more detailed understanding of the COX-independent mechanisms responsible for the chemopreventive activity of sulindac.

**Ibuprofen**
The NSAID, ibuprofen is a non-specific COX-1 and COX-2 inhibitor, which can also inhibit the Wnt/β-catenin pathway through the increased phosphorylation and degradation of β-catenin (Figure 1C) (36). Additionally, ibuprofen can inhibit activation of the nuclear factor-kappa B (NF-κB) pathway (36). NF-κB is an inducible transcription factor that regulates immune responses, cell proliferation and survival, and is critical in the activation of the pro-inflammatory pathway in CRC (2, 132). NF-κB is a master transcription factor that promotes transcription of target genes through the recruitment of coactivators and corepressors (132). Several CRC risk factors have been shown to activate NF-κB, including saturated fatty acids, fried foods, grilled meat and environmental pollutants (2). In unstimulated cells, NF-κB is sequestered in the cytoplasm bound to inhibitory IκB proteins. Upon stimulation, activation events of the IκB kinase (IKK) complex results in the proteasomal degradation of the IκB proteins and release of NF-κB. The release of NF-κB allows for nuclear translocation and transcriptional activity to be activated (82). NF-κB regulates several genes ranging in their cellular activity from immune and inflammatory (e.g. iNOS) gene expression, cell proliferation (e.g. c-myc, cyclin D1 and COX-2) and apoptosis. NF-κB also regulates genes that mediate invasion (e.g. MMP-9) and angiogenesis (e.g. VEGF and TNF) (2, 64, 132). Constitutive activation of the NF-κB pathway has been demonstrated in CRC (2). The ability of NSAIDs to suppress these COX-independent mechanisms provides alternative targets for the development of potentially safer and more efficacious drugs for CRC chemoprevention.

Celecoxib

The long-term use of NSAIDs for CRC chemoprevention is not recommended because of potentially serious side effects, including gastrointestinal ulceration and perforation that have been attributed to the inhibition of COX-1. In an effort to avoid these side effects, selective COX-2 inhibitors such as celecoxib and rofecoxib were developed to spare COX-1 (37). Clinical studies have shown that Coxibs have anti-inflammatory activities similar to traditional NSAIDs, but with less gastrointestinal toxicity (37). COX-2 inhibitors however, are associated with renal toxicity (136) and increased risk of cardiovascular side effects including increased blood pressure, stroke and myocardial infarction (37, 79). However, clinical trials with celecoxib have demonstrated contradicting results in which cardiovascular side effects were associated with long-term use or no cardiovascular side effects were found (Figure 1D) (37). Nonetheless, adverse cardiovascular effects associated with specific COX-2 inhibitors have limited their development for CRC chemoprevention because high dosages are necessary. Clinical trials have demonstrated the efficacy of celecoxib in reducing mean size and number of colorectal polyps in FAP patients (19). In 1999, celecoxib was approved by the FDA for the adjuvant treatment of FAP patients (37), but its use was suspended in 2004 by the manufacture, Pfizer (122).

Celecoxib and SS inhibit the expression and activity of survivin in human colon cancer cells (97, 121). Survivin is a bifunctional member of the inhibitor of apoptosis (IAP) family and is an essential regulator of cell proliferation and survival (4, 61). Under normal conditions, survivin is primarily present during embryonic development but is undetectable in most adult tissues. Survivin is highly tumor specific and is overexpressed in CRC and almost all other human cancers (4, 61). Survivin expression is used as a diagnostic biomarker of tumor onset and recurrence, unfavorable disease outcome and a target for cancer drug discovery (4).
V. Natural Products/Dietary Phytochemicals

Resveratrol, Curcumin, EGCG, Genistein, Baicalein

Resveratrol

Resveratrol (3,5,4’-trihydroxy-trans-stilbene) is a phytoalexin, present in grapes, red wine, peanuts, mulberries and other plants (Figure 3A) (87, 108, 114). It is an antioxidant due to its aromatic structure which acts as a free radical scavenger. Resveratrol has several mechanisms of action, which demonstrate its potent anti-inflammatory and anti-carcinogenic effects. One of the antioxidant mechanisms include increased expression of the enzyme manganese superoxide dismutase (MnSOD), which converts the highly reactive and damaging free radical, superoxide, into molecular oxygen and hydrogen peroxide (16). The anticancer activity of resveratrol in colon adenocarcinoma cells appears to involve the inhibition of matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) signaling. Inhibition of MMP-9 activation by resveratrol can inhibit metastasis and VEGF-VEGFR to inhibit angiogenesis (16, 63). In addition, resveratrol induces apoptosis and can reduce the constitutive activation of NF-κB in colon cancer cells (2).

Resveratrol has also been reported to inhibit inducible nitric oxide synthase (iNOS) expression in colon cancer cells (83). Nitric oxide (NO) is synthesized by three NO synthase isoformes, two of which are calcium/calmodulin-dependent and constitutively expressed, while iNOS is inducible by cytokines and proinflammatory agents, and is calcium independent (22). The production of NO can exert beneficial effects (i.e. anti-microbial and anti-apoptotic), however, overproduction can result in cellular injury and inflammation (64). NO plays an important role in colon tumorigenesis and increased expression and activity of iNOS has been demonstrated in human CRC tissues and animals models (85). Although the mechanism by which resveratrol acts is promising, it is rapidly metabolized following oral administration thus preventing its development as a chemopreventive agent (14).

A number of studies have shown the ability of resveratrol to induce apoptosis and inhibit cell cycle progression of colon cancer cells (87). The induction of apoptosis by resveratrol has been attributed the clustering of Fas receptors, resulting in the formation of the death inducing signaling complex, induction of ER stress, activation of caspases, and release of cytochrome C (87). In vivo studies where resveratrol was orally administered showed that it can prevent the development of intestinal tumors in the APCMin mouse model, an animal model of human colorectal cancer. Mice treated with resveratrol showed a 70% reduction of intestinal tumors in comparison to the control group. Furthermore, resveratrol inhibited the expression of cell cycle progression and cell proliferation genes but increased expression of genes involved in immune response and inhibition of tumor promoting pathways (103).

Curcumin

Curcumin [diferuloylmethane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the major yellow pigment in turmeric that is isolated from the roots (rhizomes) of Curcuma longa Lim (Figure 3B). Curcumin has been shown to have anti-inflammatory and antioxidant properties, and induce apoptosis (38, 101). Curcumin mechanism of inducing apoptotic cell death appears to vary depending on cancer type, cell type and experimental conditions. In murine models, curcumin reduced the formation of early preneoplastic lesions in
the colon and significantly inhibited the incidence and multiplicity adenocarcinomas in the azoxymethane (AOM) induced rat model of colon carcinogenesis (101). A Phase I clinical study with orally administered curcumin reported that it is a safe agent with no observed toxicity, but has low systemic bioavailability (105). Although curcumin has a low bioavailability, it has been shown in multiple human cancer cell lines to inhibit several key pathways involved in colon tumorigenesis (2).

One of curcumin’s mechanisms of action is the inhibition of the NF-κB pathway. Curcumin suppressed NF-κB activation by known inducers; TNF-α, phorbol ester and hydrogen peroxide (64, 109). In addition to inhibition of the NF-κB pathway, curcumin has been shown to inhibit the phosphatidylinositol-3-kinase (PI3K) / Akt / mammalian target of rapamycin (mTOR) (PI3K/Akt/mTOR) pathway in CRC (16). The activation of the PI3K/Akt pathway is associated with colon tumorigenesis (23). Akt is a proto-oncogene, which is activated upon phosphorylation by phosphoinositide 3-kinase (PI3K). Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene and negative regulator of Akt. PTEN inhibits the phosphorylation of Akt though its inhibition of PI3K (23). Impaired expression of PTEN by mutations or loss has been reported in 60-70% of human CRC (84). Activation of the NF-κB pathway has been shown to be regulated by the downstream signaling of activated Akt (70). In addition to inhibition of the NF-κB pathway, curcumin also induces p53-independent apoptosis and inhibits the WNT/β-catenin pathway in human colon cancer cells (64).

In combination studies involving human cancer cell lines curcumin has synergistic activity with other natural products including resveratrol (87), catechins (64), genistein and quercetin (38). Quercetin (3,3’,4’,5,7-pentahydroxyflavone) is a flavonoid with antioxidant activity found in various fruits, vegetables, tea and wine has been reported to induce apoptosis in human colon cancer cells (Figure 3C) (60). In a study of 5 patients with FAP, the combination of curcumin and quercetin effectively decreased the number (64%) and size (50.9%) of adenomatous polyps (26). The combination of resveratrol and curcumin synergistically inhibited the growth of p53-positive and p53-negative colon cancer cells in vitro and p53-positive cells in vivo (74). Curcumin in combination with chemotherapy drugs also enhances their anticancer efficacy in various cancer types (38). The combination of curcumin with 5-fluorouracil (5-FU) significantly inhibited cell growth compared to 5-FU or curcumin alone in human gastric carcinoma cells (67). In an orthotopic pancreatic cancer model curcumin enhanced the anticancer activity of gemcitabine (68).

EGCG

Epigallocatechin gallate (EGCG) is polyphenol found in green tea (Camellia sinensis L.) that has antioxidant, anti-inflammatory and chemopreventive activities (Figure 3D) (30, 114). The green tea catechins consist of (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG), which are strong antioxidants (30). Of these catechins, EGCG has been found to be the most effective chemopreventive agent and has been studied in several cancer models (20). Oxidative stress and the production of reactive oxygen species (ROS) play an important role in cancer development and progression. ROS generation performs a critical role in cancer initiation and the metabolic activation of procarcinogens (20). During oxidative stress or other toxic insult normal cells maintain homeostasis through the activation of the transcription factor nuclear factor-erythroid 2p45 (NF-E2) related factor-2 (NRF-2). NRF-2 regulates the expression of several antioxidant genes and protective enzymes (55). During oxidative stress NRF-1 and NRF-2 heterodimerize
and bind the antioxidant responsive element (ARE) sequence to activate transcription (114). Under normal conditions NRF-2 is sequestered in the cytoplasm by the negative regulator Kelch-like ECH-associated protein 1 (KEAP-1). NRF-2 target genes include glutathione S-transferase, NAD(P)H quinone oxidoreductase (NQO1), and other Phase II detoxifying and antioxidant enzymes. EGCG has been reported to enhance the activity and expression of NRF2 and its down-stream gene uridine 5’-diphosphate-glucuronosyltranferases (UGT) 1A in colon cancer cells and BALB/c mice (135). The transcriptional activation of NRF-2 by EGCG has been reported in multiple human cancer types including; colon, gastric, hepatocellular, and pancreatic carcinoma cells (38, 114). The antioxidant activity of EGCG is an important mechanism of its anticancer activity but additional mechanisms have been reported.

Alternative mechanisms of EGCG’s anticancer activity are the inhibition of lipoxygenase (LOX) and COX activity (46) as well as the inhibition of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) expression (113). EGCG and other catechins, inhibit COX-dependent AA metabolism in both human colon tumor and normal mucosal microsomes (46). EGCG inhibited protein expression of proangiogenic factors (i.e. VEGF and bFGF) in human colorectal cancer cells. Using APCMin mice, animals treated with EGCG showed significant reduction of polyp formation and tumor load in comparison to controls. The EGCG treated animals also showed a significant reduction of bFGF levels in tumors (113). Human colon cancer cells and xenograft studies have shown that EGCG can inhibit the Erk-1 and Erk-2 signaling pathway, which leads to the increased expression of VEGF mRNA (54). Inhibition of Notch2 expression and its target protein, HES1, has been suggested to be an alternative mechanism by which EGCG inhibits CRC cell proliferation, and induces apoptosis. Notch signaling plays a critical role in the renewal and differentiation of CRC stem cells (53). These studies suggest potential mechanisms of EGCG that may be explored further for strategies in the prevention of colorectal cancer.

Genistein and Baicalein

Flavonoids are a group of polyphenolic compounds, which are plant secondary metabolites and present in many fruits, vegetables and all vascular plants (13, 80). The classification of flavonoids is based upon their chemical structure and the positions of substitutes present on the parent molecule. Flavonoids are made up of flavonols, flavonones, flavanols, flavan-3-ols, and isoflavones (80). The different classes of flavonoids have been studied for their pharmacological activities and biological properties (13, 80). The activity of selective flavonoids as potential colorectal cancer chemopreventive agents has been the focus of several studies; however, their mechanism of action is still under investigation. In this review we have briefly discussed the flavonol, quercetin and its combinatorial effects with curcumin. For this section we will focus primarily on the isoflavone, genistein and briefly discuss the activity of the flavone, baicalein, and their potential mechanism(s) as colorectal chemopreventive agents.

Genistein (4’,5,7-trihydroxy-isoflavone) is one of three major soy isoflavone aglycones, the other two are daidzein and glycitein (100). Genistein is a phytoestrogen and considered to be the primary anticancer component of soybeans (Figure 3E) (86). Several epidemiological studies have supported that diets high in soy intake contribute to the lower incidence of several cancer types (i.e. breast, endometrium, prostate and colorectal cancer) (24, 86). In breast and prostate cancer, the preventive activity of genistein is believed to be through its antiestrogenic activity (5). However, genistein has been shown to inhibit growth of both hormone-dependent and hormone-independent cancer cells. The chemopreventive activity of genistein has been further
contributed to its modulation of cell cycle, induction of apoptosis, anti-oxidant activity, and anti-inflammatory activity (5).

In the AOM-induced colon cancer rat model, rats fed a diet rich in soy had approximately 76% lower colon tumor incidence in comparison to controls (41). In multiple cancer types, genistein has been shown to be an inhibitor of protein kinase C (PKC), topoisomerase II and ribosomal S6 kinase (86). PKC is a family of serine/threonine protein kinases that play central roles in cell proliferation, differentiation and apoptosis. The PKC isoyme, PKCβII has been shown to be highly induced in the preneoplastic lesions and colon tumors of AOM-treated animals compared with the normal colonic epithelium (34). In transgenic animals overexpressing PKCβII in the colonic epithelium were more susceptible to colon tumor formation after exposure to AOM (34). PKCβII has been further been shown to induce COX-2 gene expression through stabilization in colon cancer cells (133) suggesting that the inhibitory effect of genistein on tumorigenesis may involve the suppression of COX-2 expression.

Genistein has also been reported to inhibit cell growth, induce apoptosis and stimulate G2/M phase cell cycle arrest in a time and dose dependent manner through the activation of ataxia telangiectasia mutated (ATM)/p53-p21 regulatory network in multiple human CRC cell lines (134). Genistein has also been shown to inhibit cell proliferation and induce apoptosis through the inhibition of IGF-1 and the PI3K/AKT pathway (59). Another anti-proliferative mechanism of genistein has been demonstrated through its inhibition of epidermal growth factor (EGF) and activation of forkhead box O3 (FOXO3) (98). Active FOXO3 negatively regulates proliferation and is critical for EGF-mediated proliferation (98). An additional mechanism of genistein activity in colon cancer cell lines is the regulation of gene transcription through epigenetic modifications. The activity of a demethylation agent (130) and inducer of histone modifications have both been demonstrated by genistein (129). Genistein inhibits the WNT/β-catenin pathway through demethylation of multiple promoters and histone acetylation (129).

The flavonoid baicalein has been studied for its potential as colorectal cancer chemopreventive agent (Figure 3F). Baicalein (5,6,7-trihydroxyflavone) is extracted from the root of *Scutellaria baicalensis* Georgi (57). Baicalein has been reported to have anti-inflammatory and anti-tumorigenic activities in various cancer models (58). Using CRC tumor cell models treatment with baicalein has been shown to inhibit cell proliferation, induce apoptosis, and inhibit selective pathways (i.e. PI3K/AKT and NF-κB). In a study performed to determine a potential mechanism of inhibition by baicalein in HT29 colon cancer cells, baicalein induced apoptosis in a p53-dependent manner. In HT-29 xenografts baicalein inhibited the growth an activation of the PI3K/AKT pathway (62). An additional study demonstrated that treatment with baicalein inhibited cell growth, induced apoptosis, and suppressed NF-κB activation in a HCT116 colon cancer cell model (57). This study also demonstrated the inhibitory effects of baicalein on colon tumor growth in an AOM and pro-inflammatory, dextran sulfate sodium (DSS), colitis-associated colon cancer mouse model (57). Baicalein was further shown to induce expression of the Pregnane X Receptor (PXR) in human colon cancer cells (i.e. HCT116 and LS174T) and *in vivo* through a caudal type homeobox 2 (Cdx2) dependent mechanism (29). The PXR is a nuclear receptor which transcriptionally regulates several drug metabolism and transporter genes (29, 78). Treatment of colon cancer cells with baicalein induced Cdx2, an intestinal cell differentiation factor, transcriptional regulation of PXR expression (29). Utilizing a DSS model of murine colitis, a model of human inflammatory bowel disease (IBD), baicalein treatment demonstrated anti-inflammatory activities and protected from crypt loss (29). The mechanism(s) by which baicalein exerts its anti-inflammatory activity...
through PXR was shown in DSS-treated wild-type and Pxr-null mice. Baicalein significantly reduced TNF-α and IL-6 mRNA expression in DSS-treated wild-type mice but not Pxr-null mice (29). Baicalein has been shown to reduce the production of ROS by the up-regulation of peroxiredoxin-6 (PRDX6) in CRC cells (49). In these studies knock-down of PRDX6 by siRNA resulted in an increased in ROS production and cell proliferation after treatment with baicalein (49). These studies provide a better understanding of the potential anti-tumorigenic and anti-inflammatory mechanisms of baicalein in CRC, which can lead to the further development of chemopreventive strategies.

Natural products synthetic derivative

Metformin

The anti-diabetic drug, metformin (1,1dimethylbiguanide) mainly used to treat type II diabetes mellitus has been shown to decrease the risk of CRC (Figure 3G) (116). Metformin is derived from the herbaceous plant goat’s rue (Galega officinalis). Metformin directly inhibits gluconeogenesis and glycogenolysis in the liver, increases anaerobic glycolysis in peripheral tissues, and increase GLUT4 glucose transporters and insulin receptors (89). Metformin has been shown to decrease the risk of colon cancer by targeting the insulin growth factor (IGF) pathway, which plays an important role in cell proliferation, growth and differentiation. This pathway consists of three ligands (insulin, IGF-1 and IGF-2), six receptors (insulin receptor [IR] alpha [fetal], IR beta [adult], IGF-1 receptor (IGF-1R), IGF-2R, hybrid IGF-1R/IR alpha, hybrid IGF-1R/IR beta), and up to seven binding proteins (IGFBP1-7) (112). IGF-1 and IGF-2 are able to bind and activate IGF-R1 signaling and have been shown to be up-regulated in cancer (116). Upon activation, IGF-R1 undergoes autophosphorylation, recruits and phosphorylates insulin receptor substrate-1 (IRS-1), IRS-2 and src-homology/collagen (Shc), which are involved in the oncogenic processes (116). The downstream effects of IRS-1 and IRS-2 phosphorylation results in activation of PI3K leading to activation of the AKT pathway and Bcl-2 and inhibition of p27 and BAD. The RAS/MAPK pathway can also be activated by the binding of Shc to IGF-R1 (96, 116). Increased IGFs, activation of IGFRs activation of PI3K pathway can be due to insulin resistance and hyperinsulinemia (116). Hyperinsulinemia can increase the risk of pancreas, breast and bladder cancer. A potential mechanism of action of metformin is an inhibitory effect on insulin and the suppression of protein synthesis through the activation of the adenosine monophosphate activated protein kinase (AMPK) (16).

The increased risk of breast, colon and pancreatic cancers associated with Type 2 diabetes, obesity and insulin resistance may be due the influence of the insulin-IGF signaling pathway (27). However, it is still unclear if differences of circulating insulin levels influence cancer progression in diabetic patients receiving treatment (i.e. metformin). The observational studies investigating metformin use and the decreased incidence of colorectal cancer report conflicting findings (111). In a retrospective study more than 62,000 diabetic patients were taking metformin, sulfonylurea or insulin. Patients on metformin therapy had the lowest risk of developing colorectal or pancreatic cancer (27). However, in a population study the use of metformin was not associated with a decreased risk of colorectal cancer incidence (111). Although discrepancies can be explained by limitations of sample size, duration of treatment, environmental differences and additional confounding factors. The potential colorectal cancer chemopreventive mechanism of metformin through the inhibition of the insulin/IGF signaling pathway merits further exploration.
AMPK is a highly conserved heterotrimeric kinase, which coordinates several enzymes to regulate ATP conservation and synthesis (43). In a study evaluating the downstream mechanisms of metformin activation of AMPK in human endothelial cells, metformin suppressed TNF-α induced NF-κB activity (43). The suppression of NF-κB activity was attenuated by knock-down of AMPK in these cells (43) and these observations were independently supported (48). The anti-inflammatory activity of metformin through the inhibition of NF-κB, as a potential chemopreventive mechanism is still relatively new. This activity of metformin is supported by its inhibition of the NF-κB pathway in intestinal inflammation, colitis-associated colon cancer (65) and breast cancer (45). The results from these studies indicate that the anti-tumor activity of metformin may be through activity as an anti-inflammatory agent in certain cancer types through inhibition of the NF-κB signaling pathway. However, there is limited research to support this novel role of metformin and warrants further investigation.

Summary and conclusion

Increased understanding of the molecular events involved in the colorectal adenoma-carcinoma sequence provides the opportunity to develop targeted-drugs for chemoprevention. The development of CRC is strongly influenced by genetics, dietary and lifestyle risk factors. Inflammation plays a critical role in the promotion and development of cancer into a malignant phenotype. The progression into a malignant phenotype requires activation of key cellular mechanisms including uncontrolled growth, evasion of apoptotic pathways, angiogenesis, and metastasis to name a few. Early inhibition of the inflammatory state can prevent the activation of several of these pathways and thus prevent the carcinogenesis process. Although there are limitations due to side-effects attributed to the COX inhibitory activity of celecoxib and other NSAIDs (i.e. aspirin and sulindac sulfide), their promising chemopreventive activity have led investigators to study COX-independent mechanisms for the purpose of developing derivatives that lack COX inhibitory activity but retain or have improved anticancer activity. The identification of the specific therapeutic targets and an understanding of the tumorigenic pathways dependent on those targets ideally allows for the inhibition or reversal of the malignant phenotype. The multiple pharmacologic agents and natural products as discussed in this review have shown both promising results for colorectal cancer chemoprevention, but yet, limitations in both safety and efficacy. The rich diversity of the chemical structures and biological mechanism(s) of action of these agents provides opportunities to develop safer and more efficacious colorectal cancer preventive agents by chemical optimization of existing agents to improve target selectivity or the identification of new agents.
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Figure Legends

Figure 1: Selected NSAIDs chemical structures. (A) aspirin, (B) sulindac, (C) ibuprofen and (D) celecoxib are shown.

Figure 2: Sulindac and metabolites. Prodrug sulidac, which lacks COX inhibitory activity, undergoes a reversible reduction into the active COX inhibitory sulindac sulfide form. Sulindac can also be irreversibly oxidized into the sulindac sulfone form, which lacks COX inhibitory activity. Figure adapted from Gurpinar et al., Clinical Cancer Research, 2013 (40).

Figure 3: Selected natural products and synthetic derivative chemical structures. (A) resveratrol, (B) curcumin, (C) quercetin, (D) EGCG, (E) genistein, (F) baicalein, and (G) metformin are shown.

Figure 4: Simplified overview of the WNT/β-catenin signal transduction cascade.

Figure 5: Simplified overview of the NSAIDs and Coxibs, inhibition of COX signal transduction cascade.
Sulindac
Lacks COX-1 and COX-2 inhibitory activity

Sulindac Sulfide
Nonselective COX-1 and COX-2 inhibitor

Sulindac Sulfone
Lacks COX-1 and COX-2 inhibitory activity

Reversible reduction

Irreversible oxidation
Arachidonic acid

COX-1  COX-2

PGH₂

Prostaglandins

PGE₂

Proteasomal Degradation

15-PGDH

Inflammation, cell growth, gastric mucosal secretion

NSAIDs

Coxibs