Dietary Non-nutritive Factors in Targeting of Regulatory Molecules in Colorectal Cancer: An Update

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Abstract

Colorectal cancer (CRC), a complex multi-step process involving progressive disruption of homeostatic mechanisms controlling intestinal epithelial proliferation/inflammation, differentiation, and programmed cell death, is the third most common malignant neoplasm worldwide. A number of promising targets such as inducible nitric acid (iNOS), cyclooxygenase (COX)-2, NF-E2-related factor 2 (Nrf2), Wnt/β-catenin, Notch and apoptotic signaling have been identified by researchers as useful targets to prevent or therapeutically inhibit colon cancer development. In this review article, we aimed to explore the current targets available to eliminate colon cancer with an update of dietary and non-nutritional compounds that could be of potential use for interaction with regulatory molecules to prevent CRC.

Keywords: Colon cancer - COX-2 - β-catenin - Nrf2 - Notch

Introduction

Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality (Greenlee et al., 2000). Despite the development of new screening strategies, aggressive surgical and adjuvant therapy, and intensive research efforts, little progress has been made in the successful management of this disease (Williams et al., 1999). The cure rate for this cancer has remained at 50% for some decades (Burnstein, 1993). The etiology of colorectal cancer is complex and may be attributable to combined actions of inherited and environmental factors.

Colon cancer (CRC), a complex multi-step process involving progressive disruption of homeostatic mechanisms controlling intestinal epithelial proliferation/inflammation, differentiation, and programmed cell death, is the third most common malignant neoplasm worldwide (Shike et al., 1990). Several epidemiological studies have indicated the influence of lifestyle factors, particularly nutritional factors, on the development of certain forms of cancers, including the colon carcinomas (Thun et al., 1991; Giovannucci et al., 1995; Yazar et al., 2013). Epidemiological studies have indicated that CRC is strongly associated with diet (Slattery et al., 1999), and thus it may be possible to prevent the occurrence of this cancer by dietary modifications (Ashokkumar and Sudhandiran, 2011). The spectrum of AOM-induced epithelial lesions resembles those of the various types of neoplastic lesions in human CRC. In addition, AOM-induced colon cancer appears to follow the concept in which tumor initiation is followed by tumor promotion and progression in a sequential manner. Specifically, AOM induces the onset of aberrant crypt foci, as the precursor lesion, followed by the onset of adenocarcinoma most often of the distal colon, and, finally, metastasis to mesenteric lymph nodes and liver. The molecular pathogenesis is characterized by $K$-ras and/or $β$-catenin mutations. Unique to the AOM rat model is the co-occurrence of both adenomas and adenocarcinomas, and it has been estimated that 70% of colon tumors are adenocarcinomas, while the remainder are adenomas. Histologically, adenomas of the colon are non-invasive with low- to high-grade dysplasia (Reddy, 2004).

Animal Models for Colon Cancer

1, 2 Dimethyl hydrazine and azoxymethane (AOM) are frequently used to induce colon cancer in rodents (Prabhu et al., 2009; Norazalina et al., 2010; Nurul-Husna et al., 2010; Ashokkumar and Sudhandiran, 2011). The spectrum of AOM-induced epithelial lesions resembles those of the various types of neoplastic lesions in human CRC. In addition, AOM-induced colon cancer appears to follow the concept in which tumor initiation is followed by tumor promotion and progression in a sequential manner. Specifically, AOM induces the onset of aberrant crypt foci, as the precursor lesion, followed by the onset of adenocarcinoma most often of the distal colon, and, finally, metastasis to mesenteric lymph nodes and liver. The molecular pathogenesis is characterized by $K$-ras and/or $β$-catenin mutations. Unique to the AOM rat model is the co-occurrence of both adenomas and adenocarcinomas, and it has been estimated that 70% of colon tumors are adenocarcinomas, while the remainder are adenomas. Histologically, adenomas of the colon are non-invasive with low- to high-grade dysplasia (Reddy, 2004).

AOM is metabolized in the liver into methylazoxymethanol (MAM). This reaction is catalyzed by the enzyme cytochrome $P_{450}$ E1 (Sohn et al., 1991). Metabolic activation of MAM to a highly reactive

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electrophile (methyl diazonium ion) occurs in liver and colon, which is known to elicit oxidative stress. This ultimate electrophile can methylate cellular nucleophile, such as DNA, causing alkylation damage (Fiala et al., 1987; Talalay, 1992). These acquired mutations to DNA, then accumulate to cause cell proliferation leading to CRC.

**Inducible Nitric Oxide Synthase and Colon Cancer**

Nitric oxide (NO) has been shown to play an important role in colon tumorigenesis. The three isoforms of nitric oxide synthase (NOS) are encoded by distinct genes (Schmidt and Walter, 1994; Murad, 1996). NOS-1, also known as neuronal or brain NOS (nNOS) or Type I NOS, is found in high concentrations in neuronal and some non-neuronal tissues. NOS-2 is also known as Type II NOS, macrophage NOS, or inducible NOS (iNOS). Inducible nitric oxide synthase, the distinct, inducible, Ca²⁺-independent isoform of NOS can be expressed in response to pro-inflammatory agents (Forrester et al., 1996).

Several studies showed increased expression and activity of iNOS in human colon adenomas (Amba et al., 1998; Lala and Chekraborty, 2001; Cianchi et al., 2003). The expression of iNOS expression and nitro tyrosine accumulation (marker of peroxynitrite, the product of NO and superoxide) in inflamed mucosa of patients with ulcerative colitis and gastritis demonstrate the production of NO and its potential involvement in the pathogenesis of these diseases (Middleton et al., 1993). Studies in experimental models of CRC indicate that AOM-induced colon tumors have higher expression and/or activity of iNOS compared to levels found in adjacent colonic tissue (Takahashi et al., 1997; Rao et al., 1998). There are number of reports suggesting that, the expression iNOS in colorectal cancer was abundant. So it is considered as a remarkable target in CRC.

Dietary administration of scallion extract inhibits inflammation associated colon cancer by modulating the iNOS expression (Arulselvan et al., 2012). Luteolin, a bioflavonoid modulates the expression of iNOS and thereby controls the inflammation in AOM-induced colon cancer (Pandurangan et al., 2013a). Grape seed extract, a rich source of polyphenols and Yerba mate Tea both inhibits iNOS expression by modulating its upstream target NF-κB in AOM-induced colon cancer (Derry et al., 2013; Puangpraphant et al., 2013). Glycyrrhizic acid is natural and major pentacyclic triterpenoid glycosides of licorice roots extracts suppress the precancerous lesions by modulating iNOS expression in colon cancer (Khan et al., 2013). Resveratrol, a polyphenol abundantly found in grapes and red wine, exhibits beneficial health effects due to its anti-inflammatory properties by modulating iNOS expression in Caco-2 and SW480 colon cancer cell lines (Panaro et al., 2012). Gossau et al. (2011) reported that Theaflavin-2 (TF-2), a major component of black tea extract, induces apoptosis of human colon cancer cells and suppresses the expression of iNOS. The above said reports are shown considerable evidence that dietary compounds acts as a strong inhibitor of iNOS, thereby controls tumor formation in the colon.

**COX-2 and Colon Cancer**

There are two isoforms of COX, namely COX-1 and COX-2. COX-1 is constitutively expressed in a number of cell types and tissues and plays a major role in homeostasis. COXs are the rate-limiting enzymes in the conversion of arachidonic acid into prostaglandins (PGs). The precise reaction catalyzed by COXs is the conversion of arachidonic acid into PGH₂, a metabolite that then becomes the substrate of cell-specific prostaglandin and thromboxane synthases that generate PGs and thromboxane A₂, respectively. The formation of PGH₂ occurs in two steps; initially, two oxygen molecules are incorporated into arachidonate, thus generating PGG₂. The synthesis of PGH₂ is the second step and it involves reduction of PGG₂ catalyzed by the peroxidase activity of COX-2 (Figure 1). COX-2 is not detected in most normal tissues but is rapidly induced in response to mitogens, cytokines and tumor promoters, leading to increased accumulation of prostanoids in neoplastic and inflamed tissues (Subbaramaiah et al., 1996). COX-2 is highly inducible by the oncogenes ras and c-scr and other cytokines at the sites of inflammation and cancer (Kam and Lee, 2000; Turini and DuBois, 2002; Church et al., 2003). Previous studies have shown that most cancer cells are found to exhibit over-expression of COX-2, which can stimulate cellular division and angiogenesis and inhibit apoptosis (Dempke et al., 2001). Accumulated evidence suggests that COX-2 selective inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDS) induce apoptosis by suppressing the COX-2 levels (Huang et al., 2005).

Increased COX-2 expression results in the production of PGE2 and it is correlated with increased production of malonaldehydes, major product of lipid peroxidation, that can form DNA adduct in human colon and accelerates carcinogenesis (Hanif et al., 1996) or inhibit apoptosis in epithelial tumor cells (Sheng et al., 1998). A recent report is evidenced that iNOS signaling crosstalk with COX-2 in colon fibroblasts (Zhu et al., 2012). Abundant evidences supports, the role of COX-2 in the colon cancer (Tsujii

![Figure 1. Regulation of COX-2 Pathway](image)

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It is difficult to discuss the Nrf2 transcription factor without mentioning cancer prevention, since the discovery of Nrf2 is attributed greatly to studies with anti-carcinogenic compounds (Zhang, 2009). Nrf2-null mice have decreased basal and inducible expression of antioxidant genes, increased oxidative stress, and decreased reducing activity and antioxidant capacity (Chen and Kwong, 2000), suggesting that the Nrf2/ARE pathway is critical for the regulation of intracellular redox status. During colon cancer the expression of Nrf2 was limited (Patel et al., 2008; Zhang et al., 2009).

Hu et al. (2012) reported that oroxylinA modulates the Nrf2 pathway in HT-116 colorectal adenocarcinoma cells and xenograft tumors. Epigallocatechin-3-gallate and peracetylated (-)-epigallocatechin-3-gallate, an active component of Green tea also modulates the expression of NRF2 and UGT1A in colon cancer cells and BALB/c mice (Yuan et al., 2007; Zhang et al., 2009; Chiou et al., 2012). Pandurangan et al. (2013b) reported that Luteolin, a natural bioflavonoid induces Nrf2 and its downstream targets such as Glutathione-S-transferase and UDP-glucoronyltransferase in AOM-induced colon cancer. Pterostilbene is more potent than resveratrol in preventing AOM-induced colon tumorigenesis via activation of the Nrf2-mediated antioxidant signaling pathway (Chiou et al., 2011) and also in HT-29 colon cancer cell line (Harun et al., 2012). Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2 (Bat-Chen et al., 2010). Wondrak et al. (2010) reported that the cinnamon-derived dietary factor cinnamaldehyde activates the Nrf2-dependent antioxidant response in human epithelial colon cells.

Wnt/β-catenin Signaling and Colon Cancer

Wnt signaling pathway is essential in many biological processes and their downstream effectors were shown to be conserved in all metazoans (Wodarz and Nusse, 1998). The key component of the Wnt signaling is the cytoplasmic protein β-catenin, which plays a critical role in the regulation of cellular proliferation and in colon
carcinogenesis. Adenomatous polyposis coli (APC) co-operate with GSK-3β to regulate β-catenin levels in the cytoplasm through phosphorylation sites in exon 3 of the β-catenin gene (Korinek et al., 1997; Gregoireff and Clevers, 2005). In the nucleus, the β-catenin protein forms a complex with the transcription factors, T-cell factor (TCF) and lymphoid enhancer factor (LEF), and co-activates transcription (Korinek et al., 1997; Sparks et al., 1998). c-Myc and cyclin D1 have been identified as targets of the β-catenin/APC signaling pathway (He et al., 1998; Tetsu and McCormick, 1999). Frequent mutations of the β-catenin gene were found in chemically induced colon tumors in both rat and mouse carcinogenesis models (Dashwood et al., 1998; Takahashi et al., 1998; Suzui et al., 1999), suggesting that the APC-β-catenin pathway plays an important role in the development of colon carcinogenesis in rodents, as seen in humans (Figure 3).

Alterations in the APC or β-catenin gene are regarded as early critical events during colon carcinogenesis and are therefore considered to play a gate keeper role in the development of colon cancer in both humans and preclinical models (Powell et al., 1992; Polakis, 1997; Takahashi et al., 1998). Mutations in the APC or β-catenin gene were proved to repress the degradation of the protein and generate β-catenin accumulations in the cytosol (Aberle et al., 1997; Morin et al., 1997). The excessive β-catenin functions as a transcriptional activator when complexed with members of the TCF family of DNA binding proteins (Behrens et al., 1996; Molenaar et al., 1996). Furthermore, target genes of β-catenin signaling pathway, such as c-myc and cyclin D1, are growth-promoting genes, suggesting that this pathway is potentially an oncogenic pathway (He et al., 1998; Tetsu and McCormick, 1999). β-Catenin plays a critical role in the regulation of cellular proliferation and in colon carcinogenesis. APC co-operates with GSK-3β to regulate β-catenin levels in the cytoplasm through phosphorylation sites in exon 3 of the β-catenin gene (Korinek et al., 1997). About 80% of human colon tumors harbor mutations in the APC gene and half of the reminder have β-catenin gene mutations (Sparks et al., 1998). When mutations are present in either the APC or β-catenin genes, accumulation of the β-catenin protein in the cytoplasm and nucleus were observed (Korinek et al., 1997). Frequent mutations of the β-catenin gene were found in chemically induced colon tumors in both rat and mouse carcinogenesis models (Dashwood et al., 1998; Takahashi et al., 1998; Suzui et al., 1999), suggesting that the APC-β-catenin pathway plays an important role in the development of colon carcinogenesis in rodents, as seen in humans.

Chemopreventive agents such as Polymeric Black Tea (Patel et al., 2008) and Luteolin (Ashokkumar and Sudhandiran, 2011) both targets wnt/β-catenin pathway. Sphingadiene is a derivative of sphigolipid inhibits colon cancer by downregulating wnt/β-catenin pathway (Kumar et al., 2013). Phytic acid is a product of rice bran known to inhibit β-catenin during AOM-induced colon cancer (Saad et al., 2013; Shafie et al., 2013). Berberine acts as a natural inhibitor of Wnt/β-catenin signaling in HCT-116 colon carcinoma cells (Albring et al., 2013). The antiproliferative and antitumor activities of 2-hydroxycinnamaldehyde (1), a phenylpropanoid isolated from the bark of Cinnamomum cassia was found to inhibit β-catenin/T-cell factor (TCF) transcriptional activity in HCT116 colon cancer cell line (Lee et al., 2013). Pomegranate fruit extract inhibits Wnt signaling in 1, 2-Dimethylhydrazine-induced rat colon carcinogenesis (Sadik and Shaker, 2013). Plant flavonoid Isorhamnetin inhibits colorectal cancer by inhibiting the translocation of β-Catenin from the cytoplasm to nucleus in vivo and in vitro (Saad et al., 2013). Henryin is an ent-kauranederpenoid isolated from Isodon rubescens var. lushanensis, a plant that did not affect the cytosol-nuclear distribution of soluble β-catenin, but impaired the association of β-catenin/TCF4 transcriptional complex n colon cancer cells (Li et al., 2013). Cardamonin is a chalconoid isolated from Alpinia katsumadai inhibits β-catenin and downstream targets in colon cancer cells (Park et al., 2013). Genistein, a soya isoflavone, prevents AOM-induced up-regulation of Wnt/β-catenin signaling and reduces colon pre-neoplasia in rats (Zhang et al., 2012). Kang et al. (2012) reported that the growth inhibition of magnolol against human colon cancer cells can be partly attributed to the regulation of the Wnt/β-catenin signaling pathway. Allameh et al. (2012) reported that dietary caraway essential oils downregulates the expression of β-catenin during 1,2-dimethylhydrazine-induced colonic carcinogenesis. γ-Tocotrienol inhibits cell viability through suppression of β-catenin/Tcf signaling in HT-29 human colon carcinoma cells (Xu et al., 2012). From the above findings targeting wnt/β-catenin can results in the control of colon cancer formation.

### Notch Signaling and Colon Cancer

Notch signaling is a key developmental signaling pathway that plays an important role in the determination of cell fate. Recent years, the vital role of Notch signaling in regulating a balance between proliferation,
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Recent studies have shown that aberrant Notch signaling contributes to the pathogenesis of CRC. However, the potential therapeutic benefits of Notch pathway inhibitors, including γ-secretase inhibitors (GSIs) on colon carcinogenesis are still unclear (Miyamoto et al., 2013). In recent years, many researchers established the role of Notch signaling in the CRC. Notch signaling clearly plays an important role in the maintenance of the colon crypt compartment. More recently, inappropriate activation of Notch signaling has been associated with the pathogenesis of colon cancers. A significant up-regulation of Notch1 (Zagouras et al., 1995) and Hes1 (Reedijk et al., 2008) have been detected in colon adenocarcinomas, but not in normal differentiated epithelial cells. It was also reported that the activation of Notch signaling is essential for the development of adenomas in APCmin−/− mice (van Es et al., 2005) and self-renewal of tumor-initiating cells (Sikandar et al., 2010). Importantly, Hes1 is known to suppress the expression of Krüppel-like factor 4 (KLF4), a transcriptional repressor (Ghaleb et al., 2008). KLF4 is a zinc finger-containing transcription factor that is highly expressed in terminally differentiated epithelial cells of the intestine (Shields et al., 1996; Dang et al., 2000). It is also reported that overexpression of KLF4 inhibits colon cancer cell proliferation (Chen et al., 2001; Yoon et al., 2003) and haplo insufficiency of KLF4 promotes the development of intestinal adenomas in APCmin−/− mice (Ghaleb et al., 2007). KLF4 expression is reduced in colorectal neoplasia relative to normal mucosa, including both adenomas and carcinomas (Zhao et al., 2004). Notch and Wnt function together to regulate colonic progenitor cell division and differentiation. Studies in mice have also shown that Notch signaling is required for adenoma formation in response to elevated Wnt pathway signaling that occurs in the APCmin mouse model of human adenomatous polyposis coli (Reedijk et al., 2008). From this findings notch signaling is considered as a crucial in targeting colon cancer.

Withaferin-A (WA) is a bioactive compound derived from Withania somnifera, which inhibits Notch-1 signaling and downregulates pro-survival pathways, such as Akt/ NF-kappaB/Bcl-2, in colon cancer cell lines (Koduru et al., 2010). Green tea catechin, epigallocatechin-3-gallate inhibits noteh signaling (Singh et al., 2011).

**Apoptosis and CRC**

All mammalian cells contain an intrinsic program necessary to carry out cell suicide. Cell death is an evolutionarily conserved and genetically regulated process that is important for morphogenesis, embryonic development, and for the maintenance of homeostasis in adult tissues. Different modes of cell death mechanisms are defined by morphological criteria, without a clear reference to precise biochemical mechanisms. Based on these criteria several modes of cell death are known, e.g., apoptosis, necrosis, autophagy, mitotic catastrophe, anoikis, excitotoxicity, wallerian degeneration, cornification, etc. However, the molecular mechanisms involved in the first three modes have been characterized. A detailed classification of cell death was reported (Kroemer et al., 2005).

A predictable mechanism for the development of CRC is an imbalance between cell renewal and cell death, with proliferation being favored. A balance between new and old cells maintains organ size and colonic crypt structure.
Apoptosis is characterized by cell shrinkage, chromatin condensation, DNA fragmentation, and the activation of specific cysteine proteases known as caspasps. Caspasps play a critical role during apoptosis. There are at least two major mechanisms by which a caspase cascade resulting in the activation of effector caspase-3 may be initiated by the most apical caspase, one involving caspase-8 and the other involving caspase-9 (Zou et al., 1997; Srinivasula et al., 1998). The Bcl-2 family consists of more than 30 proteins, which can be divided into three subgroups: Bcl-2-like survival factors, Bax-like death factors, and BH3-only death factors. Residues from BH1, 2, and 3 form a hydrophobic groove, with which BH3-only death factors interact through their BH3-domain, whereas the N-terminal BH3-domain stabilizes this pocket (Festjens et al., 2004).

The importance of mitochondria in apoptosis was suggested by studies with a cell-free system in which spontaneous, Bcl-2-inhibitable nuclear condensation and DNA fragmentation were found to be dependent on the presence of mitochondria (Newmeyer et al., 1994). Subsequently, studies in another cell-free system showed that induction of caspase activation by addition of deoxyadenosine triphosphate depended on the presence of cyto c released from mitochondria during extract preparation (Liu et al., 1996). During apoptosis, cytochrome c is released from mitochondria and this is inhibited by the presence of Bcl-2 on these organelles (Yang et al., 1997). Cytosolic cytochrome c forms an essential part of the vertebrate “apoptosome,” which is composed of cyto c, Apaf-1, and procaspase-9 (Li et al., 1997). The result is activation of caspase-9, which then processes and activates other caspases to orchestrate the biochemical execution of cells.

There are number of novel drugs that inhibit colon cancer by modulating the apoptotic signals. Wesolowska et al. (2012) reported that multidrug resistance reversal and apoptosis induction in human colon cancer cells. Silibinin, a natural flavonoid, treatment induced the upregulation of the pro-apoptotic Bax protein and gene expression and the reversal of the Bcl-2/Bax ratio (Kauntz et al., 2012). Diallyl sulfide, a garlic derived organo sulphur compound induces apoptosis mediated through the activation of caspase (Sriram et al., 2008). Quercetin enhances hypoxia-mediated apoptosis via direct inhibition of AMPK activity in HCT116 colon cancer (Kim et al., 2012). Luteolin, a bioflavonoid induces apoptosis via modulating the expressions of Bcl-2, Bax and caspase-3 in vivo and in vitro (Pandurangan et al., 2013; Pandurangan and Ganapsam, 2013). Wen et al. (2012) reported that gelam and nenas honeys inhibit proliferation of HT-29 colon cancer cells by inducing DNA damage and apoptosis while suppressing inflammation. A ginseng metabolite induces autophagy and apoptosis via generation of reactive oxygen species and activation of JNK in human colon cancer cells (Kim et al., 2013). Epicatechin Gallate, a polyphenol from tea catechins induces cell death via p53 activation and stimulation of p38 and JNK in human colon cancer SW480 cells (Cordero-Herrera et al., 2013). Galangin is a member of flavonols and found in Alpinia officinarum induces apoptosis mediated by caspase 3 and 9 in colon cancer (HCT-15 and HT-29) cell lines (Ha et al., 2013).

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