Chemoprevention with Phytonutrients and Microalgae Products in Chronic Inflammation and Colon Cancer

Elena Talero*, Javier Ávila-Roman and Virginia Motilva

Department of Pharmacology, School of Pharmacy, University of Seville, Spain

Abstract: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder caused by deregulated immune responses in a genetically predisposed individual. This is a complex process mediated by cytokines, chemokines, adhesion molecules, cytoplasm nuclear receptors, among others. Recent data support a participation of the endoplasmic reticulum (ER) stress and mitochondrial dysfunctions in IBD. Moreover, now it is evident that chronic degenerative pathologies, including IBD, share comparable disease mechanisms at the cellular level with alteration of the autophagy mechanisms.

Mounting evidence suggests that the risk of developing colorectal cancer (CRC) is dramatically increased in patients with chronic inflammatory disease. Chronic inflammation in IBD exposes these patients to a number of signals known to have tumorigenic effects including nuclear factor kappa B (NF-κB) activation, proinflammatory cytokines and prostaglandins release and reactive oxygen species (ROS) production.

Chemoprevention consists in the use of drugs, vitamins, or nutritional supplements to reduce the risk of developing, or having a recurrence of cancer. Numerous *in vitro* and animal studies have established the potential colon cancer chemopreventive properties of phytochemicals derived from both plants (curcumin, resveratrol, epigallocatechin gallate, quercetin or genistein) and substances from marine environment, including microalgae species and their products. This review summarizes the mechanisms by which these naturally occurring compounds may mediate chemopreventive effects on cancer. These actions include induction of cell cycle arrest and apoptosis, inhibition of cell proliferation, stimulation of antimetastatic and antiangiogenic responses and increased antioxidant and anti-inflammatory activity.

Keywords: Gut, immunology, chronic inflammation, colon cancer, inflammatory bowel disease, chemoprevention, phytonutrients, microalgae.

1. INFLAMMATORY BOWEL DISEASE: IMMUNE RESPONSES AND MOLECULAR PATHWAYS IMPLICATED

The incidence of IBD continues to rise, mainly in high-incidence areas (western countries), although both incidence and prevalence are also increasing in historically low-incidence areas such as Latin America, India East or Asia. Factors associated with this "*westernization*" may be conditioning the expression of these pathologies, and the increase of the incidence among migrants from low- to high-incidence regions in just one generation suggests a strong environmental influence [1].

The hallmark of IBD, including ulcerative colitis (UC) and Crohn´s disease (CD), is chronic uncontrolled inflammation of the intestinal mucosa, which can affect any part of the gastrointestinal tract, with presence of structure alterations and superficial or transmural granulomatous infiltration [2]. IBD is associated with increased permeability of the intestinal epithelial lining resulting in continuous stimulation of the mucosal immune system. Luminal bacteria appear to further intensify the permeability defect, establishing a self-sustaining cycle of mucosal inflammation. Intestinal epithelial cells have developed control mechanisms that organize the activation of the intestinal immune system. However, under pathological conditions, bacterial products are able to cross the mucosal barrier and come into the mucosa generating a classic immune response [2, 3].

The traditional paradigm for the IBD pathogenesis was that cells from the adaptive immune system are also the mediators of intestinal inflammation (Fig. **1**). However, now the participation of the innate immune system in IBD is accepted [4-6]. In this sense, the intestinal epithelium is believed to contribute to innate immunity and to the relative sterility of the mucosal surface, playing an active role in the maintenance of the mucosal immune homeostasis [7]. Likewise, the intestinal epithelium appears to act as a "gatekeeper" that regulates the quality (type) and quantity (number) of leukocytes migrating from the intravascular to the interstitial space [8]. This is a complex process mediated by cytokines, chemokines and adhesion molecules. After exposure to the abundant intestinal bacterial antigens or environmental factors, innate immune cells in intestinal mucosa are activated, leading to the overproduction of proinflammatory cytokines. Recently, the interleukin (IL)-23/IL-12 pathway has become the subject of intensive study and the T helper type 1 (Th1) cells, driven by IL-12, and IL-17-producing T helper (Th17) cells, driven by IL-23, have been demonstrated to play an important role in IBD [9]. The Th17 pathway genes are shared between CD and UC, while others are IBD subtype-specific including autophagy genes, or epithelial barrier genes [10, 11] have demonstrated that the IL-23 receptor is vital for the maintenance of many types of CD-T cells which provide early adaptive immune responses to damage. These IL-17 producing effector T cells are crucial for protection against intercellular pathogens and for organ-specific inflammation; the therapeutic disruption of the IL-23 pathway suggests the control of auto-immune inflammation without impairing systemic immunity [12].

Many receptors are implicated in the immunopathogenesis of IBD and those located on the intestinal mucosal surface constitute an immunological barrier that is in continuous contact with a variety of commensals. Structurally distinct families of pattern recognition receptors (PRRs) are pivotal to the control of intestinal mucosal homeostasis [13]. Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-1 (NOD-1)-like receptors, both interconnected and coordinated through many signals pathways, provide an integrated system to recognize microbes and microbial molecules and to control antimicrobial effectors pathways and adaptive immune responses.

*Address correspondence to this author at the Department of Pharmacology, School of Pharmacy, University of Seville, C/Profesor García González nº2, 41012, Seville, Spain; Tel: +34-954559879; Fax: +34-954556074;
E-mail: etalero@us.es

1873-4286/12 $58.00+.00

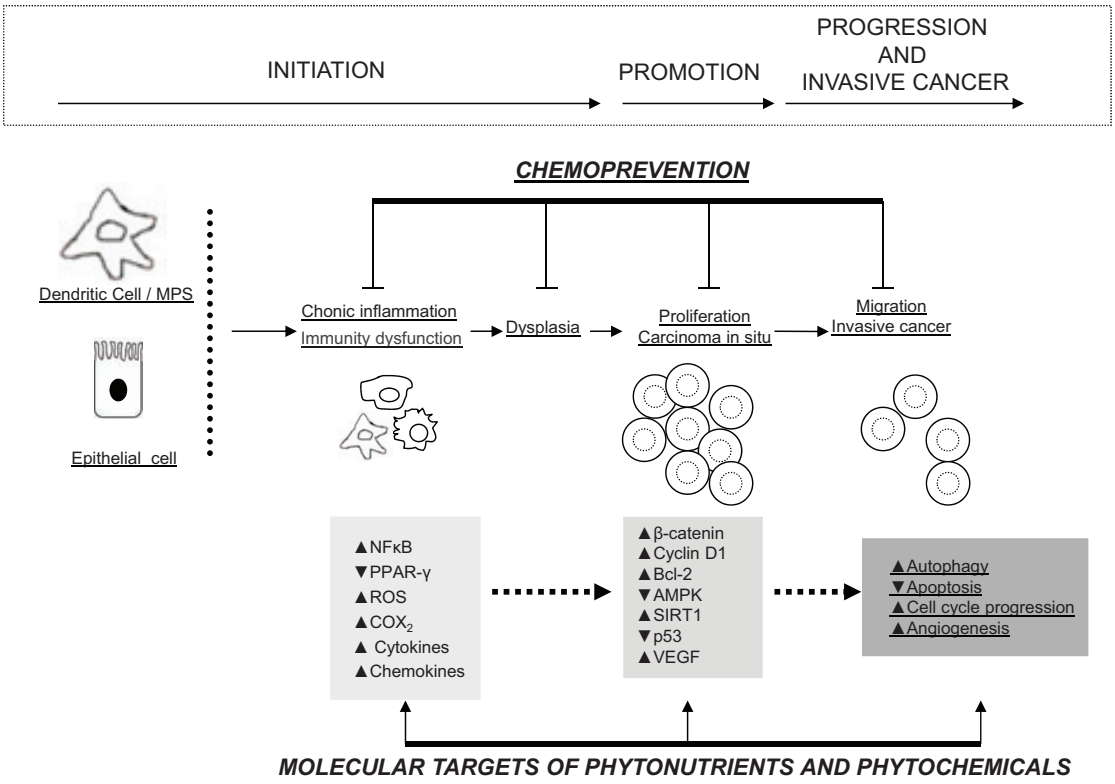

Fig. (1). Inflammation and progression of colon cancer. Potential molecular targets of chemopreventive phytochemicals and phytonutrients.
The histological changes observed in inflammatory bowel disease (IBD) that develops neoplasm correspond to the inflammation-dysplasia-cancer sequence. Chronic inflammation exposed to a number of alterations is known to have procarcinogenic effects. Carcinogenesis, divided into three phases (initiation, promotion and progression), is influenced by nuclear factors and inflammatory regulators: activation of nuclear factor kappa B (NF-κB) signalling pathway, decrease of peroxisome proliferator-activated receptor (PPAR-γ) response, production of reactive oxygen species (ROS), and cyclooxigenase-2 (COX-2) protein expression, and prostaglandin E_2 (PGE_2) production. The stimulation of NF-κB signaling pathway induces the expression of a number of genes: the angiogenic factor named vascular endothelial growth factor (VEGF), the antiapoptotic factor Bcl-2, and the proliferation factor cyclin D1. Inflammatory regulators also act in a feedback loop to modify regulative systems including AMP-activated protein kinase (AMPK) and sirtuin deacetylase enzyme (SIRT1) -1, and to weaken p53 activity. Finally, the vicious circuitry of inflammation and cancer modifies apoptosis and autophagy and promotes cell cycle progression, invasion, and angiogenesis. Endoplasmic reticulum (ER) stress and mitochondrial dysfunctions in epithelial cells are also disease-conditioning situations in IBD. The UPR/IRE1α/XBP1 pathway results in induction of autophagy. Phytochemicals and phytonutrients have been reported to directly modulate various molecular signals transduction pathways that are known to induce cancer cell death or to inhibit cancer cell proliferation. However, many of the specific molecular and cellular targets need to be confirmed.

In healthy people, normal PRR signaling protects intestinal barrier integrity and confers commensal tolerance, and different studies suggest that this kind of signalling exerts several important cytoprotective responses in the intestine epithelium, including barrier preservation, inhibition of apoptosis and inflammation, wound repair and regeneration, and autophagy control [13]. Autophagy, traditionally considered as simple as a degradation mechanism, is now believed to have numerous functions and to play complex roles in human diseases, including IBD [14]. The progress made by scientific programs such as the human genome project and genotyping technologies, have made the discovery of susceptibility loci for IBD that are shared between UC and CD, but also a number of loci that are disease-specific [15]. Association between two genes, the autophagy-related 16-like 1 gene (ATG16L1) and immunity-related GTPase family, M (IRGM), and CD was identified by a genome study and by a wide single nucleotide polymorphisms- SNP study [16, 17].

Aberrant PRR signaling, occurred in disease, leads to deleterious tissue injury associated with chronic inflammatory and autoimmune responses. Several studies have described the low expression of TLR-4 in the normal colonic mucus and the upregulation in UC [18], suggesting the possibility that abnormal bacterial sensing, microbial imbalance or dysbiosis, contribute to disease pathogenesis. Along these lines, TLR-2 recognizes a vast array of microbial components and is involved in different models of IBD [19], however TLRs do not discriminate between pathogenic and non-pathogenic microorganisms (commensal), which is important for understanding innate signalling [20]. Concerning NOD-1-like receptors (NLRs), they are expressed not only by dendritic cells [21], but also by Paneth cells and macrophages [22] and like TLRs, they do not differentiate between pathogenic microorganisms and commensal flora [20]. The first innate receptor strongly linked to the development of chronic intestinal inflammation in a subset of CD patients is the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), also known as caspase recruitment domain-containing protein 15 (CARD15) or also as inflammatory bowel disease protein 1 (IBD1) NOD2/CARD15 [23, 24]. NOD2 mutations are associated with an increased risk for CD, which suggests that the deregulated recognition of intestinal microbes leads to disease in a genetically predisposed individual. Recently, two independent groups have linked NOD2 and autophagy [25, 26]: NOD2 stimulation induces autophagy in dendritic cells and requires ATG5, ATG7 and ATG16L1. NOD2-mediated autophagy affects bacterial handling and antigen presentation in dendritic cells and mutant NOD2 is retained in the cytosol and therefore fails to bring ATG16L1 to the plasma membrane, impairing autophagy targeting of bacteria. Autophagy has been identified as a key process in host resistence to bacterial infection, although little is known about the

steps by which pathogens manipulate the cell to evade the autophagy pathway. The connection between autophagy and IBD development can exist on multiple levels, including intestinal homeostasis, bacterial clearance, cytokine production, and Paneth cell functions [15], whereas some genetic alterations of autophagy promote the development of IBD. In addition, other processes related to IBD pathology (responses to pathogens, oxidative damage and other stresses) would be presumably altered by malfunction of the autophagic process.

On the other hand, upon contact of microbial components with both NOD2 and TLR, NF-κB signaling pathway stimulates the expression of multiple molecules relevant to the pathogenesis of various diseases including IBD and CRC [27, 28]. NF-κB signalling pathway is a complex network that regulates a cellular pathway involved in the expression of a wide variety of genes that play critical roles in immune responses [29]. NF-κB is regulated by the IκB family, with seven IκB members including IκBα, IκBβ, IκBγ, IκBε, Bcl-3 and the precursor proteins p100 and p105. Briefly, following stimulation with various inflammatory stimuli, such as certain members of the tumor necrosis factor alpha (TNF-α) cytokine family, IL-1, TLR ligands, and the p50 subunit mostly, who translocates to the nucleus and activates the transcription of various target genes [30]. The result is the increase production of proinflammatory molecules during active IBD, including those encoding cytokines such as IL-1, IL-2, IL-6, IL-12, and TNF-α [31]. These cytokines are primarily secreted by monocytes and macrophages upon activation and then induce intestinal macrophages, neutrophils, fibroblasts and smooth-muscle cells to produce prostanoids, proteases and many other mediators of inflammatory tissue responses, and promote the production of other chemotactic cytokines affecting innate as well as acquired immune response at mucosal sites [32].

In response to NF-κB activation, commensal bacteria dampen inflammation via nucleocytoplasmic redistribution of peroxisome proliferator-activated receptor (PPAR)-γ, a member of the nuclear receptor group of transcription factors. PPAR-γ is highly expressed in the intestinal epithelium, immune cells and adipocytes, and regulates a number of genes participating in metabolism, proliferation, signal transduction and cellular motility. The role of PPAR-γ in the immune response is through its ability to down-modulate the expression of inflammatory cytokines such as IL-1β, TNF-α, and interferon-gamma (IFN-γ) and also to direct immune cell differentiation towards anti-inflammatory phenotypes [33]. In addition, other mechanisms involved in the anti-inflammatory effects of PPAR-γ ligands include modulation of NF-κB, c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) signalling pathways [34] (Fig. **2**). PPAR-γ activation contributes to the maintenance of intestinal homeostasis in several experimental assays. A recent *in vivo* study by Guri *et al.* [35] investigated the underlying mechanisms by which the deletion of PPAR-γ in intestinal epithelial cells modulates the severity of experimental IBD, immune cell distribution and global gene expression. These authors observed that PPAR-γ expression is required for preventing colonic inflammatory lesions, up-regulating lysosomal pathway genes and increasing the production of the anti-inflammatory cytokine IL-10. In a different experimental model of IBD, activation of PPAR-γ by different agonists suppressed gut inflammatory lesions, weight loss and inflammatory mediators expression [36-38]. Most notably, the PPAR-γ agonist rosiglitazone showed therapeutic efficacy in humans with UC, although this molecule and other drugs belonging to the thiazolidinedione class of anti-diabetic drugs, are unlikely to be adopted for the treatment of IBD because of their side effects [39].

It is now evident that chronic degenerative disorders, such as type 2 diabetes or IBD, share comparable disease mechanisms at cellular level, including ER and mitochondrial dysfunctions, with inflammatory processes as a key disease-conditioning situation in different tissues [40, 41]. Stress in mitochondria, or in the ER independently, causes cell death, but these process may be also connected: it has been reported that ER stress causes mitochondrial dysfunction via p53-upregulated modulator of apoptosis (PUMA) and tumor necrosis factor receptor-associated protein 1 (TRAP1), located in the mitochondria and associated with the unfolded protein response (UPR) in the ER [42]. Genetic and environmental factors can affect ER stress in the intestinal epithelium and consequently inflammation [43]; genetic factors include either primary or secondary ER stress and environmental factors include bacteria, diet or drugs. The hypothesis that ER and mitochondria share common mechanisms in triggering the UPR and a protective signalling pathway from the ER to the nucleus controls cell-stress-response caused by unfolded and/or misfolded proteins. Accumulation of UPR or aggregated proteins in the ER results in increased chaperone expression, translational arrest and induction of autophagy and UPR signaling is mainly driven by inositol-requiring endoplasmic reticulum-to-nucleus signaling protein 1α (IRE1α), X-box-binding-1 (XBP1) pathway [44]. IREα is a transmembrane kinase/endoribonuclease, which initiates the non-conventional splicing of the messenger RNA (*m*RNA) encoding the key transcription activator XBP1 which is a key component of the ER stress response and is required for the differentiation and function of certain secretory epithelial cells [45, 46]. IREα is ubiquitous, whereas IRE1β is specifically expressed in the intestinal epithelium. IRE1α exhibits both endoribonuclease activity, with XBP1 being the only known substrate, and kinase activity that engages both JNK and classical NF-κB pathways. XBP1 deletion causes ER stress in the epithelium, enteritis, increased susceptibility to dextran sodium sulfate (DSS) colitis, lacks of Paneth cells in the intestinal epithelia, and decreases crypt bactericidal function, among others.

The full understanding of the different immunological mechanisms implicated in the development and perpetuation of chronic inflammatory diseases, such as IBD, is very important as therapeutic interventions are subject to these mechanisms. In this way, treatments could be customized for each specific group or subgroup of patients.

2. INTESTINAL CANCER AS A CONSEQUENCE OF CHRONIC INFLAMMATORY DISORDER

At present, reasons for the elevated cancer risk in IBD patients are not clear. Genetic predisposition and data from familial cases of intestinal cancers could indicate their connections, although it is not clear which heritable genetic factors contribute to increased CRC risk in IBD.

Since Hinton *et al.* [47] reported in 1966 that patients with ulcerous colitis were at greater risk of developing CRC, many papers have reported that the length of the disease, its extent and association with other inflammatory diseases such as sclerosant colangitis, and anti-inflammatory treatment are factors that concede inflammation an advantageous role, at least, in carcinogenesis [48, 49]. Intestinal neoplasias, originated under inflammatory conditions, have been the object of numerous clinical, anatomopathological, genetical and molecular studies in both humans and animals. The association between UC and elevated risk for CRC is clear; however, there are some debates about whether CD possesses a similar risk [50]. Earlier studies did not find a significant increase in the risk, although several other studies support the association between CD and cancer. Therefore, although most of the knowledge about colon cancer from chronic intestinal conditions comes from UC evidence, the data suggest that both of these conditions confer increased intestinal cancer risk.

Experimental models in animals have been tested, but only a few are applicable in the study of the inflammation-dysplasia-cancer sequence, which is evident in histological studies of the affected intestinal mucosa [51, 52]. All histological studies are based on the histological classification proposed by Riddle *et al.* [53] and Pascal [54]: i) Undefined for dysplasia/probably negative.

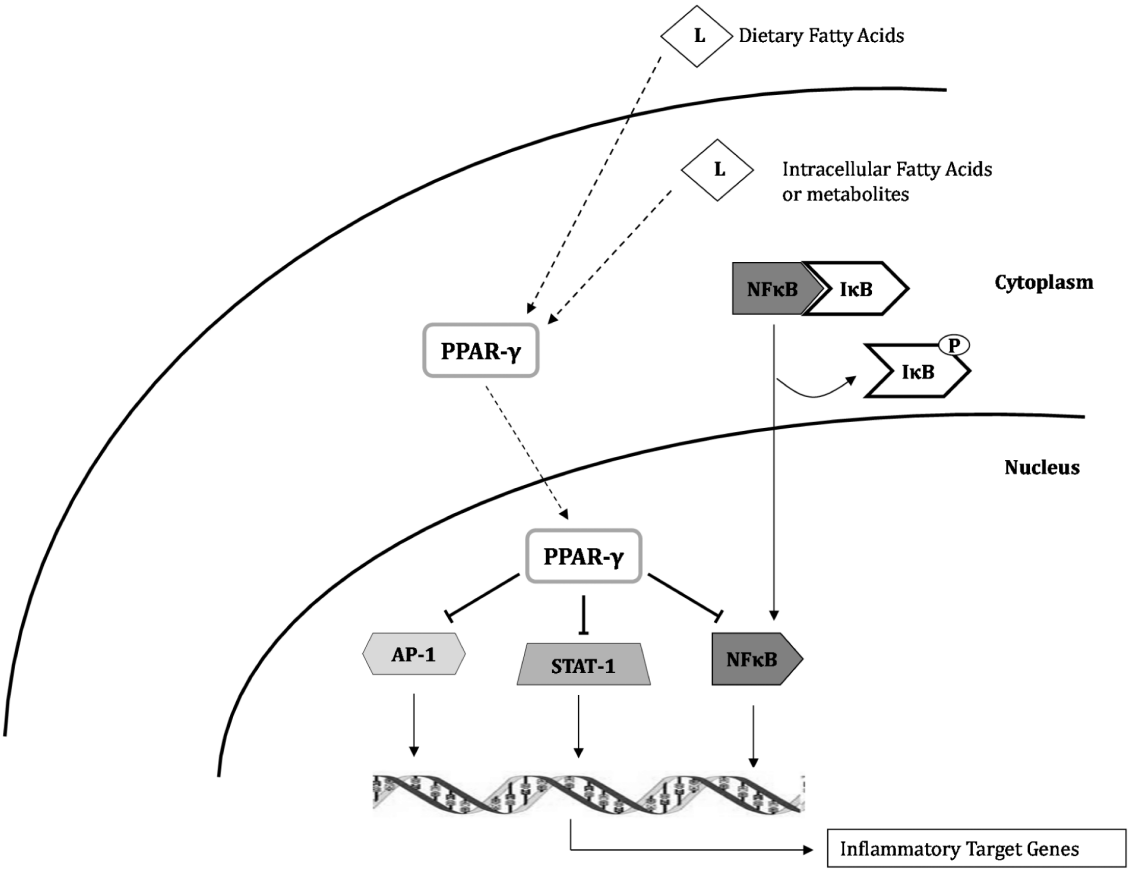

Fig. (2). The mechanisms by which peroxisome proliferator-activated receptor (PPAR)-γ exerts its anti-inflammatory effects are represented. These mechanisms include negative interference between PPAR-γ and other nuclear factors such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 1 (STAT-1) and activator protein-1 (AP-1). PPAR-γ ligands (L) including dietary fatty acids and endogenous ligands such as arachidonic acid derivatives obtained via the lipoxygenase pathway or via the cyclooxygenase pathway are also shown.

ii) Undefined-defined for dysplasia/probably positive. iii) Low grade dysplasia. iv) High grade dysplasia. v) Carcinoma. However, the identification of dysplasia in intestinal inflammatory diseases represents a huge challenge for both clinicians and pathologists, so a clear diagnosis of dysplasia in IBD is not always possible. Possible markers, such as p53 and alpha-methylacyl coenzyme, have been used. The combination of these two markers is positive in 75.8% for cancers and 30.3% for undefined biopsies for dysplasia, while only in 0.6% for non-neoplastic epithelium.

The suspect lesions can be visible macroscopically or only microscopically. Bird and Good [55], in 1987, described focal points of aberrant crypts (AC) such as preneoplastic lesions in rodents treated with carcinogen. The AC can be high or flat lesions: "high AC" are microscopical large lesions that raise above the surrounding epithelium, presenting a round, elongated, open lights. "Flat AC" do not raise above the epithelium, showing a brilliant methylene blue and presenting small or slightly enlarged as well as compressed open lights. Their detection results from the combination of methylene blue staining and transilumination [55]. β-catenin and cycline D1 expression has been studied in both, high and flat ACs; while β-catenin is found in the cytoplasm of flat lesions, it moves to the cytoplasm and nucleus in polypoidal lesions. Thus, inflammation seems to play an important role in the dysplasia-cancer sequence both in flat lesions and in mass, while the translocation of β-catenin to the cytoplasm and nucleus is an early event that occurs in polypoidal lesions [56].

Several lines of evidence implicate chronic intestinal inflammation as a key predisposing factor to modulate tumorigenesis. The relationship and mechanisms through which infection and inflammation increase cancer risk and promote tumor development have been recently the object of important revisions [57, 48]. Chronic inflammation and repeated events of inflammatory relapse in IBD expose a number of alterations known to have procarcinogenic effects. Carcinogenesis, divided into three phases (initiation, promotion and progression), is influenced by different inflammatory mediators (Fig. **1**).

The contribution of the inducible isoform of cyclooxygenase (COX-2) to carcinogenesis is well established. COX-2 can activate procarcinogens and indirectly increase free-radical production. The relationship between ROS and COX-2 expression has been addressed in a recent study which demonstrated that the chemopreventive sulindac and nitric oxide-donating aspirin (NO-ASA) generate ROS to induce COX-2 expression, which can be abolished by two antioxidants and an inhibitor of NADPH oxidase [58]. Recent data implicate COX-2 production downstream of TLR4 activation in the development of inflammation-associated colorectal neoplasia [48, 59]. Activation of TLR4 initiates a signaling cascade that culminates in NF-κB activation and subsequent transcription of a number of proinflammatory mediators, including COX-2. Whether NF-κB is required for ROS-induced COX-2 expression, however, needs further elucidation. Nonsteroidal anti-inflammatory drugs (NSAID) may inhibit the development of gastrointestinal cancers through inhibition of COX-2 activity. Nevertheless, COX-2-independent mechanisms also contribute significantly to the chemopreventive effects of NSAID. In any case, the available data reinforce the role of COX-2 in chronic inflammation and in the development of CRC [48].

There is growing evidence of the connection between inflammation, tumor development, and NF-κB. Viral oncogenes, hepatitis B and C proteins, and human papillomavirus infection have been implicated in NF-κB activation. Besides, some chemical and physical carcinogens, especially nicotine and carcinogens in tobacco, can promote cell proliferation, survival and inflammation via NF-κB activation [60]. The role of NF-κB in promoting carcinogenesis is evidenced by numerous studies which indicate that this factor can block apoptosis by regulating anti-apoptotic proteins, including inhibitor of apoptosis proteins (IAPs) or by inhibiting JNK activation and the accumulation of ROS [61]. In chronic inflammation, the cytokines and chemokines produced by inflammatory cells activate NF-κB, which translocates into the nucleus, inducing the expression of certain tumorigenic, adhesion proteins, chemokines and inhibitors of apoptosis that promote cell survival. Therefore, NF-κB may contribute to the development of CRC by sustaining the ongoing inflammatory process in the gut mucosa NF-κB is also connected with the regulation of many genes differently expressed in invasion and metastasis: cyclin D1 and cMyc oncogenes, vascular endothelial growth factor (VEGF), and IL-8 [62]. Several products have been suggested to inhibit NF-κB activation, including curcumin, ginseng extract, resveratrol, green tea extract, among others, being known by their antiproliferative properties [63]. At present, there is significant enthusiasm for the use of specific NF-κB inhibitors as new anti-cancer therapy [64, 65]; however, it is important to introduce some considerations about this kind of new drugs because an ideal inhibitor should prevent NF-κB activation without any apparent effects on other signaling pathways or immunological effects [31].

In colon tumour tissue, expression of PPAR-γ has also been detected in several studies using clinical samples [66]. Activation of PPAR-γ leads to cell differentiation and apoptosis. In addition, PPAR-γ ligands have been shown to be potent inhibitors of angiogenesis, a process necessary for tumour growth and metastasis, and also to protect against cellular transformation. A recent study on colon induced carcinogenesis by dimethylhydrazine showed a decrease of PPAR-γ expression after tumour induction and that the diclofenac chemopreventive action may be through PPAR-γ (activation or overexpression), regulation of COX-2 and the subsequent start of apoptosis [67]. There are very interesting data from the lab of Bassaganya-Riera which have recently observed the beneficial effects of dietary n-3 polyunsaturated fatty acids (PUFAs) in experimental IBD and inflammation-induced CRC through, at least in part, PPAR-γ-dependent mechanism [68]. Further studies are needed to fully elucidate the anti-proliferative and pro-differentiation mechanisms of PPAR-γ activators and their expedient evaluation in the clinical management of CRC.

A leading theory is that the oxidative stress accompanying chronic inflammation contributes to neoplastic transformation. ROS, like the superoxide anion and NO species such as nitric oxide radical ($NO^{\bullet}$) and its metabolite peroxynitrite, can interact with DNA in proliferating epithelium resulting in permanent genomic alterations. In addition, in colitis-associated colon carcinogenesis, ROS may contribute to the *p53* mutations and can functionally impair the protein components of the DNA mismatch repair system [14, 69]. Inducible nitric oxide synthase (iNOS) expression is induced during inflammation and catalyzes the production of NO, however depending on the concentration, genetic background, and type of NOs enzyme, NO may induce protective effects [70]. Clinical data show that iNOS levels are elevated in actively inflamed mucosa from IBD; however, there is controversy about its role in intestinal carcinogenesis [71, 52].

Autophagy plays a dual role in tumorigenesis: the promotion of cell death as a tumor suppressor and the prevention of cell death as an oncogenic mechanism [14]. Mitochondrial DNA is more susceptible to damage due to the lack of repair systems and histone protein protection, and it is thought that autophagy removes damaged mitochondria, thus alleviating oxidative stress to prevent carcinogenesis. However, autophagy is also a cytoprotective mechanism that prevents cells from death under starvation or stress conditions. Studies have shown that ROS can induce autophagy, which instead of causing cell death, protects cells from apoptosis or necrosis, suggesting that autophagy plays both promotion and suppression roles in tumorigenesis [72]. The coordinated regulation of autophagy and apoptosis is essential for cells to make a dynamic choice between death and survival when under stress. These processes are regulated by common factors (p53) although they also share some key autophagy genes (Beclin-1 and Atg5) [73-75]. The tumor-suppressor protein p53 is altered in more than 50% of human cancers and mutation of the p53 gene is one of the most common genetic changes in the development of human colorectal cancer. Recently, this protein has been shown to regulate autophagy [76, 77]: nuclear p53 stimulates autophagy, by this means sustaining the challenge of cells to deal with stress. Meanwhile, cytoplasmic p53 inhibites autophagy by managing the AMP-activated protein kinase (AMPK), a positive regulator of autophagy, and activates mammalian target of rapamycin (mTOR), the main negative regulator [78]. In HCT-116 human CRC cells, loss of p53 impaires autophagic flux upon starvation, culminating in apoptosis [79]. This could explain why some cancer cells retain p53. Although the interplay between autophagy and p53 is complex, the understanding of it would supply new strategies to deal with cancer. Experimental evidence suggests that autophagy is activated by tumor cells as a pro-survival mechanism against cytotoxic agents. Thus, inhibition of autophagy can be used as a tumor cell sensitizing strategy. Along these lines, several CRC cells and others cell lines (HT-29, HTC-116, DLD-1, SW480, WiDr, LoVo, SW620), treated with pharmacological inhibitors of autophagy or subjected to siRNA-mediated downregulation, show increased sensibility to COX inhibition [80], tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced cell death [81], aminoacid and glucose deprivation [82], sulphoraphane-induced apoptosis [83] and 5-fluorouracil chemotherapy [84].

Sirtuins (SIRT) are a group of highly phylogenetically conserved proteins that occur in organisms from bacteria to human beings, and which catalyze the deacetilation of target proteins. The deacetylation reaction spends NAD+, a key molecule in energy metabolism, thus linking protein regulatory control to metabolic conditions [85, 86]. SIRT1 deacetylation of p53 causes a weakening of its apoptotic effect. As a consequence, SIRT1 might be considered a facilitator for cancer development. Nevertheless, although pro-oncogenic effects of SIRT1 have been reported in a number of studies, there are also reports showing a tumor suppressor role for this protein [87].

On the other hand, sirtuins have been implicated in circadian rhythms by deacetilating proteins in the clock mechanism of circadian control [88], which establishes a link between sirtuins and melatonin. Interestingly, sleep disruption has been associated with IBD [89] and although information about the role of sirtuins in IBD is limited, there are several reports that show an anti-inflammatory effect for these molecules. Resveratrol, the best known SIRT1 activator, reverses colitis-associated decrease in SIRT-1 gene expression, activation of NF-κB, increase of COX-2 expression, and other changes, in a DSS-induced colitis, and in a spontaneous IL-$10^{-/-}$ mouse model of colitis [90, 91]. The same authors show that resveratrol suppresses colon cancer associated with colitis [92]. In addition, SIRT1 is a negative regulator of NF-κB activity through the deacetylation of the p65 lysine 310 [93]. Several studies support the notion that SIRT1 could be involved in carcinogenesis [94], and SIRT1 has been found to be up-regulated in various human cancers, including colon cancer [95]. SIRT1 expression is associated with microsatellite instability and CpG island methylator phenotype in human CRC [96]. Conversely, there are also studies showing that SIRT1 can act as tumor suppressor. SIRT1 suppresses intestinal tumorigenesis and colon cancer growth in a β-catenin-driven mouse

model of colon cancer [97]. Abnormal levels of β-catenin may contribute to neoplastic transformation in colon cells [98] and the stability of this protein increases by acetylation [99]. SIRT1 deacetylates β-catenin and promotes cytoplasmic localization of the otherwise oncogenic form of β-catenin [97]. On the other hand, defects in the Wnt signalling, which is upstream of β-catenin, have also been associated to human cancers [100]. SIRT-1 has been shown to regulate Wnt signalling in four colon cancers cell lines (HT-29, HCT-116, RKO and DLD-1) [101]; in this study, SIRT1 promotes constitutive Wnt signaling and Wnt-induced cell migration, therefore presenting more pro-tumor than an anti-tumor effect. In another study, SIRT1 inhibites proliferation of the colon cancer cell line HCT-116 and SIRT1 inhibition promotes colon tumor formation in a tumor xenograft assay [102]. These results show that sirtuins have pleitropic effects on cancer development demonstrating that there is a wide therapeutic potential for both activators and inhibitors of sirtuins.

Caloric restriction (CR) has long been the only factor able to extend maximum life span in diverse species [103], including primates [104]. Studies on yeast, worms, flies and mice point to a role for nutrient responsive molecules, such as SIRT1 and mTOR, in aging and CR [105]. SIRT1 is proposed to mediate the health benefits of CR showing its capacity to ameliorate degenerative diseases associated with aging [106]. Recently, Firestein *et al*. [97] have demonstrated that CR induces an increase of SIRT1 expression in the intestine of rodents. Moreover, this study shows that the ectopic SIRT1-induction in a mouse model of colon cancer significantly reduces tumor formation, proliferation and animal morbidity in absence of CR. Autophagy relationship with sirtuins have been demonstrated through AMPK, which senses the intracellular AMP/ATP ratio and inhibits mTOR-dependent signalling [107] while rising cellular NAD+ levels [108]. Consequently, AMPK activation switches on autophagy and increases sirtuin activity. In addition, SIRT1 regulates autophagy by deacetylation of several components of the autophagic cascade [109] and the life span-prolonging effect of sirtuins has been proposed to be mediated by autophagy [110]. Up to date, there are no studies connecting autophagy-sirtuin activity and colon cancer.

The identification of these signals has led to a greater mechanistic understanding of IBD pathogenesis and its evolution to colon cancer, and points to potentially new therapeutic targets.

3. CHEMOPREVENTION AND BIOMARKERS

Chemoprevention is an old concept that consists in the practice of using drugs, vitamins or nutritional supplements to reduce the risk of developing, or having a recurrence of cancer [111, 112]. A number of genetic changes can trigger cells to become cancerous although studying the changes as they occur is difficult, especially in humans. The carcinogenesis is a slow process that could start twenty years before the symptoms appear. That long spam of time offers several opportunities for intervention: the original mutation, the transforming and dedifferentiated process or the migration and metastatic movement. Recently, Nature dedicated a supplement to analyze current data of interest for cancer prevention; in this publication Professor Sporn discussed about the state of the art for chemoprevention [113]. The vast majority of cancer research is devoted to find cures, rather than finding new ways to prevent disease. However, common epithelial cancers including lung, colon, pancreas, ovary, skin, prostate and breast, which are responsible for most deaths, have a long latency period of twenty years or more. This latency period is a sufficient occurrence to use preventive drugs that block the development of invasive and/or metastatic disease. With these objectives, cancer chemoprevention uses natural, synthetic or biological substances to reverse, suppress or prevent either the initial phase of carcinogenesis or the progression of neoplastic cells to cancer. In this line, induction of apoptosis represents a potent cellular mechanism against cancer. Zhang *et al*. [114] have recently shown how the selective elimination of premalignant tumour cells by transcription-regulating factors, such as TRAIL and all-trans retinyl acetate (RAC), is an effective method for chemoprevention. In *in vivo* experimental models of carcinogenesis, it is possible to prevent the onset of cancer in many organs in which carcinoma occurs [113]. Furthermore, chemoprevention has now been clinically validated: selective estrogen receptor modulators (SERMs) can reduce in five-fold the incidence of estrogen receptor-positive breast cancer. In men, antiandrogenic drugs have shown effectiveness in long-term clinical trials for prostate cancer.

Topics to debate include potential toxicity for long term treatments, cost-effective or target population. In such a way, it is suggested stopping doing clinical chemoprevention trials in large populations of people at relatively low risk and focus to the highest risk such as smokers for lung cancer [113]. Individuals identified as being high risk, through use of biomarkers, could receive counselling for life style changes and they might be eligible for chemoprevention [115]. Moreover, using biomarkers to select people for cancer clinical studies would allow for more powerful trials. Anywhere, here the difficulty is finding reliable biomarkers to detect the real process and not false starts; sensitivity and specificity are two essential features and the key to both is finding better biomarkers (genes, proteins and cellular metabolites) that can be measured and associated with the development of cancer. Phosphatidylinositol-3-kinase (PI3K) signalling pathway might be used for chemoprevention and early trials have shown that the administration of a drug that decreases PI3K activity causes regression of abnormal lesions in the airways of high-risk smokers [116]. Other proposals include alteration in gene expression, levels of death-associated-protein kinase or DAR kinase (enzyme implicated in apoptosis), antibodies to mutant p53 (as signal of damage of cell tumour suppressor system), changes in cancer-related genes or markers of inflammation [115].

Many researchers in chemoprevention are beginning to think that perhaps the best way to catch cancer is to target inflammation. Chronic inflammation appears to encourage tumours by prompting the growth of new blood vessels and remodelling extracellular matrix. Thus, it is created a prime setting for normal cell growth to turn malignant [117]. Targeting inflammation might prevent not only cancer but a number of other syndromes or diseases including diabetes, cardiovascular diseases or aging; epidemiological studies with NSAIDs, COX inhibitors (COX-1 or COX-2), may reduce the risk of colon, lung, prostate, brain or skin cancers [118, 119]. Other findings propose that statins, used for cholesterol control, might disrupt the growth and proliferation of cancer cells [120]. Similarly, retrospective studies that may be impractical to confirm prospectively suggest that diabetics treated with metformin have a substantially reduced cancer burden compared with other diabetics. It is unclear if this reflects a chemopreventive effect and thus, developing clinical trials may define important new indications for biguanides in the prevention and/or treatment of many common cancers [121].

4. USE OF DIETARY PHYTONUTRIENTS IN IBD AND COLON CANCER

4.1. Phytonutrients Derived from Fruits and Vegetables

Epidemiological studies have suggested that dietary agents identified from fruits and vegetables contribute to keep balanced cell proliferation and prevent cell carcinogenesis. Moreover, phytonutrients have received considerable attention because of their low cost and wide safety margin. A substantial amount of evidence from animal and cell culture experiments and some human studies have shown cancer chemopreventive effects from these natural products. However, single-agent intervention has failed to produce the expected outcome in clinical trials; therefore, combinations of them are gaining increasing popularity [122]. A recent study from European Prospective Investigation into Cancer and Nutrition observed

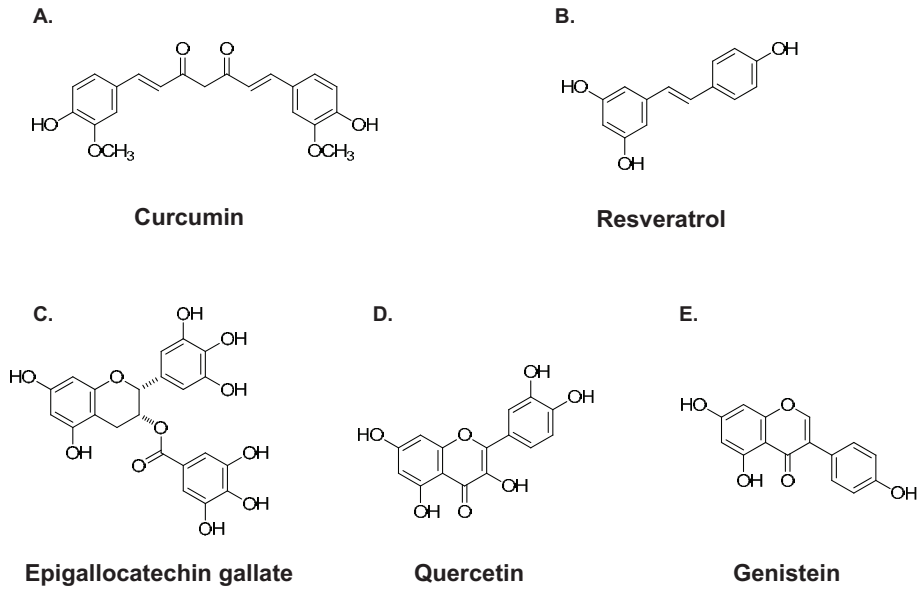

Fig. (3). Chemical structures of some phytonutrients derived from fruits and vegetables.

inverse associations between the consumption of vegetables and fruits and risk of lung cancer. Moreover, this study suggests that in current smokers, consumption of vegetables and fruits may reduce lung cancer risk, especially squamous cell carcinomas [123]. However, the chemopreventive properties of plant foods are complicated to demonstrate and several considerations should be taken into account in order to confirm which phytonutrients are cancer preventive agents [124].

Next paragraphs summarize recent advancements on potential colon cancer chemoprevention and mechanistic insight into the actions of the most commonly used and highly researched phytochemicals derived from diet, including curcumin, resveratrol, epigallocatechin gallate (EGCG), quercetin and genistein.

Curcumin

Curcumin (diferuloylmethane) (Fig. **3A**) is a bioactive substance present in the rhizomes of the herb *Curcuma longa* that has been used for centuries in Asia, both in traditional medicine and in cooking as turmeric which gives food an exotic natural yellow color. This perennial herb has multiple ingredients, including curcuminoids, the most active ingredients for medicinal use [125]. Curcumin has been used for a variety of diseases, including respiratory conditions, inflammation, liver and hepatic disorders, obesity, diabetic wounds, rheumatism and certain tumors [126-130]. To date, no studies in animals or humans have discovered significant toxicity related to curcumin, even at very high doses [131, 132].

IBD Animal Models and Clinical Studies with Curcumin

Curcumin has been shown to have preventive as well as therapeutic effects in different experimental models of colitis. Along these lines, treatment with curcumin prior to the induction of colitis mitigated the injurious effects of DSS or trinitrobenzene sulfonic acid (TNBS) via regulation of oxidant/anti-oxidant balance and reduction of serum TNF-α and colonic NO levels [133, 134]. In addition, curcumin blocked NF-κB activation [135, 136] and inhibited TLR-4 expression, which has been shown to induce NF-κB leading to inflammation in the colonic mucosa [137]. Curcumin has also been reported to ameliorate TNBS-induced chronic colitis through decrease of COX-2 expression [138], as well as via suppression of Th1 cytokines expression (IL-12, IFN-γ, TNF-α, IL-1) and increase of Th2 cytokines profile (IL-4 and IL-10) in colon mucosa [139]. Other mechanisms by which the dietary polyphenol curcumin exerts its anti-inflammatory effects in the development of chronic UC include reduction in iNOS expression and activity, inhibition of p38 MAPK signaling [140] and activation of PPAR-γ and its ligands [141]. A recent study has reported the anti-inflammatory effect of the diferuloylmethane administered for two weeks in the spontaneous colitis in IL-10 gene-deficient mice through a reduced production of potent pro-inflammatory mucosal cytokines [142].

Clinical data evaluating the use of curcumin in IBD (including UC and CD) is limited to two studies comprising data for only 99 patients. The first one, conducted by Holt *et al.* [143] was a small, open-label study, in which curcumin was administered to five patients with UC and five with CD. All proctitis patients improved, with reductions in concomitant medications in four of them and four of five CD patients experienced a reduction in disease activity index scores. Hanai *et al.* [144] conducted a randomized, multicenter, double-blind, placebo-controlled trial of curcumin plus sulfasalazine or mesalamine compared to placebo plus sulfasalazine or mesalamine in 89 patients with quiescent UC. The relapse rate was significantly higher in the placebo group (20.5%) than in the curcumin-treated group (4.7%). The polyphenol also improved both clinical activity index and endoscopic index scores.

In vitro Studies with Curcumin in Colon Cancer

Although the exact mode of action of curcumin is not yet published, pre-clinical studies have shown that curcumin inhibits a number of signaling pathways and molecular targets involved in cancer progression and inflammation [130]. This dietary compound has been reported to exhibit potent *in vitro* antiproliferative and apoptosis-inducing activities in colon cancer cell lines. Along these lines, curcumin treatment induced apoptosis and inhibited colon cancer cell growth via an increase in PPAR-γ, resulting in suppression of cyclin D1 and epidermal growth factor receptor (EGFR) [145] as well as through inhibition of PPAR-δ expression, leading to down-regulation of VEGF [146]. Curcumin has been shown to act synergistically with 5-fluorouracil plus oxaliplatin, a standard chemotherapy for this malignancy, in inhibiting the growth of HCT-116 and HT-29 cells through attenuation of surface growth factor pathways, specifically EGFRs and insulin-like growth factor-1 receptor (IGF-1R), which are considered to play important role in the progression of CRC [147]. Moreover, the diferuloylmethane

induced caspase-3-mediated β-catenin cleavage and c-Myc down-regulation in the Wnt signaling pathway, resulting in G2/M phase arrest as well as increased apoptosis by enhancing the degradation of β-catenin, E-cadherin and APC in HCT-116 cells [148].

Oxidative stress caused by exposure to curcumin has also been implicated in its apoptotic effect [149]. Moreover, Milacic *et al.* [150] reported that curcumin has proteasome-inhibitory activity, which is a strong apoptotic stimulus for both colon cancer HCT-116 and SW480 cell lines. In addition, Lee *et al.* [151] demonstrated the apoptotic effects of curcumin on HT-29 colon cancer cells through the AMPK activation and the decrease in *p*Akt and COX-2 proteins. Other proapoptotic mechanisms of curcumin include induction of p53, overexpression of Bax, down-regulation of Bcl-2 and caspase-3 activation in human colon cancer cells [146, 152].

Biological mechanisms by which curcumin may prevent cancer are also thought to involve the inhibition of NF-κB activation, which may lead to suppression of inducible enzymes expression, such as COX-2, adhesion molecules and inflammatory cytokines [153]. In this line, curcumin inhibited TNF-α-induced COX-2 expression through suppression of NF-κB activity in colon cancer cells. This effect was due to the inhibition of the I(kappa) B kinase (IKK) signaling complex that is responsible for the phosphorylation of I(kappa) B (IkB) [154]. In a similar report, this substance suppressed cytokine-mediated NF-κB activation with concomitant down-regulation of intercellular adhesion molecule-1 (ICAM-1) and IL-8 gene expression [155]. The pro-inflammatory cytokine IL-8 has been shown to affect cancer progression through angiogenic and mitogenic effects [156]. Curcumin effectively inhibited neurotensin-mediated NF-κB induction and subsequent IL-8 gene induction and migration of HCT-116 colon cancer cells [157].

In addition, curcumin has been reported to inhibit chenodeoxycholate or phorbol ester-mediated induction of COX-2 and the subsequent synthesis of prostaglandin E2 in a number of gastrointestinal cell lines [158]. Goel *et al.* [159] showed that curcumin specifically inhibited COX-2 but not COX-1 expression in HT-29 human colon cancer cells. Cotreatment with curcumin and celecoxib, a selective COX-2 inhibitor, synergistically inhibited colon cancer cell growth and induced apoptosis through mechanisms involving down-regulation of COX-2 expression [160]. Du *et al.* [161] reported similar results after combination of curcumin and 5-fluorouracil. The overexpression of metalloproteinases (MMPs) has been reported in colorectal cancer and plays an important role in neoplastic cell proliferation and metastasis. Curcumin suppressed cell migration of the human colon cancer cell line, colo 205, by downregulating COX-2 and matrix metalloproteinase-2 expressions [162]. Curcumin has been shown to inhibit vascular tube formation, which may explain its chemopreventive effect at the level of tumor growth and metastasis. These antiangiogenic properties have been shown to be mediated in part by down regulation of COX-2 expression and prostaglandin E_2 (PGE_2) production in human intestinal microvascular endothelial cells [163].

Animal Studies with Curcumin in Colon Cancer

Curcumin has also shown promise as a chemopreventive agent because of its *in vivo* regression of various animal models of colorectal carcinogenesis. Along these lines, curcumin treatment to mice bearing xenografts of colon cancer HCT-116 cells resulted in tumor growth inhibition, associated with proteasomal inhibition, growth arrest and stimulation of apoptosis [150]. Li *et al.* [164] demonstrated that liposomal curcumin inhibited colon carcinoma growth in Colo205 and LoVo xenografts and these effects were accompanied by a potent antiangiogenic response, as shown by reduction in CD31 (an endothelial marker), VEGF and IL-8 expression. Another interesting study reported that cotreatment of curcumin and resveratrol was more effective than either agent alone in inhibiting growth of p53-positive (wt) and p53-negative colon cancer HCT-116 cells *in vitro* and *in vivo* in SCID xenografts of colon cancer HCT-116 (wt) cells [165].

Since adenomas are generally regarded as precursor lesions of colorectal cancer, several reports have examined the effect of dietary curcumin in the adenomatous polyposis coli (Apc^{Min+}) mouse, a model of human familial adenomatous polyposis. These animals are genetically predisposed to develop intestinal tumors as a result of a mutation of the Apc gene [166]. The results of these studies have shown that curcumin retarded adenoma growth, reflected by total number of adenomas and mean adenoma size [167, 168]. This effect may be mediated by the inhibition of COX-2 protein expression and the reduction of levels of two oxidative DNA adducts in intestinal adenoma tissue [169]. Curcumin also decreased expression of the oncoprotein β-catenin in the enterocytes of the Apc^{Min+} mouse [167]. Another paper examining the effects of curcumin on intestinal inflammation in this mouse model, has reported that this compound decreased total intestinal polyps by 75% as well as mRNA and protein expression of IL-1β and chemokine ligand 2 (CCL2), suggesting that the benefits of curcumin in colon cancer may be, at least in part, mediated by its antiinflammatory activity [170].

The colon carcinogens 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM) produce their effects through a series of early steps involving inflammation, increased cell proliferation in colon crypts, epigenetic and genetic alterations and late events such as decreased sensitivity to apoptosis induction resulting in the development of cancer [171]. Curcumin administration during initiation, postinitiation and promotion/progression phases of AOM-induced colon carcinogenesis in rats significantly inhibited tumor development in a dose-dependent manner and increased apoptosis in the colonic tumors [172, 173]. It has also been shown that combination of curcumin and celecoxib suppressed DMH-induced colon carcinogenesis in rats by decreasing the average number of aberrant crypt foci (ACF) [174]. Similarly, cotreatment of dietary curcumin and green tea catechins had a preventive effect in this model of carcinogenesis since this combination inhibited the total number of ACF and the proliferation index as well as increased the apoptotic index [175].

It has been shown that cyclic administration of DSS in drinking water results in the establishment of chronic colitis and the development of colorectal dysplasias and cancers with pathological features that resemble those of human colitis-associated neoplasia [51]. Villegas *et al.* [176] have recently demonstrated the protective effect of dietary curcumin in this model of chronic colitis-associated CRC through a reduction in β-catenin expression and in the production of the proinflammatory cytokines TNF-α and IFN-γ.

Clinical Studies with Curcumin in Colon Cancer

Curcumin has been evaluated as a potential chemopreventive and/or chemotherapeutic agent in several different clinical trials [177, 178]. In a phase I clinical trial, fifteen patients with advanced CRC refractory to standard chemotherapies received curcumin doses between 0.45 and 3.6 g daily for up to 4 months. Consumption of 3.6 g of curcumin daily generated detectable levels of curcumin and its glucuronide and sulfate metabolites in plasma and urine as well as caused inhibition of PGE_2 production in blood leukocytes measured *ex vivo* [179]. More recently, Carroll *et al.* [180] evaluated the effects of oral curcumin (2 g or 4 g per day for 30 days), in a nonrandomized and open-label clinical trial, in 44 eligible smokers with eight or more ACF on screening colonoscopy. The results showed a significant 40% reduction in ACF number occurred with the 4-g dose, whereas ACF were not reduced in the 2-g group. Another study showed that curcumin treatment improved the general health of patients with CRC by decreasing serum TNF-α levels and increasing apoptotic tumor cells and expression of p53 in tumor tissue [181]. The combination of curcumin and quercetin reduced the number and size of ileal and rectal adenomas in five

patients with familial adenomatous polyposis without appreciable toxicity, after six months of treatment [182].

Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) (Fig. **3B**) is a polyphenolic phytoalexin synthesized by plants in response to stress and invasion of other pathogens. It is found in grape skins, red wine, peanuts, mulberries and cranberries. Resveratrol is formed by a condensation reaction between 3 molecules of malonyl coA and a molecule of 4-coumaroyl coacatalyzed by resveratrol synthase [183]. Both the cis and trans forms coexist; however, trans is the biologically active form [184]. Numerous *in vitro* and animal studies show that resveratrol has potent antioxidant, anti-aging, anti-inflammatory and anti-cancer effects, promotes vascular endothelial function and enhances lipid metabolism [185-187].

IBD Animal Models with Resveratrol

Resveratrol has been evaluated for its beneficial effects in several UC models. This natural compound ameliorated DSS-induced acute inflammation in mouse colonic mucosa through inhibition of iNOS expression and NF-κB, signal transducer and activator of transcription-3 (STAT3) and extracellular signal-regulated kinase (ERK) activity [188]. The inhibitory effects of resveratrol in this model of UC were also associated with its antioxidative effect and the reduction in the expression levels of TNF-α, IL-8 and IFN-γ [189]. In addition, this polyphenol decreased COX-2 expression and PGD_2 concentration and enhanced apoptosis in rats with TNBS-induced acute colitis [190]. Resveratrol has also been reported to abrogate chronic colitis by inducing SIRT1 expression with consequent decrease in NF-κB activity [90]. Other mechanisms involved in the beneficial effects of resveratrol in DSS-induced colitis model include inhibition of pro-inflammatory factors, such as TNF-α, IL-1β and NO levels and prostaglandin E synthase-1 (PGES-1), COX-2 and iNOS expression, as well as increase of the anti-inflammatory cytokine IL-10 [191, 192]. In addition, oral administration of resveratrol ameliorated the established chronic colitis in IL-10 (-/-) mice by inducing immunosuppressive CD11b(+) Gr-1(+) cells in the colon. This study also reported that resveratrol decreased local and systemic inflammatory cytokine concentrations, including IFN-γ, TNF-α, IL-6, RANTES, IL-12 and IL-1β [193].

In vitro Studies with Resveratrol in Colon Cancer

A significant number of *in vitro* studies have shown that induction of apoptotic cell death and inhibition of cell cycle progression are the two major pathways responsible for the chemopreventive role of resveratrol in colon cancer. This compound has been shown to elicit apoptotic signaling via mitochondria. Mohan *et al.* [194] reported that caspase-2 activation occurred upstream of mitochondria in the colon cancer cells HCT-116 treated with resveratrol. The activated caspase-2 triggered mitochondrial apoptotic events by inducing conformational changes in Bax/Bak with subsequent release of cytochrome c, apoptosis-inducing factor and endonuclease G. Lee *et al.* [195] identified caspase-6 activation and lamin-A cleavage as a major signaling pathway in resveratrol-evoked apoptosis in HCT-116 human colon carcinoma cells. The antiapoptotic effects of resveratrol were significantly mitigated in the absence of the tumor suppressor protein p53. These authors also reported that the polyphenol, at high concentrations, synergistically promoted 5-Fluoruracil-mediated colon cancer cell apoptosis through enhancement of caspase-6 activity irrespective of p53 status. Conversely, lower doses of resveratrol displayed antagonistic effects on cell apoptosis [196]. In another study, resveratrol induced apoptosis in the colon carcinoma cell line HCT-116 regardless of p53 status and at concentrations comparable to those found in wine and grapes [197]. Park *et al.* [198] demonstrated that resveratrol-induced apoptotic cell death in HT-29 cells was associated with its ability to induce ER stress response.

Insulin-like growth factor-I receptor (IGF-IR) is overexpressed during colon carcinogenesis. Upon IGF-1 binding, IGF-1R activates the PI3K/Akt cascade [199], which stimulates the Wnt/β-catenin pathway, among others [200]. This signaling pathway plays a central role in elevating colonocyte proliferation and suppressing apoptosis in colon cancer [201]. Resveratrol has been shown to suppress colon cancer cell proliferation and elevate apoptosis after IGF-1 exposure through suppression of IGF-1R/Akt/Wnt signaling pathways as well as activation of p53 [202]. Moreover, this polyphenol significantly decreased the amount and proportion of β-catenin in the nucleus of colon cancer cell line RKO [203].

It was reported the involvement of mitochondrial ROS on antitumoral activity of this bioactive compound in the human colorectal carcinoma cell line HT-29 [204]. Resveratrol has also demonstrated anti-inflammatory effects, through inhibition of NOS enzyme activity [205] and increase of PPAR-γ activity in CaCo-2 cells [206]. In addition, Zykova *et al.* [207] showed that the anticancer effects of resveratrol were mediated directly through COX-2 and that this polyphenol decreased COX-2-mediated PGE_2 production in HT-29 cells.

Resveratrol has also been shown to exhibit antimetastatic effects through its potent inhibition of cell adhesion, migration and invasion, as well as reduction of secretion of MMP-9 and MMP-2 in Lovo cells cultured under normoxia and hypoxia [208]. Kimura *et al.* [209] reported that antimetastatic actions of resveratrol were probably due to the inhibition of VEGF-induced angiogenesis.

Animal Studies with Resveratrol in Colon Cancer

Schneider *et al.* [210] showed that orally administered resveratrol decreased the number of tumors in the small intestine and prevented tumor formation in the colon of Apc^{Min+} mice. Moreover, these authors demonstrated that resveratrol downregulated genes known to be implicated in cell cycle progression, such as cyclins D1 and D2, as well as upregulated a panel of genes controlling the activation of immune response and the inhibition of the carcinogenic process and tumor expansion.

Chronic resveratrol supplementation has been shown to suppress DMH-induced colon carcinogenesis at various stages through reduction of the ACF incidence, tumor development, free radical-mediated oxidative stress, cell proliferation and cancer-associated bacterial enzyme activities, when supplemented throughout the experimental period [211, 212]. Moreover, these authors reported that mechanisms underlying the potential chemopreventive effect of resveratrol in this model of colon carcinogenesis included down-regulation of the expression of inflammatory, cell proliferative and apoptotic biomarkers such as COX-2, ornithine decarboxylase, caspase-3 and heat shock proteins 70 and 27 [213].

Studies on the effects of resveratrol on AOM-induced carcinogenesis revealed a significant reduction in the number and multiplicity of ACF in the colorectal mucosa by modulation of Bax and p21 expression [214]. Similarly, a recent paper has reported that this polyphenol prevented the onset of colon cancer in a mouse model of colitis-driven colon cancer [91]. It has also been reported the inhibitory effect of dietary administration of pterostilbene, a natural dimethylated analogue of resveratrol, against the formation of AOM-induced ACF and adenomas, by suppressing the proliferation of malignant colonocytes and abnormal expression of iNOS and COX-2 as well as inducing apoptosis in the colonic crypts [215, 216]. In addition, this compound downregulated the expression of β-catenin and cyclin D1, and reduced the levels of TNF-α, IL-1 β and IL-4 and nuclear staining for phospho-p65, a key molecule in the NF-κB pathway [217].

Clinical Studies with Resveratrol in Colon Cancer

Nguyen *et al.* [218] conducted a phase I pilot clinical trial in patients with colon cancer to evaluate the effects of a low dose of plant-derived resveratrol and resveratrol containing freeze-dried

grape powder (GP) on Wnt signaling in the colon. The results showed that resveratrol in combination with other bioactive compounds in GP inhibited Wnt pathway in normal colonic mucosa, as indicated by a reduction in the expression of a panel of Wnt target genes. In another recent study, twenty patients with confirmed colorectal cancer, who were to undergo surgical resection of their malignancy, received eight daily doses of resveratrol at 0.5 or 1.0 g before surgery. Consumption of resveratrol significantly reduced colon tumor cell proliferation, as reflected by reduction in Ki-67 staining, a surrogate marker of cell growth [219]. Further clinical studies of dietary supplementation with resveratrol as a potential colon cancer preventive strategy are needed.

Epigallocatechin Gallate

Green tea (*Camellia sinensis)* contains several polyphenolic compounds, including the catechins (-)-epigallocatechin gallate (EGCG) (Fig. **3C**), (-)-epigallocatechin (EGC), epicatechin-3-gallate (ECG) and (-)-epicatechin (EC) [220]. Among the green tea constituents, EGCG is the major biologically active compound, which has been shown to possess *in vitro* and *in vivo* anti-inflammatory and anti-oxidant properties, responsible for their cancer chemopreventive potency [221, 222].

IBD Animal Studies with EGCG

Recent studies show the beneficial effects of EGCG in different experimental models of colitis. These effects were associated with a significant reduction of NF-κB and (AP-1) activation [223], as well as an inhibition of TNF-α and IFN-γ production. EGCG also showed antioxidant activity via decreasing NO and malondialdehyde (MDA) and increasing superoxide dismutase (SOD) in colonic mucosa [224]. Furthermore, EGCG ameliorated acute experimental colitis by the suppression of mast cells and macrophage activities [225].

In vitro Studies with EGCG in Colon Cancer

Previous *in vitro* studies have demonstrated that EGCG inhibits human CRC cells growth and induces apoptosis in cancer cells [226-228]. The overexpression of EGFR and human epidermal growth factor receptor-2 (HER2) has been reported in colorectal carcinoma and has shown to play a critical role in neoplastic cell proliferation [229]. EGCG inhibited activation of EGFR, HER2 and HER3 receptors as well as the downstream effectors ERK and Akt in HT-29 and SW837 human colon cancer cells [226, 227]. The inhibitory effect of EGCG on activation of EGFR has been attributed to changes in the organization of the plasma membrane and, as a result, the inhibition of EGF binding to its receptor in HT-29 colon cancer cells [230]. This compound also inhibited the transcriptional activity of the AP-1, c-fos, NF-κB and cyclin D1 promoters in HT-29 cells [226].

In addition, EGCG has shown to induce apoptosis by suppression of COX-2 expression and the subsequent production of PGE_2 in different colon cancer cells, such as SW837, HT-29 and HCA-7 cells [227, 228]. The inhibitory effect of EGCG on the expression of this inducible enzyme was mediated by the reduction of NF-κB activity [231] and activation of AMPK [228, 232]. The stimulation of AMPK was accompanied by the reduction of VEGF and glucose transporter, Glut-1, in EGCG-treated cancer cells [228]. Another study reported that the anti-inflammatory properties of EGCG may be related to its antifolate activity. EGCG, by inhibiting folic acid uptake, can disturb the metabolism of this vitamin in Caco-2 cells, producing the release of adenosine and the suppression of NF-κB activation [233]. Moreover, EGCG has been reported to improve the inflammatory response in the colon adenocarcinoma cell lines HT-29 and T84 by down-regulating the secretion of the chemokines IL-8 and macrophage inflammatory protein (MIP)-3alpha [234].

Animal Studies with EGCG in Colon Cancer

It has been shown the chemopreventive ability of EGCG and Polyphenon E (Poly E), a decaffeinated extract of green tea that contains 60% EGCG and lesser amounts of other tea catechins, in a colitis-related mouse CRC model induced by AOM and DSS. Treatment with EGCG or Poly E suppressed the multiplicity and volume of colonic neoplasms as well as reduced colonic expression of COX-2 and inflammatory cytokines, such as TNF-α, IFN-γ, IL-6, IL-12 and IL-18 [235]. EGCG has been also reported to prevent the formation of colon ACF induced by 2-amino-3-methylimidazo[4,5-f] quinoline in mice [236] and rats [237]. Another study examined the effects of EGCG on the development of AOM-induced colonic premalignant lesions in C57BL/KsJ-db/db (db/db) mice, which are obese and develop diabetes mellitus. EGCG administration in drinking water effectively suppressed the development of aberrant crypt foci, thus suggesting the inhibitory effects of EGCG on the early phase of obesity-related mouse colon carcinogenesis. In addition, this suppressing effect of EGCG was associated with the improvement of hyperlipidemia and hyperinsulinemia and a depressant effect on the IGF/IGF-IR axis [238], which is involved in the development, progression and metastatic potential of CRC [239]. Furthermore, Ju *et al*. [240] reported that EGCG effectively inhibited intestinal tumorigenesis in Apc^{Min+} mice, possibly through the attenuation of the carcinogenic events, which include aberrant nuclear β-catenin and activated Akt and ERK signaling.

Clinical Studies with EGCG in Colon Cancer

Hoensch *et al*. [241] conducted a controlled, prospective and observational pilot study where long-term treatment with a tea-based flavonoid mixture (daily standard dose 20 mg apigenin and 20 mg EGCG) reduced the recurrence rate of colon neoplasia in high risk patients particularly in those with resected colorectal cancer. Since data evaluating the efficacy of this phytochemical in cancer chemoprevention are limited, future clinical studies are needed.

Quercetin

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Fig. **3D**) is a typical flavonoid, the most well defined group of polyphenolic compounds and is abundant in many commonly consumed fruits and vegetables, including apples, cranberries, blueberries, leek and onions [242, 243]. It has been reported that the average intake of flavonoids is 23 mg per day with quercetin contributing with almost 70% [244, 245]. Flavonoids in general are considered as non-absorbable due to being bound to sugars as beta-glycosides. Hydrolysis causing their absorption can only occur in the colon by microorganisms [244]. Quercetin is usually present in glycosylated forms, such as quercitrin (3-rhamnosylquercetin) and rutin (3-rhamnosyl-glucosylquercetin). Quercetin and its glycosides have been reported to display a number of biochemical and pharmacological activities, including anti-viral, anti-microbial, anti-allergic, anti-inflammatory and anti-cancer effects [246, 247].

IBD Animal Studies with Quercetin

Previous papers have shown that quercitrin and rutin exert intestinal anti-inflammatory effects in experimental models of rat colitis, whereas no clear effects have been demonstrated for the aglycone form quercetin. The beneficial effects of quercitrin were associated with a reduction in colonic MDA levels and alkaline phosphatase activity as well as with an improvement in the colonic oxidative status [248, 249]. Moreover, an inhibition of NOS activity, probably related to a decreased expression in the inducible form of the enzyme via downregulation of NF-κB activity was also reported after chronic treatment with quercitrin [250, 251]. Kwon *et al.* [252] demonstrated that a diet containing rutin, but not quercetin, ameliorated DSS-induced colitis, presumably by suppressing the induction of pro-inflammatory cytokines, including IL-1β and IL-6. These authors suggested that rutin may be useful for the prevention and treatment of IBD and colorectal carcinogenesis via attenuation of pro-inflammatory cytokine production.

In vitro Studies with Quercetin in Colon Cancer

The effects of quercetin on colon cancer have been largely analyzed in cell culture models. This flavonoid inhibited cell growth and induced apoptosis in a number of colon cancer cells, including HT-29, Caco-2, SW480 and T84 [253-255]. Kim *et al.* [256] reported that these anticancer effects may be mediated, in part, by the ability of quercetin to downregulate ErbB2/ErbB3 signaling and the Akt pathway, which have been associated with the development of human colon cancer. In addition, quercetin supressed cell growth, arrested the cell cycle at the sub-G1 phase and induced apoptotic death in both breast and colon cancer cells through the activation of AMPK signaling. This activation seemed to be closely related to a decrease in COX-2 expression [257, 258]. It has been reported that some chemopreventive agents, including quercetin, kaempferol, genistein, resveratrol and resorcinol suppressed both transforming growth factor (TGF)-α-stimulated and non-stimulated COX-2 promoter activities in human colon cancer DLD-1 cells [259]. Furthermore, the antitumor effects of quercetin has been related to the inhibition of cyclin D1 and survivin expression as well as the down-regulation of transcriptional activity of β-catenin/Tcf in SW480 colon cancer cells transiently transfected with the TCF-4 reporter gene. This signaling pathway has been shown to play a pivotal role in cellular developmental processes and human carcinogenesis [260].

This flavonoid has also shown to have cancer-preventive effects through its strong antioxidative properties [261]. In this respect, the cytoprotective ability of certain dietary flavonoids, including quercetin, against oxidative DNA damage induced by hydrogen peroxide in Caco-2 cells was reported [262]. In human lymphocytes the flavonoids quercetin and rutin showed a dose-dependently protective effect against DNA damage caused by the mutagenic anticancer drug mitomycin C [263]. In the same line, a significant protective effect of these two flavonoids has been recently demonstrated after DNA damage had been induced *in vitro* by two food mutagens in lymphocytes obtained from colon cancer patients, which had increased basal DNA damage possibly due to an overproduction of ROS [264]. Thus, dietary supplementation with flavonoid-rich vegetables and fruits may prove very effective in protecting against oxidative stress.

Animal Studies with Quercetin in Colon Cancer

The liposomal quercetin significantly inhibited solid tumors growth in different murine models, including BALB/c mice bearing CT26 colon adenocarcinoma. The mechanisms underlying this antitumor effect were related with inhibition of tumor angiogenesis, induction of apoptosis and downregulation of heat shock protein 70 expression in tumor tissues [265]. Dietary quercetin has been also described to reduce AOM-induced colorectal carcinogenesis in rats [266-269]. The flavonoids quercetin, curcumin, rutin and silymarin suppressed the amount of ACF in this model of colon cancer. Moreover, all the herbal compounds, except silymarin, evoked apoptosis in the large intestine, with quercetin being the most potent [270]. Choi *et al.* [271] evaluated the effect of quercetin intake in the AOM model as well as inflammatory response in rats fed with high-fat diet rich in omega-6 fatty acids. These authors reported that quercetin supplementation reduced the number of ACF in animals fed high-fat diet; however, no significant changes in COX-2 and iNOS expression were found after quercetin administration. In a similar study, this flavonoid inhibited the formation of early preneoplastic lesions in the AOM model, which was associated with reductions in proliferation and increases in apoptosis. The protection conferred by quercetin may be in part due to a suppression of proinflammatory mediators expression typically upregulated at disease onset, such as COX-2 [272]. A recent study has reported that quercetin decreased polyp number and size in the Apc^{Min+} mouse model of intestinal tumorigenesis and that these effects may be related to a reduction in macrophage infiltration [273].

Clinical Studies with Quercetin in Colon Cancer

Bobe *et al.* [274] have recently conducted a 4-year randomized, multi-center, nutritional intervention trial to evaluate the effect of intake of flavonols, especially isorhamnetin, kaempferol and quercetin, in 872 patients presenting at least one histologically confirmed colorectal adenoma. This study reported a reduction in serum IL-6 concentrations, associated with a decreased risk of adenoma recurrence and higher flavonol intake.

Genistein

Asian diet contains high amounts of soy and soy products, which are rich in isoflavones, plant-derived polyphenols. Soy intake has long been recognized to reduce the incidence of different cancers, cardiovascular disease, postmenopausal syndrome, diabetes mellitus and osteoporosis [275-277]. One of the most abundant and the best-characterized dietary isoflavones is genistein (4,5,7-trihydroxyisoflavone) (Fig. **3E**). Like other isoflavones, genistein is capable of binding to the estrogen receptor, with a preference for receptor β, the predominantly expressed receptor subtype in the gastrointestinal tract and to trigger mechanisms of estrogen action [278]. Genistein reproduced the health benefits of soy, lowering the incidence of cardiovascular diseases [279], preventing osteoporosis and attenuating postmenopausal problems [280]. Furthermore, it has been reported that genistein exerts important metabolic and hormonal changes, including decrease of body mass and fat tissue, accompanied by a decreased appetite and alteration of expression of genes engaged in lipid metabolism and glucose transport into cells affecting lipolysis, lipogenesis and ATP synthesis [281].

IBD Animal Studies with Genistein

Genistein has shown beneficial anti-inflammatory effects in a rodent model of TNBS-induced chronic colitis through reduction in mieloperoxidase (MPO) activity and COX-2 mRNA and protein expression [282].

In vitro Studies with Genistein in Colon Cancer

Several *in vitro* studies have demonstrated that genistein has anti-cancer effects in prostate, breast, colon, gastric, lung and pancreatic adenocarcinomas and in lymphoma [276]. Genistein has been reported to inhibit cell growth and induce apoptosis in a number of colon cancer cell lines [283, 284]. The mechanisms involved in genistein's anti-cancer properties include inhibition of IGF-IR signaling and attenuation of the PI3K/Akt pathway activity, which is known to be critical in the regulation of colon cancer progression [285]. Additionally, the inhibitory effect of genistein in mouse colon cancer MC-26 cells involved activation of transforming growth factor-beta1 (TGF-β1)/Smad signaling [286], which has been shown to be associated with cell proliferation inhibition and induction of growth arrest and apoptosis in epithelial tissue [287]. Genistein has also been reported to induce apoptosis through the overexpression of Bax and the down-regulation of Bcl-2 in human colon cancer HT-29 [288]. The protective effects of genistein have been also attributed, in large part, to their antioxidant properties. Besides scavenging free radicals, this isoflavone stimulated antioxidant protein gene expression, such as metallothionein and catalase in Caco-2 cells [289].

Genistein has been shown to suppress metastasis in different cancer types, including colon cancer. Genistein reduced experimental lung metastasis of murine colon 26-L5 carcinoma cells by 44% [290]. It has been reported that epigenetic modifications, such as DNA promoter methylation and histone modification, play crucial roles in regulating the expression of many metastasis suppressor genes, which indicates the association between aberrant epigenetic alterations and cancer metastasis [291]. Genistein has been shown to regulate epigenetic alterations of various genes in cancer cell lines [292, 293]. It has been proposed that those changes may contribute to the function of this isoflavone in the inhibition of CRC metastasis [291]. Genistein has been reported to regulate specific

genes in the Wnt signaling pathway in colon cancer cells, by inducing Wnt5a expression, which was accompanied by a decrease in DNA methylation level at the analyzed CpG island [294].

A recent study has demonstrated that a mixture of soy isoflavones (genistein, daidzein and glycitein) inhibited proliferation and growth of DLD-1 human colon adenocarcinoma cells by causing arrests at G2/M phase of the cell cycle. Moreover, these authors showed that estrogen receptor-β is involved in the growth-suppressive effects of soy isoflavones [295]. Since the loss of the expression of this receptor is a common step in estrogen-dependent tumor progression in colon [296], upregulation and activation of receptor estrogen-β status by specific food-borne-ligands such as soy isoflavones could potentially be a dietary prevention or therapeutic strategy for colon cancer.

Furthermore, combination of 5-fluorouracil and genistein inhibited cell growth, induced apoptosis and ROS generation as well as abrogated COX-2 expression and PGE_2 production in colon cancer cells through phosphorylation of AMPK [297]. Co-treatment with indole-3-carbinol, derived from Cruciferous vegetables and genistein synergistically induced apoptosis in human colon cancer HT-29 cells by simultaneously inhibiting the phosphorylation of Akt and progression of the autophagic process [298]. Another study reported that genistein increased the cytosolic levels and growth inhibitory activities of EGCG against human colon cancer cells

Animal Studies with Genistein in Colon Cancer

The potential of soy isoflavones to reduce colon cancer has also been investigated in animal experiments. These studies have found controversial results depending on soy isoflavones dose and the animal model. In this line, a recent study showed that a diet containing different isoflavones (genistein, daidzein and equol) reduced ACF formation and COX-2 expression in a dose-independent manner in model of colon carcinogenesis induced by DMH [299]. However, other studies reported that genistein or soy isoflavones did not protect animals from colon tumor development [300, 301]. Moreover, the combination of 0.01% EGCG in the drinking fluid and 0.2% genistein in the diet enhanced intestinal tumorigenesis in male Apc^{Min+} mice [302].

Clinical Studies with Genistein in Colon Cancer

Data from epidemiologic studies evaluating the preventive effects of soy or isoflavones on colon cancer are inconsistent [303]. Adams *et al*. [304] found no reduction in colorectal epithelial cell proliferation after a 12-month dietary soy isoflavone intervention in a randomized controlled trial conduced in men and women with previous adenomatous polyps. In relation to colonic adenoma development two studies have examined the effect of soyabean food consumption on polyp growth [305, 306]. Although the findings from both studies suggest an inverse relationship between the intake of miso soup [305] or tofu [306] and the development of colorectal adenomas, this relationship was not found to be significant.

4.2. Marine Natural Products: Microalgae as a Source of Biomolecules with Potential in IBD and Cancer

Traditionally, earth's natural products have been studied to a great extent and have been used therapeutically. Early research focused on inland compounds, but in the last 30 years the need of new therapeutic molecules has given rise to a vast number of studies in marine invertebrates and microbes. We have witnessed the discovery of many new interesting compounds, which are commonly referred to as marine natural products. The large diversity found in the marine environment allows for the recollection of multiple molecules as well as it represents a huge source to isolate new microbes such as bacteria, cyanobacteria, fungi, algae or small invertebrates such as sponges, tunicates, bryozoans and mollusc [307]. It has been demonstrated that many of these molecular structures may be potentially useful to be used as drugs in the treatment of diseases such as cancer, chronic inflammation, infections or neurological disorders. Therefore, these organisms constitute extremely important oceanic resources, covering more than 90% of the oceanic biomass [308]; they are taxonomically diverse, largely productive and biologically active.

Biotechnology of microalgae was first developed during the 1960's and now is a major field of study for both research and industry. Currently, about 30,000 species of microalgae are known and only twelve are cultivated on an industrial scale, thus constituting a natural source of secondary metabolites. Being photoautotrophics, their simple growth requirements, in contrast to macroalgae, make them attractive for demand by the pharmaceutical, food, cosmetic and biodiesel industry. Algae, including both prokaryotic and eukaryotic organisms, can perform photosynthesis more efficiently than the plants themselves. They have a great capacity to adapt to different environmental conditions that make them suitable for living in different types of terrestrial or aquatic environment. In addition to their varying evolutionary histories or environmental features specific to the oceans, these organisms adapt their metabolism to different environmental conditions, producing marine natural products which possess different structural characteristics regarding to their terrestrial counterparts. The marine life forms have evolved into different secondary metabolites to some extent, besides hand their natural products are also unique in their frequent incorporation of halogen atoms from the surrounding sea water [309].

Nowadays, microalgae are considered rich sources of bioactive molecules, including carotenoids, polysaccharides and long-chain polyunsaturated fatty acids. These compounds have been shown to possess promising pharmaceutical potential because of their anti-inflammatory, antimicrobial, antiviral and antitumoral activities [310, 311]. Along these lines, β-carotene, a known antioxidant whose main sources are *Dunaliella and Haematococcus*, has been used as chemopreventive agent and for the treatment of neurodegenerative diseases. Other interesting molecules synthesized by microalgae with pharmacological applications include fatty acids, used as anti-inflammatory agents or for cardiovascular diseases (*Chlorella*); phycobiliproteins used as inmunomodulatory, antioxidant or anticancer factors (*Spirulina*); sulfated polysaccharides used as anti-viral (*Porphyridium*); vitamins, phenolic or volatile compounds, used as anti-microbial and antioxidants (*Spirulina*) (see Table **1**). The most studied bioactive compounds synthesized by microalgae are described below.

Carotenoids

Carotenoids have received increasing attention because of the decreased incidence of cancers associated with their consumption. The main carotenoids are carotenes and xanthophylls. They are lipid components which can be found in plants and algae as well as in non-photosynthetic organisms such as animals, fungi and bacteria. They are recognized by the red, orange or yellow appearing on the leaves, flowers or fruits, the color of bird feathers, shells of crustaceans and the scales and skin of fish [312]. It has been shown the antioxidant activity of many pigments including carotenoids that confers a protective role against oxidative stress in multiple contexts. In the human case, this protection is presumably the cause of improved health associated with consumption of these compounds. On the other hand, these products are widely used as colorants in the food industry and in aquaculture as essential components for proper growth and reproduction of species with commercial value. Marine microalgae contain up to 0.2% of carotenoids (w:w dry weight) and may thus be of high interest as functional food to prevent cancer. For this reason, microalgae are currently being studied as chemopreventive agents in numerous *in vitro* and *in vivo* inflammation models [313-315].

Microalgae as *Dunaliella, Haematococcus* or *Chlorella* are an important source of carotenoids [316] and for this reason, they are currently being studied for their potent antioxidant activity (food

Table 1. Potential Functional Ingredients found in Microalgae, Biologic Activity and Examples of these Bioactivities

Compounds	Biologic Activity	Microalgae	Reference
Carotenoids			
β-carotene	Antioxidant activity Anticancer (lunch, mouth, stomach, breast)	*Dunaliella salina* *Haematococcus pluvialis*	[405] [406]
Astaxanthin	Antioxidant and anti-inflammatory activity and cancer prevention	*Haematococcus pluvialis* *Chlorella vulgaris*	[406] [407]
Lutein	Antioxidant activity	*Chlorella pyrenoidosa* *Haematococcus pluvialis*	[408]
Violaxanthin	Antioxidant activity	*Chlorella elipsoidea* *Dunaliella tertiolecta*	[326] [313]
Zeaxanthin	Antioxidant activity	*Picochlorum sp* *Nannochloris* *Haematococcus pluvialis*	[409]
Fatty acids			
Oleic acid	Antioxidant activity	*Haematococcus pluvialis* *Chlorella vulgaris* *Dunaliella salina* *Spirulina platensis*	[410] [411] [412] [413]
Linoleic acid	Antioxidant and antimicrobial activity	*Dunaliella salina* *Spirulina platensis*	[412] [414]
Docosahexanoic acid (DHA)	Anti-inflammatory, reduce risk of certain heart diseases and anti-proliferative	*Spirulina platensis* *Nannochloropsis oculata* *Crypthecodinium cohnii, Schizochytrium sp.* *Nitzschia laevis*	[415] [416] [37]
Eicosapentaenoic acid (EPA)	Anti-inflammatory, reduce risk of certain heart diseases and anti-proliferative	*Nannochloropsis oculata* *Spirulina platensis* *Porphyridium cruentum* *Crypthecodinium cohnii, Schizochytrium sp.* *Nitzschia laevis*	 [416] [417] [378]
Proteins			
Phycobiliproteins (phycocyanin and allophhycocianin)	Antioxidant activity	*Spirulina plantensis*	[418]
Polyssacharides			
Sulfated polysaccharide	Antiviral, anticancer, anti-hyperlipidemia, and anticoagulant	*Porphyridium* *Chlorella autotrophica* *Isochrysis sp*	 [361] [362]
Insoluble fiber	Reduce total and LDL cholesterol	*Chlorella vulgarisup*	[419]

(Table 1) Contd....

Compounds	Biologic Activity	Microalgae	Reference
Vitamins			
Tocopherols	Antioxidant activity	*Porphyridium* *Spirulina platensis*	[420]
Phenolic compounds			
Benzoic acid derivatives, cinnamic acid derivatives and hydroxybenzaldehydes	Antioxidant activity	*Spirulina maxima* *Chlorella ellipsoidea* *Nannochloropsis sp.*	[421] [422]
Volatile compounds			
Phytol, neophytadiene, etc	Antimicrobial and antioxidante activity	*Synechocystis sp.*	[423]

and cosmetic industry) and anti-proliferative properties (pharmaceutical industry). Bio-guided fractionation of microalgae extracts, followed by studies on human cells, have demonstrated that many pigments, beyond their ecological function as light harvesting molecules, also act as potent bioactive compounds on cancer cells and may have great potential in the prevention and treatment of cancers [317]. It has generated interest in many groups to purify original compounds, understand their biological activity, and also identify the pharmacological targets of molecules previously known for their ecological function.

β-carotene

β-carotene (Fig. **4A**) is the most studied and known carotenoid. It is ingested and processed in the liver and intestine to retinol, antioxidant component that favors the non-appearance of certain cancers as lung, breast, mouth and stomach. Currently, the most important marine source of β-carotene is *Dunaliella salina*, microalgae grown on large outdoor terrain in some countries like Israel, producing large amount for the market [318]. Epidemiologic studies indicate that an increased intake of fruits and vegetables that contain carotenoids as β-carotene and retinol is associated with a decreased risk of many types of cancer and degenerative diseases due to its antioxidant and anti-inflammatory activities [319-321].

Previous *in vitro* studies showed that β-carotene exhibited growth-inhibitory and proapoptotic effects in human colon adenocarcinoma cells, at least in part, by reduction in the expression of COX-2 [322]. In addition, data from animal models of colon carcinogenesis have shown that dietary supplementation of β-carotene reduced the number of ACF induced by AOM and colonic COX-2 expression [271].

The retinoids are a class of over 4,000 natural and synthetic molecules structurally and/or functionally related to fat-soluble vitamin A [323]. These compounds participate in a broad spectrum of biological activities, such as reproduction, embryogenesis, growth, differentiation, proliferation, apoptosis, vision, bone formation, metabolism, hematopoiesis and immunological processes [324]. Several studies have demonstrated the important role of vitamin A and retinoids in the oncogenesis of many tissues. In 1995 Lotan [325] showed, through *in vitro* and *in vivo* applications, that these compounds can influence malignant cell growth in a number of ways, by producing growth arrest and apoptosis, in a variety of cell lines [326].

Xanthophylls

Xanthophylls are yellow or brown pigments belonging to the carotenoids group, and they were first isolated from crab *Astacus astacus*. They are also found in plants, yeast, trout, salmon and shellfish, feathers of some birds and specially, in microalgae.

Different xanthophylls have been described, including flavoxanthin, lutein, criptoxanthin, rubixanthin, violaxanthin, rodoxanthin, cantaxanthin, zeaxanthin and astaxanthin, all of them known for their antioxidant activity. Among them, the most studied are astaxanthin, lutein, violaxanthin and zeaxanthin. Their antioxidant properties are related with their anti-free radical activities. Free radicals and ROS are constantly produced by cells as part of their metabolic processes [327]. Humans have well-developed defence systems that generally maintain an equilibrium between free radicals and antioxidants [328]. However, under conditions of elevated oxidative stress, for example, aging [329] and chronic diseases [330] or the phagocytes when generate an excess of free radicals to aid in their defensive degradation of the invader, defences may be overwhelmed [331], causing an imbalance in cellular processes that results in the attack of cellular components. Oxidative stress can lead to the oxidative damage of DNA, producing significant base damage, strand breaks, altered gene expression, and, ultimately mutagenesis [332, 333], which is the first step for development cancer. Due to their antioxidant properties, xanthophylls have many applications in nutraceutical, cosmetic and food industry. In the last years, these industries have used them as dietary supplement to treat or prevent cancer (breast, colon, or endometrial…) and several inflammatory disorders. These natural products obtained from different organisms have been reported to be interesting molecules by its potent capacity to prevent or modulate colitis, cardiovascular disease, respiratory inflammation, thrombosis as well as enhance immune response in several *in vitro* and *in vivo* studies [334-337].

1. Astaxanthin

A lot of studies have reported important functions played by natural xanthophylls, including astaxanthin, in regulating immunity and disease aetiology.

Astaxanthin (Fig. **4B**) has important applications in the nutraceutical, cosmetics, food and feed industries. Interest in the biological activity of astaxanthin, an oxycarotenoid found in high amounts in the carapace of crustaceans and in the flesh of salmon and trout, has increased in recent years. Astaxanthin has several essential biological functions including protection against oxidation of essential polyunsaturated fatty acids; protection against UV light effects or immune response. In addition, *in vitro* studies have demonstrated that astaxanthin is several fold more active as a free radical antioxidant than β-carotene and α-tocopherol [314].

Some microorganisms such as the Chlorophyte alga *Haematococcus pluvialis* are rich in astaxanthin. This alga is believed to accumulate the highest levels of astaxanthin in nature and commercially grown *H. pluvialis* can produce 30 g of astaxanthin kg^{-1} dry

biomass [338]. Currently, microalgae as *H. pluvialis* suppose an important source of astaxanthin and relative-molecules, which are used for preventing inflammatory process, such as colitis, and some types of cancer, including colon cancer [314, 339, 340]. In this regards, dietary astaxanthin ameliorated the colonic inflammation induced by DSS. These beneficial effects were due to the reduction of the inflammatory factors TNF-α, IL-1β, COX-2 and NF-κB [341].

Previous *in vitro* studies reported that this compound exhibited growth-inhibitory effects by arresting cycle cell progression and promoting apoptosis in a number of colon cancer cells, including HCT-116, HT-29, LS-174, WiDr and SW-480 [314]. Astaxanthin has also been described to reduce AOM/DSS-induced colorectal carcinogenesis in mice though inhibition of TNF-α, IL-1β and NF-κB expression as well as by suppressing proliferation and inducing apoptosis in the colonic adenocarcinomas [341]. In addition, this natural product stimulated apoptosis in DMH-induced rat colon carcinogenesis through the regulated expressions of NF-κB, COX-2, MMP-2 and 9, proliferating cell nuclear antigen (PCNA) and ERK-2 [342].

The anti-inflammatory and antioxidative effects of astaxanthin has also been showed in a randomized double-blind, placebo-controlled study. This natural product enhanced immune response, and decreased oxidative status and inflammation in young healthy women [343].

2. Lutein

Lutein (Fig. **4C**) is an important xanthophyll known by its potent antioxidant activity, being the second most prevalent carotenoid in human serum [344], and dark, leafy green vegetables such as spinach and kale are the major lutein sources in food [345].

Like other carotenoids, the protective effects of lutein are linked to its ability to act as an antioxidant, because it is a potent quencher of singlet oxygen molecules and an excellent direct free radical scavenger [346]. For instance, lutein has been recommended for cancer prevention and diseases related to retinal degeneration [347, 348]. Moreover, this compound has shown protective effects against the development of early atherosclerosis [349].

Several epidemiological and experimental studies have reported that lutein could be used as a dietary supplement for reducing injury in tissues by antioxidant and anti-inflammatory pathways. This compound may protect against alterations of the redox status, DNA damage and inflammatory process by ROS-mediated induction. ROS are closely related to both cancer and the inflammatory process by family of oxidant-sensitive proteins, such as transcriptional modulator NF-κB [350]. It has been showed that several mycotoxins, for example deoxynivalenol (DON), generate free radicals that induce lipid peroxidation and cellular redox signalling, leading to changes in membrane integrity and in the antioxidant status of the cell. A recent *in vitro* study on a human colon adenocarcinoma cell line (HT-29) showed the cytoprotective effect of lutein on DON-induced oxidative stress and inflammation, through a reduction in oxidative stress, by maintaining glutathione levels, inhibition of nuclear migration of NF-κB, downregulation of COX-2 expression and prevention of apoptosis [351]. These antioxidant actions of lutein have been also recently evaluated in a human derived liver cell line (HepG2) using cisplatin as a control. It was showed that cisplatin induced pronounced oxidative stress related to DNA damage and that the supplementation with lutein protected these adverse effects caused by the exposure of the cells to platinum compound [352]. Moreover, lutein has been reported to inhibit hydrogen peroxide-induced activation of NF-κB and IL-8 expression in gastric epithelial AGS cells [353]. It has been also shown that a balance of dietary lutein and PUFAs may reduce the inflammatory process through PPARs pathways, IL-1 and retinoic acid X receptor (RXR) in chickens [354].

High lutein intake is associated with a reduction in cancer risk including colon or pancreas cancer [355, 356]. *In vivo* studies have recently shown that dietary supplementation of lutein reduced colon carcinogenesis in rats by modulation of proteins involved in cellular proliferation, including K-ras, the protein kinase B (PKB) and β-catenin [357]. In addition, another paper reported that the chemopreventive activity of β-carotene and lutein against colon carcinogenesis depends on the dose level. In this way, low doses of β-carotene and lutein inhibited AOM-induced rat colonic ACF formation but high doses augmented ACF incidence [358].

Some marine microalgae as *Chlorella ellipsoidea* and *Chlorella vulgaris* have showed to have anti-proliferative effects on a human colon cell line (HCT-116) by early apoptosis induction. This effect was due to their vast content in carotenoids, although the main of them was lutein [326]. For this reason, microalgae could be a lutein alternative source by biotechnological advances [359].

3. Violaxanthin

Violaxanthin (Fig. **4D**) is a natural xanthophyll pigment with an orange colour found in a variety of plants, macro- and micro-algae. This product has been isolated from several microalgae including *Dunaliella tertiolecta*. Recently, this microalgae has been chemically investigated to isolate molecules inhibiting cancer cell proliferation and inducing apoptosis in MCF-7 (breast) and LNCaP (prostate) cancer cell lines.

In this study, violaxanthin showed strong antiproliferative activity by inhibiting cancer cell proliferation and inducing apoptosis on MCF-7 cell line [313]. An extract of *Chlorella ellipsoidea* containing mainly violaxanthin exerted strong antiproliferative effects, including induction of apoptosis in HCT-116 human colon cancer cells [326]. These results suggest that studying the pharmacology of violaxanthin and pharmacomodulated derivatives on cancer cells and *in vivo* models may allow potent antiproliferative drugs. Moreover, currently many groups are studying how to increase violaxanthin from microalgae production taking advantage of their easy culture using nuclear transformation or UVA mediated modulation of photosynthetic efficiency for producing high amount [360].

Polysaccharides

Polysaccharides are natural polymers with a variety of properties that may be translated into significant commercial applications. Marine microorganisms, such as bacteria or microalgae, are considered a vast source of polysaccharides due to the enormous marine diversity. Since 1990's, polysaccharides from microalgae have been studied because of their structural diversity, which leads to a wide diversity of applications such as *in vitro* inhibition of viral replication [361, 362]. More recently, they have been reported to possess many biological activities, such as antiviral, antitumor, antimicrobial, anticoagulant [363] and inmunomodulating effects. These compounds are susceptible to be modified chemically in order to tailor new properties and hence to broaden the spectrum of potential applications. A recent paper has reported the structural determination of a cell wall glycoprotein from the red microalga *Porphyridium sp*. [364]. The additional importance of this research lies in its potential for biotechnological applications, especially in evaluating the use of microalgae as cell factories for the production of therapeutic proteins.

Microalgae have recently gained increasing interest as bioreactors for recombinant protein production, for example, overexpression of the phytoene synthase gene from the green algae *Chlorella zorigiensis* in *Chlamydomonas reinhardtii* led to a two-fold increase in the content of violaxanthin and lutein [360] which are very interesting in the antioxidant and antiinflammatory process. Proteins from microalgae are interesting because has been showed to have immune modulating activities. In this line, bioactive phycocyanin and water soluble polysaccharides of *Spirulina* are responsible for its enhanced biological defense activity against infectious

diseases and reduction of allergic inflammation through sustaining the functions of the mucosal immune system. However, use of polysaccharides is restricted for various reasons, including a lack of simple methods for isolating them from extracts. Despite several methods for isolation of seaweed polysaccharides have been reported, all of them are rather labour intensive and time consuming.

In 2001, Pugh *et al*. [365] reported that immulina, a high-molecular weight polysaccharide fraction from *Spirulina*, was a potent activator of NF-κB and induced both IL-1β and TNF-α mRNAs in THP-1 human monocytes. Balachandran *et al*. [366] showed that immulina derived from *Spirulina platensis* activated monocytes THP-1 and NF-κB through a CD14- and TLR2- (receptors which recognizes pathogens and active innate immunity) - dependent process. On the other hand, in an *ex vivo* experiment, spleens and Peyer's patches from mice fed with immulina showed an IgA production enhanced in Peyer's patch cells and similary, IFN-γ production was enhanced in cells isolated from the spleens of mice consuming the *Spirulina* extract [366].

For this reason, science is developing biotechnological transformation methods of microorganisms such as microalgae, to use them as pharmaceutical bio-factories [367-370].

Fatty Acids

Fatty acids are considered an important source of biological componentes for the treatment of several pathologies, much of them inflammatory diseases, including IBD, atherosclerosis, Parkinson's and Alzheimer's diseases and cancer. From a chemical point of view, many of these compounds are long chain fatty acids where they can be either saturated or unsaturated, PUFAs being the most studied for their pharmacological potential. Data from epidemiological, clinical and experimental studies have reported that dietary fish oil containing n-3 PUFAs have benefits for human health, including protection against colon cancer development and other cancers such as endometrial, breast and prostate cancer [371-373]. Fish oils are the main sources of n-3 PUFAs because of their high levels of docosahexaenoic acid (DHA) (Fig. **4E**), eicosapentaenoic acid (EPA) (Fig. **4F**) and docosapentaenoic acid (DPA). However, their clinical use is often limited by their unpleasant fishy taste and its adverse effects such as flatulence, halitosis, heartburn, diarrhoea and belching [374, 375].

Since there is a high demand for n-3 PUFAs in the pharmaceutical and food industries, alternative processes for PUFAs production are currently being studied, as for example exploitation of microalgae [376]. The marine environmental and specially microalgae are a huge source of different fatty acids, including monounsaturated fatty acids, such as oleic acid (OLA) and PUFAs, including linoleic acid (LNA), α-linolenic acid (ALA), EPA and DHA. Among them, EPA and DHA are the major PUFAs in microalgae. Moreover, conjugated linoleic acid (CLA) is common in cooked meat, pasteurized dairy products or processed cheeses. It has been shown that microalgae can modulate the CLA content; in this line, diet supplemented with DHA-enriched microalgae (i.e. *Schizochytrium* sp) reduced the milk fat content and modified the milk fatty acid composition toward increased CLA cis-9 trans-11, C18:1 trans and DHA concentrations [377]. All of these fatty acids have been shown to be involved in inflammatory signaling pathways and many studies have reported their antioxidant, anti-inflammatory and anti-cancer activities, both *in vitro* and *in vivo* [68, 378, 379].

The interest in studying the role of fatty acids on immune response began in 1970 [380]; since then, a lot of studies have reported their beneficial effects in the treatment of inflammatory diseases including IBD, among others. Since IBD therapies have modest results for the long-term management and significant side-effects, diet and nutritional factors may play a key role in IBD and thus, developing nutritional interventions against this pathology remains important. In this regard, the antiinflammatory effects of diets enriched in PUFA are very interesting.

A promising avenue for developing such immunonutrition-based therapies for IBD is by targeting PPAR-γ. Moreover, the anti-inflammatory mechanisms of fatty acids have been shown to be related, in part, to their regulatory effect on PPARs. PPAR-γ has been shown to produce beneficial effects in experimental IBD by repression of NF-κB thereby reducing inflammation. At the same time, PPAR-γ interacts with NF-κB and modulates the intensity, duration and consequences of inflammation (Fig. **2**) [381, 382].

As mentioned previously, the beneficial effects of PPAR-γ ligands in both human and animals suffering from colitis has been previously demonstrated. At present, there is a need to discover novel naturally agonists of PPARs that exert therapeutic and prophylactic actions against inflammatory disorders, with limited side-effects. Fatty acids are known to bind to PPAR-γ, having PUFAs higher binding affinity than saturated or monounsaturated fatty acids [383]. In this line, our research group is currently working on PUFAs isolated from microalgae. These compounds, called oxilipins, have shown to have an interesting anti-inflammatory activity *in vitro* by decreasing TNF-α production and their action may be due, in part, to the regulation of PPAR-γ and NFκB pathway [384].

In animal models of IBD, a large body of evidence supports a protective effect of PUFAs through PPAR-γ-dependent mechanisms. Along these lines, CLA induced colonic PPAR-γ expression and provided protection against the disease in a pig model of bacterial-induced colitis [385], as well as in mouse and pig models of DSS colitis [36, 37]. Similarly, DHA and EPA diet administration attenuated colonic mucosal inflammation in rats; this effect was associated with reduced levels of IκBα/β and high PPAR-γ expression [386]. A recent study has reported the anti-inflammatory activity of EPA in IL-10 gene-deficient mice through the PPAR signaling pathway [387].

In addition to PPAR-γ modulation, other mechanisms by which PUFAs exert their beneficial effects in colitis have been described. In this respect, dietary olive oil administration supplemented with EPA and DHA in rats with DSS-induced colitis reduced colonic inflammatory response through the reduction of levels of leukotriene B(4), TNF-α and NO [388]. Interestingly, the same diet combined with quercitrin caused a significantly greater inhibitory effect of the proinflammatory mediators in comparison with rats receiving the diet without this flavonoid. Those effects were also associated with a lower colonic iNOS and COX-2 expression [389]. In addition, oral administration of pure DHA in BALB/c mice with colitis ameliorated the inflammatory changes of the colon through downregulation of inflammatory cytokines such as IL-1β, CD14 antigen and TNF-α, as well as membrane remodeling genes such as MMP-3, -10 and -13 [390]. Varnalidis *et al*. [391] have recently reported that animals fed a diet containing high levels of EPA exhibited a reduction in colonic epithelial erosion after 8 days of DSS-induced colitis despite the increased colonic neutrophil infiltration. The preventive effects of these compounds have also been shown in human studies. Along these lines, a prospective cohort study reported that dietary intake of the n-3 PUFAs EPA and DHA, was associated with protection from colitis in a cohort aged over 45 years [392].

On the other hand, PUFAs have been reported to have beneficial biological functions in carcinogenic processes. *In vitro* studies have demonstrated that EPA and DHA induced apoptosis in the colon cancer cell lines HT-29, Caco-2, and DLD-1 through an increase in caspase-3 activity and inhibition of Bcl-2 [393]. In addition, the antiproliferative properties of oils containing EPA and DHA, derived from three microalgas (*Crypthecodinium cohnii*, *Schizochytrium* sp. and *Nitzschia laevis*), have been previously shown in human colon adenocarcinoma Caco-2 cells [378]. These n-3 PUFAs also suppressed cell growth and VEGF expression in HT-29 cells cultured *in vitro* as well as *in vivo* when implanted in nude mice. The anti-angiogenic properties of these fatty acids were

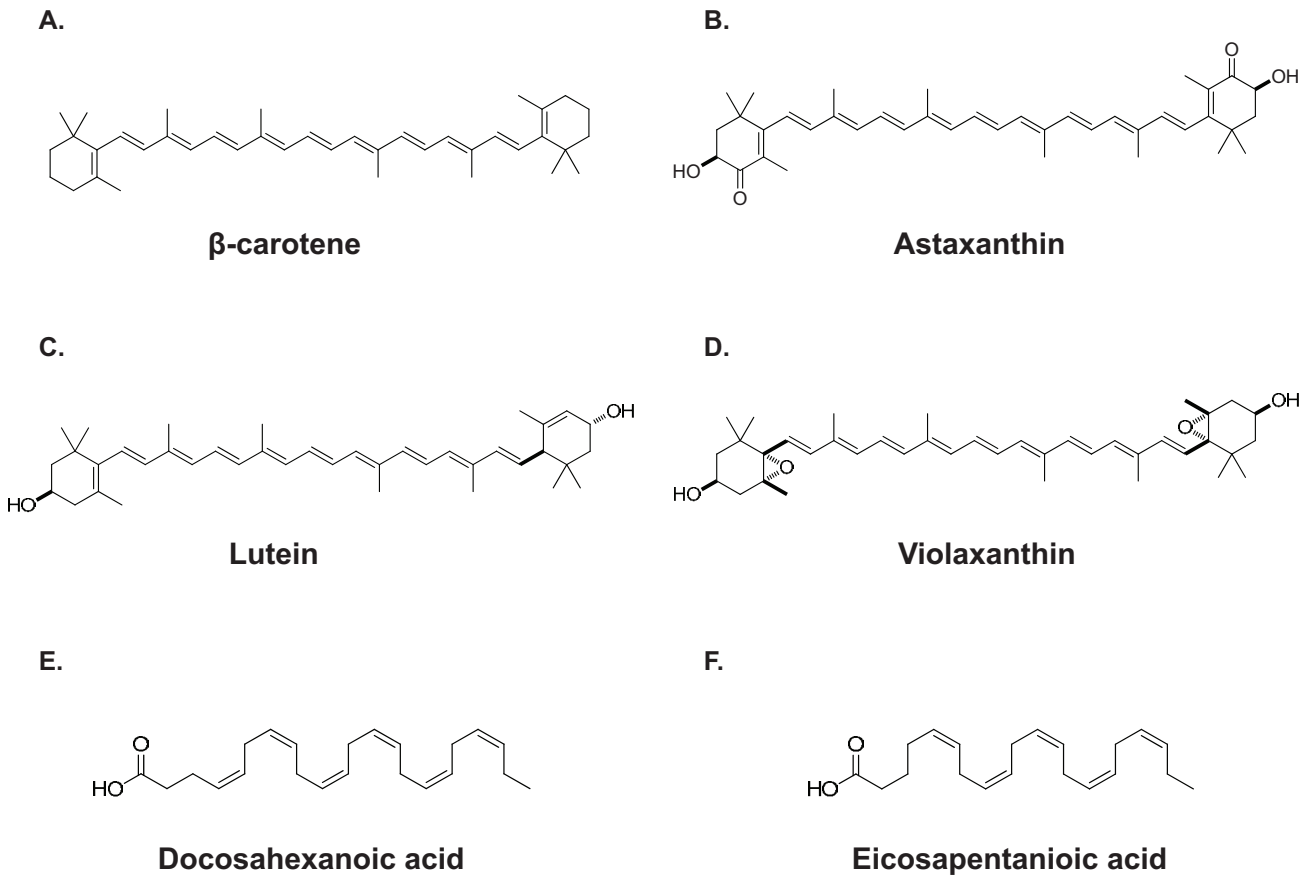

Fig. (4). Chemical structures of some functional ingredients found in microalgae.

due in part to the downregulation of the COX-2/PGE_2 pathway [394]. Another study reported that lycopene and EPA synergistically inhibited the proliferation of human colon cancer HT-29 cells by downregulation of PI3K/Akt/mTOR signaling pathway [395]. Allred *et al*. [396] found that growth inhibitory effects of EPA in these cells were the result of a PPARγ-mediated pathway. DHA depressed colon tumor growth *in vitro* through p53 dependant-apoptosis and p53 independent pathways. In addition, these authors reported an inhibition of the growth of human adenocarcinoma COLO 205 in nude mice by a diet supplemented by golden algae oil containing DHA [397].

Further experiments in animals have shown that chemical induction of colon tumors by AOM or DMH in rats decreased when the animals were fed diets containing DHA and EPA [398-400]. In 2009, van Beelen *et al*. [401] studied the chemopreventive effects of high fat microalgal oil diet on AOM-induced colon carcinogenesis in rats following eight weeks of dietary treatment. These effects were compared to the effects of high fat fish oil. The results showed that both these oils gave the same 50% reduction of AOM-induced ACF when compared to corn oil, suggesting that microalgal oil is a good alternative for fish oil regarding protection against colorectal cancer. Another study reported that EPA diet administration caused a significant suppression of polyp number and growth in Apc^{Min+} mice. This effect was associated to a significant reduction in COX-2 expression and β-catenin nuclear translocation [402].

The chemopreventive efficacy of EPA in patients with familial adenomatous polyposis (FAP) has been recently reported in a randomised, double-blind, placebo-controlled trial. Treatment with this fatty acid for six months reduced the polyp number and the sum of polyp diameters, showing a favorable safety profile [403]. In another study, supplementation with EPA (2 g/day for 3 months) significantly increased apoptosis and reduced cell proliferation in normal colonic mucosa in patients with colorectal adenomas [404]. Therefore, the marked inhibitory effect of these PUFAs on cancer development, in the absence of toxicity, makes these fatty acids excellent candidates for both CRC chemoprevention and treatment.

CONCLUSION

Clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation and cancer. In this regard, intestinal neoplasias, originated under chronic inflammatory conditions have been the object of numerous studies in both humans and animals, and the association between IBD and elevated risk for CRC was established.

There are two different pathways linking inflammation and tumorigenesis: an extrinsic pathway mediated by chronic inflammation and oxidative environment (inflammation-cancer) and an intrinsic pathway in which genetic alterations induce alterations in cell cycle and proliferation, apoptosis, or metastatic and angiogenic effects and in the absence of an underlying inflammation, initiate a tumour-driven host immune response leading to a microenvironment composed of inflammatory cells (cancer-inflammation). These data suggest that inflammatory response represent a basic mechanism for cancer origin and development.

Numerous *in vitro* and *in vivo* studies and some clinical data have well established the antioxidant, anti-inflammatory and anticancer potential of the dietary nutrients from plants and from microorganisms such as microalgae. In this line, the simple growth requirements of microalgae make them attractive for demand by the pharmaceutical, food and cosmetic industry. Complementary basic

studies and well-controlled randomized clinical trials are required to ascertain the optimal dose for chemopreventive efficacy, excluding toxic effects, all with the objective to be validated for a good clinical practice.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Latella G, Fiocchi C, Caprili Rr. News from the "5th International Meeting on Inflammatory Bowel Diseases" CAPRI 2010. J Crohns Colitis 2010; 4: 690-702.

[2] Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis 2006; 12: S3-S9.

[3] Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-29.

[4] Abreu MT. The pathogenesis of inflammatory bowel disease: translational implications for clinicians. Curr Gastroenterol Rep 2002; 4: 481-89.

[5] Neurath MF, Weigmann B, Finotto S, *et al*. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. J Exp Med 2002; 195: 1129-43.

[6] Montgomery SM, Ekbom A. Epidemiology of inflammatory bowel disease. Curr Opin Gastroenterol 2002; 18: 416-20.

[7] Itzkowitz SH. Molecular biology of dysplasia and cancer in inflammatory bowel disease. Gastroenterol Clin North Am 2006; 35: 553-71.

[8] Chapkin Rs, Davidson LA, Ly L, Weeks Br, Lupton JR, Mcmurray DN. Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. J Nutr 2007; 137: 200S-204S.

[9] Sarra M, Monteleone I, Stolfi C, *et al*. Interferon-gamma-expressing cells are a major source of interleukin-21 in inflammatory bowel diseases. Inflamm Bowel Dis. 2010; 16: 1332-39.

[10] Waterman M, Xu W, Stempak JM, *et al*. Distinct and overlapping genetic loci in crohn's disease and ulcerative colitis: Correlations with pathogenesis. Inflamm Bowel Dis. 2011; 17: 1936-42.

[11] Stappenbeck TS, Rioux JD, Mizoguchi A, *et al*. Crohn disease: A current perspective on genetics, autophagy and immunity. Autophagy. 2010. PMID: 20729636

[12] Martin B, Hirota K, Cua DJ, *et al*. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity. 2009; 31:321-30.

[13] Cario E. Innate immune signalling at intestinal mucosal surfaces: a fine line between host protection and destruction. Curr Opin Gastroenterol. 2008; 24: 725-32.

[14] Huang J, Lam GY, Brumell JH. Autophagy Signaling Through Reactive Oxygen Species. Antioxid Redox Signal. 2011. PMID: 20874258

[15] Brant SR, Shugart YY. Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. Inflamm Bowel Dis. 2004; 10: 300-11.

[16] Hampe J, Franke A, Rosenstiel P, *et al*. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. 2007; 39: 207-11.

[17] Rioux JD, Xavier RJ, Taylor KD, *et al*. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet. 2007; 39: 596-604.

[18] Zhang S, Zhao X, Zhang D. Cellular and molecular immunopathogenesis of ulcerative colitis. Cell Mol Immunol 2006; 3: 35-40.

[19] Pasare C, Medzhitov R. Toll-like receptors and acquired immunity. Semin Immunol 2004; 16: 23-26.

[20] Haller D. Intestinal epithelial cell signalling and host- derived negative regulators under chronic inflammation: to be or not to be activated determines the balance towards commensal bacteria. Neurogastroenterol Motil 2006; 18: 184-99.

[21] Fritz JH, Girardin SE, Fitting C, *et al*. Synergistic stimulation of human monocytes and dendritic cells by Toll-like receptor 4 and NOD1- and NOD2-activating agonists. Eur J Immunol 2005; 35: 2459–70.

[22] Lala S, Ogura Y, Osborne C, *et al*. Crohn's disease and the NOD2 gene: a role for paneth cells. Gastroenterology 2003; 125: 47-57.

[23] Hugot JP, Chamaillard M, Zouali H, *et al*. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411: 599–603.

[24] Ogura Y, Bonen DK, Inohara N, *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411: 603-06.

[25] Travassos LH, Carneiro LA, Ramjeet M, *et al*. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol. 2010; 11 :55-62.

[26] Cooney R, Baker J, Brain O, *et al*. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med. 2010; 16: 90-97.

[27] Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; 3: 390-407.

[28] Gutierrez O, Pipaon C, Inohara N, *et al*. Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. J Biol Chem 2002; 277: 41701-05.

[29] Pasparakis M. Regulation of tissue homeostasis by NF-kappaB signalling: implications for inflammatory diseases. Nat Rev Immunol. 2009; 9: 778-88.

[30] Tergaonkar V. NFkappaB pathway: a good signaling paradigm and therapeutic target. Int J Biochem Cell Biol 2006; 38: 1647-53.

[31] Wang S, Liu Z, Wang L, *et al*. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol. 2009; 6: 327-34.

[32] Sartor RB, Hoentjen F. Proinflammatory cytokines and signaling pathways in intestinal innate immune cells. In: Mestecky J *et al*. Eds, Mucosal Immunology. Philadelphia, Elsevier. 2005; 681–701.

[33] Martin H. Role of PPAR-gamma in inflammation. Prospects for therapeutic intervention by food components. Mutat Res. 2009; 669: 1-7.

[34] Saubermann LJ, Nakajima A, Wada K, *et al*. Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis. Inflamm Bowel Dis 2002; 8: 330-9.

[35] Guri AJ, Mohapatra SK, Horne WT *et al*. The role of T cell PPAR gamma in mice with experimental inflammatory bowel disease. BMC Gastroenterol. 2010; 10: 60.

[36] Bassaganya-Riera J, Hontecillas R. CLA and n-3 PUFA differentially modulate clinical activity and colonic PPAR-responsive gene expression in a pig model of experimental IBD. Clin Nutr. 2006; 25: 454-65.

[37] Bassaganya-Riera J, Reynolds K, Martino-Catt S, *et al*. Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology. 2004; 127: 777–91.

[38] Tanaka T, Kohno H, Yoshitani S, *et al*. Ligands for peroxisome proliferator-activated receptors alpha and gamma inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res 2001; 61: 2424-8.

[39] Lewis JD, Lichtenstein GR, Deren JJ, *et al*. Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial. Gastroenterology. 2008; 134: 688-95.

[40] Lee JW, Kim WH, Yeo J, *et al*. ER stress is implicated in mitochondrial dysfunction-induced apoptosis of pancreatic beta cells. Mol Cells. 2010; 30: 545-49.

[41] Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol. 2010; 28: 573-621.

[42] Takemoto K, Miyata S, Takamura H, *et al*. Mitochondrial TRAP1 regulates the unfolded protein response in the endoplasmic reticulum. Neurochem Int. 2011. PMID: 21338643

[43] Kaser A, Martínez-Naves E, Blumberg RS. Endoplasmic reticulum stress: implications for inflammatory bowel disease pathogenesis. Curr Opin Gastroenterol. 2010; 26: 318-26.

[44] Back SH, Lee K, Vink E, *et al*. Cytoplasmic IRE1alpha-mediated XBP1 mRNA splicing in the absence of nuclear processing and endoplasmic reticulum stress. J Biol Chem. 2006; 281:18691-706.

[45] Kaser A, Blumberg RS. Endoplasmic reticulum stress and intestinal inflammation. Mucosal Immunol. 2010; 3: 11-16.

[46] Nakamura T, Furuhashi M, Li P, *et al*. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell. 2010; 140: 338-48.
[47] Hinton JM. Risk of malignant change in ulcerative colitis. Gut 1966; 7: 427-32.
[48] Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am.J.Physiol. Gastrointest. Liver Physiol 2004; 287: G7-G17.
[49] Grenstein AJ. Cancer in inflammatory bowel disease. Mt Sinai J. Med 2000; 67: 227-40.
[50] O'connor PM, Lapointe TK, Beck PL, *et al*. Mechanisms by which inflammation may increase intestinal cancer risk in inflammatory bowel disease. Inflamm Bowel Dis. 2010; 16: 1411-20.
[51] Lea MA. Recently identified and potential targets for colon cancer treatment. Future Oncol. 2010; 6: 993-1002.
[52] Talero E, Sánchez-Fidalgo S, Villegas I, *et al*. Role of different inflammatory and tumor biomarkers in the development of ulcerative colitis-associated carcinogenesis. Inflamm Bowel Dis. 2011; 17: 696-710.
[53] Riddle RH, Goldman H, Ransohof DF, *et al*. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical application. Human Pathol 1983; 14: 931-68.
[54] Pascal RR. Dysplasia and early carcinoma in inflammatory bowel disease and colorectal carcinomas. Human Pathol 1994; 25: 1160-71.
[55] Bird PR, Good CK. The significance of aberrant crypt foci in understanding the pathogenesis of colon cancer. Toxico Lett 2000; 112-113: 295-402.
[56] Cooper HS, Murthy S, Kido K, *et al*. Dysplasia and cancer in the dextran sulphate sodium mouse colitis model. Relevance to colitis-associated neoplasia in the human: a study of histopathology, β-catenin and expression and the role of inflammation. Carcinogenesis 2000; 21: 757-68.
[57] Terzić J, Grivennikov S, Karin E, *et al*. Inflammation and colon cancer. Gastroenterology. 2010; 138: 2101-2114.e5.
[58] Sun Y, Chen J, Rigas B. Chemopreventive agents induce oxidative stress in cancer cells leading to COX-2 overexpression and COX-2-independent cell death. Carcinogenesis. 2009; 30: 93-100.
[59] Fukata M, Chen A, Klepper A, *et al*. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine. Gastroenterology. 2006; 131: 862-77.
[60] Aggarwal BB, Shishodia S, Sandur SK, *et al*. Inflammation and cancer: how hot is the link? Biochem Pharmacol. 2006; 72: 1605-21.
[61] Luo JL, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. J Clin Invest. 2005; 115: 2625-32.
[62] Richmond A. Nf-kappa B, chemokine gene transcription and tumour growth. Nat Rev Immunol. 2002; 2: 664-74.
[63] Bharti AC, Aggarwal BB. Chemopreventive agents induce suppression of nuclear factor-kappaB leading to chemosensitization. Ann N Y Acad Sci. 2002; 973: 392-95.
[64] Katsman A, Umezawa K, Bonavida B. Chemosensitization and immunosensitization of resistant cancer cells to apoptosis and inhibition of metastasis by the specific NF-kappaB inhibitor DHMEQ. Curr Pharm Des. 2009; 15: 792-808.
[65] Salminen A, Lehtonen M, Suuronen T, *et al*. Terpenoids: natural inhibitors of NF-kappaB signaling with anti-inflammatory and anticancer potential. Cell Mol Life Sci. 2008; 65: 2979-99.
[66] Schmidt MV, Brüne B, Von Knethen A. The nuclear hormone receptor PPARγ as a therapeutic target in major diseases. Scientific World Journal. 2010; 10: 2181-97.
[67] Kaur J, Sanyal SN. Modulation of inflammatory changes in early stages of colon cancer through activation of PPARgamma by diclofenac. Eur J Cancer Prev. 2010; 19: 319-27.
[68] Bassaganya-Riera J, Hontecillas R. Dietary conjugated linoleic acid and n-3 polyunsaturated fatty acids in inflammatory bowel disease. Curr Opin Clin Nutr Metab Care. 2010; 13: 569-73.
[69] Meira LB, Bugni JM, Green SL, *et al*. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest. 2008; 118: 2516-25.
[70] Martín MJ, Jiménez MD, Motilva V. New issues about nitric oxide and its effects on the gastrointestinal tract. Curr Pharm Des. 2001; 7: 881-908.
[71] Seril DN, Liao J, Yang GY. Colorectal carcinoma development in inducible nitric oxide synthase-deficient mice with dextran sulfate sodium-induced ulcerative colitis. Mol Carcinog 2007; 46: 341-53.
[72] Azab MB, Chen Y, Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. Antioxid Redox Signal. 2009; 11: 777-90.
[73] Huett A, Goel G, Xavier RJ. A systems biology viewpoint on autophagy in health and disease. Curr Opin Gastroenterol. 2010l; 26: 302-09.
[74] Wirawan E, Vande Walle L, Kersse K, *et al*. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Dis. 2010; 1: e18.
[75] Yousefi S, Perozzo R, Schimd I, *et al*. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol. 2006; 8: 1124-32.
[76] Balaburski GM, Hontz RD, Murphy ME. p53 and ARF: unexpected players in autophagy. Trends Cell Biol 2010; 20: 363-69.
[77] Maiuri MC, Galluzzi L, Morselli E, *et al*. Autophagy regulation by p53. Curr Opin Cell Biol 2010; 22: 181-85.
[78] Tasdemir E, Maiuri MC, Galluzzi L, *et al*. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol. 2008; 10: 676-87.
[79] Scherz-Shouval R, Weidberg H, Gonen C, *et al*. p53-dependent regulation of autophagy protein LC3 supports cancer cell survival under prolonged starvation. Proc Natl Acad Sci U S A. 2010; 107: 18511-16.
[80] Bauvy C, Gane P, Arico S, *et al*. Autophagy delays sulindac sulphide-induced apoptosis in the human intestinal colon cancer cell line HT-29. Exp Cell Res 2001; 268: 139-49.
[81] Han J, Hou W, Goldstein LA, *et al*. Involvement of protective autophagy in TRAIL resistance of apoptosis-defective tumor cells. J Biol Chem 2008; 283: 19665-77.
[82] Sato K, Tsuchihara S, Fujii M, *et al*. Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. Cancer Res 2007; 67: 9677-84.
[83] Nishikawa T, Tsuno NH, Okaji Y, *et al*. Inhibition of autophagy potentiates sulphorane-induced apoptosis in human colon cancer cells. Ann Surg Oncol 2010; 17: 592-602.
[84] Li J, Hou N, Faried A, *et al*. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. Eur J Cancer 2010; 46: 1900-09.
[85] Lu SP, Lin SJ. Regulation of yeast sirtuins by NAD+ metabolism and calorie restriction. Biochim Biophys Acta 2010; 1804: 1567-75.
[86] Zhang T, Kraus WL. SIRT1-dependent regulation of chromatin and transcription: linking NAD+ metabolism and signalling to the control of cellular functions. Biochim Biophys Acta 2010; 1804: 1666-75.
[87] Dominy JE, Lee y, Gerhart-Hines Z, *et al*. Nutrient-dependent regulation of PGC-1α′s acetylation state and metabolic function through the enzymatic activities of Sirt1/GCN5. Biophys Acta 2010; 1804: 1684-89.
[88] Imai SI. "Clocks" in the NAD world: NAD as a metabolic oscillator for the regulation of metabolism and aging. Biochim Biophys Acta 2010; 1804: 1584-90.
[89] Swanson GR, Burgess HJ, Keshavarzian A. Sleep disturbances and inflammatory bowel disease: a potential trigger for disease flare? Expert Rev Clin Immunol 2011; 7: 29-36.
[90] Singh UP, Singh NP, Balwan S, *et al*. Resveratrol (trans-3,5,4′-trihydroxystilbene) induces silent mating type information regulation-1 and down-regulates nuclear transcription factor-kappaB activation to abrogate dextran sulphate sodium-induced colitis. J Pharmacol Exp Ther 2010; 332: 829-39.
[91] Cui X, Jin Y, Hofseth AB, *et al*. Resveratrol suppresses colitis and colon cancer associated with colitis. Cancer Prev. Res. 2010; 3: 549-59.
[92] Hofseth LJ, Singh UP, Singh NP, *et al*. Taming the beast within: resveratrol suppresses colitis and prevents colon cancer. Aging 2010; 2: 183-84.
[93] Yeung F, Hoberg JE, Ramsey CS, *et al*. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. Embo J 2004; 23: 2369-80.
[94] Ford J, Jiang M, Milner J. Cancer-specific functions of SIRT1 enables human epithelial cancer cell growth and survival. Cancer Res 2005; 65: 10457-63.
[95] Stünkel W, Peh BK, Tan Yc, *et al*. Function of the SIRT1 protein deacetylase in cancer. Biotechnol J 2007; 2: 1360-68.

[96] Nosho K, Shima K, Irahara N, *et al.* SIRT1 histonedeacetylaseexpression is associatedwith microsatellite instability and CpG island methylator phenotype in colorectal cancer. Mod Pathol 209; 22: 922–32.

[97] Firestein R, Blander G, Michan S, *et al*. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS ONE 2008; 3: e2020.

[98] Tetsu O, McCormick F. β-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 1999; 398: 422-26.

[99] Ge X, Jin Q, Zhang F, *et al*. PCAF acetylates β-catenin and improves its stability. Mol Biol Cell 2009; 20; 419-27.

[100] Polakis P. Wnt signalling and cancer. Genes Dev 2000; 14: 1837-51.

[101] Holloway KR, Calhoun TN, Saxena MF, *et al*. SIRT1 regulates Dishevalled proteins and promotes transient and constitutive Wnt signalling. Proc Natl Acad Sci USA 2010; 107: 9216-21.

[102] Kabra N, Li Z, Chen L F, *et al*. SirT1 is an inhibitor of proliferation and tumor formation in colon cancer. J Biol Chem 2009; 284: 18210-17.

[103] Kennedy BK, Steffen KK, Kaeberlein M. Ruminations on dietary restriction and aging. Cell Mol Life 2007; 64: 1323-28.

[104] Colan RJ, Anderson RM, Johnson ST F, *et al*. Caloric restriction delays disease onset and mortality in Rhesus monkeys. Science 2009; 325: 201-04.

[105] Harrison DE, Strong R, Sharp ZD F, *et al*. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009; 460: 392-95.

[106] Campisi J, Yaswen P. Aging and cancer cell biology. Aging Cell. 2009; 8: 221-25.

[107] Meley D, Bauvy C, Houben-Weerts JHPM F, *et al*. AMP-activated protein kinase and the regulation of autophagic proteolysis. J Biol Chem 2006; 281: 34870-79.

[108] Cantó C, Gerhart-Hines Z, Feige JN F, *et al*. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009; 458: 1056-60.

[109] Lee IH, Cao L, Mostoslavsky R F, *et al*. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc Natl Acad Sci USA 2008; 105: 3374-79.

[110] Morselli E, Maiuri MC, Markaki M F, *et al*. The life span-prolonging effect of sirtuin-1 is mediated by autophagy. Autophagy 2010; 6: 1-3.

[111] De Flora S, Ferguson LR. Overview of mechanisms of cancer chemopreventive agents. Mutat Res 2005; 591: 8-15.

[112] Kelloff GJ, Crowell JA, Steele VE *et al*. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. J Nutr 2000; 130: 467S-71S.

[113] Sporn B. The Big C for chemoprevention. Nature 2011; 471: S10-S11.

[114] Zhang L, Ren X, Alt E *et al*. Chemoprevention of colorectal cancer by targeting APC-deficient cells for apoptosis. Nature; 464: 1058-63.

[115] Brower V. Portents of malignancy. Nature 2011; 471: S19-S21.

[116] Malhotra S, Lam S, Man SF, *et al*. The relationship between stage 1 and 2 non-small cell lung cancer and lung function in men and women. BMC Pulm Med 2006; 6: 2.

[117] Gravitz L. Fist Line of defence. Nature 2011; 471: S5-S7.

[118] Tan XL, Lombardo KM, Bamlet WR, *et al*. Aspirin, Nonsteroidal Anti-inflammatory Drugs, Acetaminophen, and Pancreatic Cancer Risk: a Clinic-Based Case-Control Study. Cancer Prev Res 2011; 4:1835-41.

[119] Ming ME. The search for a chemoprevention agent effective against melanoma: considerations and challenges. J Invest Dermatol 2011; 131: 1835-41.

[120] Baron JA. Statins and the colorectum: hope for chemoprevention? Cancer Prev Res 2010; 3:573-75.

[121] Pollak M. Metformin and other biguanides in oncology: advancing the research agenda. Cancer Prev Res. 2010; 3:1060-65.

[122] Shukla Y, George J. Combinatorial strategies employing nutraceuticals for cancer development. Ann N Y Acad Sci 2011; 1229: 162-75.

[123] Büchner F L, Bueno-de-Mesquita HB, Linseisen J, *et al*. Fruits and vegetables consumption and the risk of histological subtypes of lung cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Causes Control 2010; 21: 357-71.

[124] Deweerdt S. The omnivore´s labyrinth. Nature 2011; 471: S22-S24. 70.

[125] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008; 65: 1631-52.

[126] Srivastava RM, Singh S, Dubey SK, Misra K, Khar A. Immunomodulatory and therapeutic activity of curcumin. Int Immunopharmacol 2011; 11: 331-41.

[127] Shehzad A, Ha T, Subhan F, Lee YS. New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. Eur J Nutr 2011; 50: 151-61.

[128] Abdel Fattah EA, Hashem HE, Ahmed FA, Ghallab MA, Varga I, Polak S. Prophylactic role of curcumin against cyclosporine-induced nephrotoxicity: histological and immunohistological study. Gen Physiol Biophys 2010; 29: 85-94.

[129] Mathy-Hartert M, Jacquemond-Collet I, Priem F, Sanchez C, Lambert C, Henrotin Y. Curcumin inhibits pro-inflammatory mediators and metalloproteinase-3 production by chondrocytes. Inflamm Res 2009; 58: 899-908.

[130] Villegas I, Sánchez-Fidalgo S, Alarcón de la Lastra C. New mechanisms and therapeutic potential of curcumin for colorectal cancer. Mol Nutr Food Res 2008; 52: 1040-61.

[131] Dadhaniya P, Patel C, Muchhara J, *et al*. Safety assessment of a solid lipid curcumin particle preparation: acute and subchronic toxicity studies. Food Chem Toxicol 2011; 49: 1834-42.

[132] Kanai M, Imaizumi A, Otsuka Y, et al. Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. Cancer Chemother Pharmacol. 2012; 69: 65-70.

[133] Arafa HM, Hemeida RA, El-Bahrawy AI, Hamada FM. Prophylactic role of curcumin in dextran sulfate sodium (DSS)-induced ulcerative colitis murine model. Food Chem Toxicol 2009; 47: 1311-7.

[134] Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR, Das PK. Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. Br J Pharmacol 2003; 139: 209-18.

[135] Deguchi Y, Andoh A, Inatomi O, *et al*. Curcumin prevents the development of dextran sulfate Sodium (DSS)-induced experimental colitis. Dig Dis Sci 2007; 52: 2993-8.

[136] Jian YT, Mai GF, Wang JD, Zhang YL, Luo RC, Fang YX. Preventive and therapeutic effects of NF-kappaB inhibitor curcumin in rats colitis induced by trinitrobenzene sulfonic acid. World J Gastroenterol 2005; 11: 1747-52.

[137] Lubbad A, Oriowo MA, Khan I. Curcumin attenuates inflammation through inhibition of TLR-4 receptor in experimental colitis. Mol Cell Biochem 2009; 322: 127-35.

[138] Jiang H, Deng CS, Zhang M, Xia J. Curcumin-attenuated trinitrobenzene sulphonic acid induces chronic colitis by inhibiting expression of cyclooxygenase-2. World J Gastroenterol 2006; 12: 3848-53.

[139] Zhang M, Deng CS, Zheng JJ, Xia J. Curcumin regulated shift from Th1 to Th2 in trinitrobenzene sulphonic acid-induced chronic colitis. Acta Pharmacol Sin 2006; 27: 1071-7.

[140] Camacho-Barquero L, Villegas I, Sánchez-Calvo JM, *et al*. Curcumin, a Curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. Int Immunopharmacol 2007; 7: 333-42.

[141] Zhang M, Deng C, Zheng J, Xia J, Sheng D. Curcumin inhibits trinitrobenzene sulphonic acid-induced colitis in rats by activation of peroxisome proliferator-activated receptor gamma. Int Immunopharmacol 2006; 6: 1233-42.

[142] Ung VY, Foshaug RR, MacFarlane SM, *et al*. Oral administration of curcumin emulsified in carboxymethyl cellulose has a potent anti-inflammatory effect in the IL-10 gene-deficient mouse model of IBD. Dig Dis Sci 2010; 55: 1272-7.

[143] Holt PR, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. Dig Dis Sci 2005; 50: 2191-3

[144] Hanai H, Iida T, Takeuchi K, *et al*. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol 2006; 4: 1502-6.

[145] Chen A, Xu J. Activation of PPAR gamma by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR. Am J Physiol Gastrointest Liver Physiol 2005; 288: G447-56.

[146] Wang JB, Qi LL, Zheng SD, Wang HZ, Wu TX. Curcumin suppresses PPARdelta expression and related genes in HT-29 cells. World J Gastroenterol 2009; 15: 1346-52.

[147] Patel BB, Sengupta R, Qazi S, *et al*. Curcumin enhances the effects of 5-fluorouracil and oxaliplatin in mediating growth inhibition of colon cancer cells by modulating EGFR and IGF-1R. Int J Cancer 2008; 122: 267-73.

[148] Jaiswal AS, Marlow BP, Gupta N, Narayan S. Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. Oncogene 2002; 21: 8414-27.

[149] Watson JL, Hill R, Yaffe PB, *et al*. Curcumin causes superoxide anion production and p53-independent apoptosis in human colon cancer cells. Cancer Lett 2010; 297: 1-8.

[150] Milacic V, Banerjee S, Landis-Piwowar KR, Sarkar FH, Majumdar AP, Dou QP. Curcumin inhibits the proteasome activity in human colon cancer cells in vitro and in vivo. Cancer Res 2008; 68: 7283-92.

[151] Lee YK, Park SY, Kim YM, Park OJ. Regulatory effect of the AMPK-COX-2 signaling pathway in curcumin-induced apoptosis in HT-29 colon cancer cells. Ann N Y Acad Sci 2009; 1171: 489-94.

[152] Su CC, Lin JG, Li TM, *et al*. Curcumin-induced apoptosis of human colon cancer colo 205 cells through the production of ROS, Ca2+ and the activation of caspase-3. Anticancer Res 2006; 26: 4379-89.

[153] Gupta SC, Kim JH, Kannappan R, Reuter S, Dougherty PM, Aggarwal BB. Role of nuclear factor κB-mediated inflammatory pathways in cancer-related symptoms and their regulation by nutritional agents. Exp Biol Med (Maywood) 2011; 236: 658-71.

[154] Plummer SM, Holloway KA, Manson MM, *et al*. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. Oncogene 1999; 18: 6013-20.

[155] Jobin C, Bradham CA, Russo MP, *et al*. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. J Immunol 1999; 163: 3474-83.

[156] Martin D, Galisteo R, Gutkind JS. CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFkappaB through the CBM (Carma3/Bcl10/Malt1) complex. J Biol Chem 2009; 284: 6038-42.

[157] Wang X, Wang Q, Ives KL, Evers BM. Curcumin inhibits neurotensin-mediated interleukin-8 production and migration of HCT116 human colon cancer cells. Clin Cancer Res 2006; 12: 5346-55.

[158] Zhang F, Altorki NK, Mestre JR, Subbaramaiah K, Dannenberg AJ. Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. Carcinogenesis 1999; 20: 445-51.

[159] Goel A, Boland CR, Chauhan DP. Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. Cancer Lett 2001; 172: 111-8.

[160] Lev-Ari S, Strier L, Kazanov D, *et al*. Celecoxib and curcumin synergistically inhibit the growth of colorectal cancer cells. Clin Cancer Res 2005; 11: 6738-44.

[161] Du B, Jiang L, Xia Q, Zhong L. Synergistic inhibitory effects of curcumin and 5-fluorouracil on the growth of the human colon cancer cell line HT-29. Chemotherapy 2006; 52: 23-8.

[162] Su CC, Chen GW, Lin JG, Wu LT, Chung JG. Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B /p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. Anticancer Res 2006; 26: 1281-8.

[163] Binion DG, Otterson MF, Rafiee P. Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition. Gut 2008; 57: 1509-17.

[164] Li L, Ahmed B, Mehta K, Kurzrock R. Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. Mol Cancer Ther 2007; 6: 1276-82.

[165] Majumdar AP, Banerjee S, Nautiyal J, *et al*. Curcumin synergizes with resveratrol to inhibit colon cancer. Nutr Cancer 2009; 61: 544-53.

[166] Senda T, Iizuka-Kogo A, Onouchi T, Shimomura A. Adenomatous polyposis coli (APC) plays multiple roles in the intestinal and colorectal epithelia. Med Mol Morphol 2007; 40: 68-81.

[167] Mahmoud NN, Carothers AM, Grunberger D, *et al*. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis 2000; 21: 921-7.

[168] Perkins S, Verschoyle RD, Hill KA, *et al*. Chemopreventive efficacy and pharmacokinetics of curcumin in the Min/+ mouse, a model of familial adenomatous polyposis. Cancer Epidemiol Biomarkers Prev 2002; 11: 535-40.

[169] Tunstall RG, Sharma RA, Perkins S, *et al*. Cyclooxygenase-2 expression and oxidative DNA adducts in murine intestinal adenomas: modification by dietary curcumin and implications for clinical trials. Eur J Cancer 2006; 42: 415-21.

[170] Murphy EA, Davis JM, McClellan JL, Gordon BT, Carmichael MD. Curcumin's effect on intestinal inflammation and tumorigenesis in the ApcMin/+ mouse. J Interferon Cytokine Res 2011; 31: 219-26.

[171] Perše M, Cerar A. Morphological and molecular alterations in 1,2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats. J Biomed Biotechnol. 2011;2011:473964.

[172] Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res 1995; 55: 259-66.

[173] Kawamori T, Lubet R, Steele VE, *et al*. Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. Cancer Res 1999; 59: 597-601.

[174] Shpitz B, Giladi N, Sagiv E, *et al*. Celecoxib and curcumin additively inhibit the growth of colorectal cancer in a rat model. Digestion 2006; 74: 140-4.

[175] Xu G, Ren G, Xu X, *et al*. Combination of curcumin and green tea catechins. Food Chem Toxicol 2010; 48: 390-5.

[176] Villegas I, Sánchez-Fidalgo S, de la Lastra CA. Chemopreventive effect of dietary curcumin on inflammation-induced colorectal carcinogenesis in mice. Mol Nutr Food Res 2011; 55: 259-67.

[177] Cheng AL, Hsu CH, Lin JK, *et al*. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or premalignant lesions. Anticancer Res 2001; 21: 2895-900.

[178] Dhillon N, Aggarwal BB, Newman RA, *et al*. Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res 2008; 14: 4491-9.

[179] Sharma RA, Euden SA, Platton SL, *et al*. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clin Cancer Res 2004; 10: 6847-54.

[180] Carroll RE, Benya RV, Turgeon DK, *et al*. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. Cancer Prev Res (Phila) 2011; 4: 354-64.

[181] He ZY, Shi CB, Wen H, Li FL, Wang BL, Wang J. Upregulation of p53 expression in patients with colorectal cancer by administration of curcumin. Cancer Invest 2011; 29: 208-13.

[182] Cruz-Correa M, Shoskes DA, Sanchez P, *et al*. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. Clin Gastroenterol Hepatol 2006; 4: 1035-8.

[183] Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. Plant foods and herbal sources of resveratrol. J Agric Food Chem 2002; 50: 3337-40.

[184] Ovesná Z, Horváthová-Kozics K. Structure-activity relationship of trans-resveratrol and its analogues. Neoplasma 2005; 52: 450-5.

[185] Athar M, Back JH, Kopelovich L, Bickers DR, Kim AL. Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms. Arch Biochem Biophys 2009; 486: 95-102.

[186] Kroon PA, Iyer A, Chunduri P, Chan V, Brown L. The cardiovascular nutrapharmacology of resveratrol: pharmacokinetics, molecular mechanisms and therapeutic potential. Curr Med Chem 2010; 17: 2442-55.

[187] Marques FZ, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. Int J Biochem Cell Biol 2009; 41: 2125-8.

[188] Youn J, Lee JS, Na HK, Kundu JK, Surh YJ. Resveratrol and piceatannol inhibit iNOS expression and NF-kappaB activation in dextran sulfate sodium-induced mouse colitis. Nutr Cancer 2009; 61: 847-54.

[189] Yao J, Wang JY, Liu L, *et al*. Anti-oxidant effects of resveratrol on mice with DSS-induced ulcerative colitis. Arch Med Res 2010; 41: 288-94.

[190] Martín AR, Villegas I, La Casa C, de la Lastra CA. Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. Biochem Pharmacol 2004; 67: 1399-410.

[191] Sánchez-Fidalgo S, Cárdeno A, Villegas I, Talero E, de la Lastra CA. Dietary supplementation of resveratrol attenuates chronic colonic inflammation in mice. Eur J Pharmacol 2010; 633: 78-84.

[192] Larrosa M, Yañéz-Gascón MJ, Selma MV, *et al*. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. J Agric Food Chem 2009; 57: 2211-20.

[193] Singh UP, Singh NP, Singh B, et al. Role of resveratrol-induced CD11b(+) Gr-1(+) myeloid derived suppressor cells (MDSCs) in the reduction of CXCR3(+) T cells and amelioration of chronic colitis in IL-10(-/-) mice. Brain Behav Immun. 2012; 26: 72-82.

[194] Mohan J, Gandhi AA, Bhavya BC, *et al*. Caspase-2 triggers Bax-Bak-dependent and -independent cell death in colon cancer cells treated with resveratrol. J Biol Chem 2006; 281: 17599-611.

[195] Lee SC, Chan J, Clement MV, Pervaiz S. Functional proteomics of resveratrol-induced colon cancer cell apoptosis: Caspase-6-mediated cleavage of lamin A is a major signaling loop. Proteomics 2006; 6: 2386-94.

[196] Chan JY, Phoo MS, Clement MV, Pervaiz S, Lee SC. Resveratrol displays converse dose-related effects on 5-fluorouracil-evoked colon cancer cell apoptosis: the roles of caspase-6 and p53. Cancer Biol Ther 2008; 7: 1305-12.

[197] Mahyar-Roemer M, Katsen A, Mestres P, Roemer K. Resveratrol induces colon tumor cell apoptosis independently of p53 and precede by epithelial differentiation, mitochondrial proliferation and membrane potential collapse. Int J Cancer 2001; 94: 615-22.

[198] Park JW, Woo KJ, Lee JT, *et al*. Resveratrol induces pro-apoptotic endoplasmic reticulum stress in human colon cancer cells. Oncol Rep 2007; 18: 1269-73.

[199] Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 2005; 4 :988-1004.

[200] Desbois-Mouthon C, Cadoret A, Blivet-Van Eggelpoel MJ, *et al*. Insulin and IGF-1 stimulate the beta-catenin pathway through two signalling cascades involving GSK-3beta inhibition and Ras activation. Oncogene 2001; 20: 252-9.

[201] Polakis P. The many ways of Wnt in cancer. Curr Opin Genet Dev 2007; 17: 45-51.

[202] Vanamala J, Reddivari L, Radhakrishnan S, Tarver C. Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways. BMC Cancer 2010; 10: 238.

[203] Hope C, Planutis K, Planutiene M, *et al*. Low concentrations of resveratrol inhibit Wnt signal throughput in colon-derived cells: implications for colon cancer prevention. Mol Nutr Food Res 2008; 52: S52-61.

[204] Juan ME, Wenzel U, Daniel H, Planas JM. Resveratrol induces apoptosis through ROS-dependent mitochondria pathway in HT-29 human colorectal carcinoma cells. J Agric Food Chem 2008; 56: 4813-8.

[205] Cosan DT, Bayram B, Soyocak A, *et al*. Role of phenolic compounds in nitric oxide synthase activity in colon and breast adenocarcinoma. Cancer Biother Radiopharm 2010; 25: 577-80.

[206] Ulrich S, Loitsch SM, Rau O, *et al*. Peroxisome proliferator-activated receptor gamma as a molecular target of resveratrol-induced modulation of polyamine metabolism. Cancer Res 2006; 66: 7348-54.

[207] Zykova TA, Zhu F, Zhai X, *et al*. Resveratrol directly targets COX-2 to inhibit carcinogenesis. Mol Carcinog 2008; 47: 797-805.

[208] Wu H, Liang X, Fang Y, Qin X, Zhang Y, Liu J. Resveratrol inhibits hypoxia-induced metastasis potential enhancement by restricting hypoxia-induced factor-1 alpha expression in colon carcinoma cells. Biomed Pharmacother 2008; 62: 613-21.

[209] Kimura Y, Sumiyoshi M, Baba K. Antitumor activities of synthetic and natural stilbenes through antiangiogenic action. Cancer Sci 2008; 99: 2083-96.

[210] Schneider Y, Duranton B, Gossé F, Schleiffer R, Seiler N, Raul F. Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. Nutr Cancer 2001; 39: 102-7.

[211] Sengottuvelan M, Viswanathan P, Nalini N. Chemopreventive effect of trans-resveratrol--a phytoalexin against colonic aberrant crypt foci and cell proliferation in 1,2-dimethylhydrazine induced colon carcinogenesis. Carcinogenesis 2006; 27: 1038-46.

[212] Sengottuvelan M, Nalini N. Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. Br J Nutr 2006; 96: 145-53.

[213] Sengottuvelan M, Deeptha K, Nalini N. Influence of dietary resveratrol on early and late molecular markers of 1,2-dimethylhydrazine-induced colon carcinogenesis. Nutrition 2009; 25: 1169-76.

[214] Tessitore L, Davit A, Sarotto I, Caderni G. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. Carcinogenesis 2000; 21: 1619-22.

[215] Suh N, Paul S, Hao X, *et al*. Pterostilbene, an active constituent of blueberries, suppresses aberrant crypt foci formation in the azoxymethane-induced colon carcinogenesis model in rats. Clin Cancer Res 2007; 13: 350-5.

[216] Chiou YS, Tsai ML, Wang YJ, *et al*. Pterostilbene inhibits colorectal aberrant crypt foci (ACF) and colon carcinogenesis via suppression of multiple signal transduction pathways in azoxymethane-treated mice. J Agric Food Chem 2010; 58: 8833-41.

[217] Paul S, DeCastro AJ, Lee HJ, *et al*. Dietary intake of pterostilbene, a constituent of blueberries, inhibits the beta-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats. Carcinogenesis 2010; 31: 1272-8.

[218] Nguyen AV, Martinez M, Stamos MJ, *et al*. Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res 2009; 1: 25-37.

[219] Patel KR, Brown VA, Jones DJ, *et al*. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res 2010; 70: 7392-9.

[220] Lambert JD, Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 2010; 501: 65-72.

[221] de Mejia EG, Ramirez-Mares MV, Puangpraphant S. Bioactive components of tea: cancer, inflammation and behavior. Brain Behav Immun 2009; 23: 721-31.

[222] Yang CS, Wang H. Mechanistic issues concerning cancer prevention by tea catechins. Mol Nutr Food Res 2011; 55: 819-31.

[223] Abboud PA, Hake PW, Burroughs TJ, *et al*. Therapeutic effect of epigallocatechin-3-gallate in a mouse model of colitis. Eur J Pharmacol 2008; 579: 411-7.

[224] Ran ZH, Chen C, Xiao SD. Epigallocatechin-3-gallate ameliorates rats colitis induced by acetic acid. Biomed Pharmacother 2008; 62: 189-96.

[225] Mochizuki M, Hasegawa N. (-)-Epigallocatechin-3-gallate reduces experimental colon injury in rats by regulating macrophage and mast cell. Phytother Res 2010; 24: S120-2.

[226] Shimizu M, Deguchi A, Lim JT, Moriwaki H, Kopelovich L, Weinstein IB. (-)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. Clin Cancer Res 2005; 11: 2735-46.

[227] Shimizu M, Deguchi A, Joe AK, Mckoy JF, Moriwaki H, Weinstein IB. EGCG inhibits activation of HER3 and expression of cyclooxygenase-2 in human colon cancer cells. J Exp Ther Oncol 2005; 5: 69-78.

[228] Hwang JT, Ha J, Park IJ, *et al*. Apoptotic effect of EGCG in HT-29 colon cancer cells via AMPK signal pathway. Cancer Lett 2007; 247: 115-21.

[229] Wang J, Hollingshead J, El-Masry N, et al. Expression of EGFR, HER2, Phosphorylated ERK and Phosphorylated MEK in Colonic Neoplasms of Familial Adenomatous Polyposis Patients. J Gastrointest Cancer. 2011; DOI: 10.1007/s12029-011-9330-9

[230] Adachi S, Nagao T, Ingolfsson HI, *et al*. The inhibitory effect of (-)-epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. Cancer Res 2007; 67: 6493-501.

[231] Peng G, Dixon DA, Muga SJ, Smith TJ, Wargovich MJ. Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog 2006; 45: 309-19.

[232] Park IJ, Lee YK, Hwang JT, Kwon DY, Ha J, Park OJ. Green tea catechin controls apoptosis in colon cancer cells by attenuation of H2O2-stimulated COX-2 expression via the AMPK signaling pathway at low-dose H2O2. Ann N Y Acad Sci 2009; 1171: 538-44.

[233] Navarro-Perán E, Cabezas-Herrera J, Sánchez-Del-Campo L, García-Cánovas F, Rodríguez-López JN. The anti-inflammatory and

anti-cancer properties of epigallocatechin-3-gallate are mediated by folate cycle disruption, adenosine release and NF-kappaB suppression. Inflamm Res 2008; 57: 472-8.

[234] Porath D, Riegger C, Drewe J, Schwager J. Epigallocatechin-3-gallate impairs chemokine production in human colon epithelial cell lines. J Pharmacol Exp Ther 2005; 315: 1172-80.

[235] Shirakami Y, Shimizu M, Tsurumi H, Hara Y, Tanaka T, Moriwaki H. EGCG and Polyphenon E attenuate inflammation-related mouse colon carcinogenesis induced by AOM plus DDS. Mol Med Report 2008; 1: 355-61.

[236] Yuan JH, Li YQ, Yang XY. Protective effects of epigallocatechin gallate on colon preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f] quinoline in mice. Mol Med 2008; 14: 590-8.

[237] Carter O, Dashwood RH, Wang R, *et al*. Comparison of white tea, green tea, epigallocatechin-3-gallate, and caffeine as inhibitors of PhIP-induced colonic aberrant crypts. Nutr Cancer 2007; 58: 60-5.

[238] Shimizu M, Shirakami Y, Sakai H, *et al*. (-)-Epigallocatechin gallate suppresses azoxymethane-induced colonic premalignant lesions in male C57BL/KsJ-db/db mice. Cancer Prev Res (Phila) 2008; 1: 298-304.

[239] Durai R, Yang W, Gupta S, Seifalian AM, Winslet MC. The role of the insulin-like growth factor system in colorectal cancer: review of current knowledge. Int J Colorectal Dis 2005; 20: 203-20.

[240] Ju J, Hong J, Zhou JN, *et al*. Inhibition of intestinal tumorigenesis in Apcmin/+ mice by (-)-epigallocatechin-3-gallate, the major catechin in green tea. Cancer Res 2005; 65: 10623-31.

[241] Hoensch H, Groh B, Edler L, Kirch W. Prospective cohort comparison of flavonoid treatment in patients with resected colorectal cancer to prevent recurrence. World J Gastroenterol 2008; 14: 2187-93.

[242] Slimestad R, Fossen T, Vågen IM. Onions: a source of unique dietary flavonoids. J Agric Food Chem 2007; 55: 10067-80.

[243] Gerhauser C. Cancer chemopreventive potential of apples, apple juice, and apple components. Planta Med 2008; 74: 1608-24.

[244] Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol 1999; 37: 937-42.

[245] Wach A, Pyrzynska K, Biesaga M. Quercetin content in some food and 561 herbal samples. Food Chemistry 2007; 100: 699-704.

[246] Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. Cancer Lett 2008; 269: 315-25.

[247] Chirumbolo S. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. Inflamm Allergy Drug Targets 2010; 9: 263-85.

[248] Sánchez de Medina F, Gálvez J, Romero JA, Zarzuelo A. Effect of quercitrin on acute and chronic experimental colitis in the rat. J Pharmacol Exp Ther 1996; 278: 771-9.

[249] Sánchez de Medina F, Vera B, Gálvez J, Zarzuelo A. Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. Life Sci 2002; 70: 3097-108.

[250] Camuesco D, Comalada M, Rodríguez-Cabezas ME, *et al*. The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. Br J Pharmacol 2004; 143: 908-18.

[251] Comalada M, Camuesco D, Sierra S, *et al*. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. Eur J Immunol 2005; 35: 584-92.

[252] Kwon KH, Murakami A, Tanaka T, Ohigashi H. Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. Biochem Pharmacol 2005; 69: 395-406.

[253] Kuo SM. Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells. Cancer Lett 1996; 110: 41-8.

[254] Richter M, Ebermann R, Marian B. Quercetin-induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling. Nutr Cancer 1999; 34: 88-99.

[255] Agullo G, Gamet-Payrastre L, Fernandez Y, Anciaux N, Demigne C, Remesy C. Comparative effects of flavonoids on the growth, viability and metabolism of a colonic adenocarcinoma cell line (HT29 cells). Cancer Lett 1996; 105: 61-70.

[256] Kim WK, Bang MH, Kim ES, *et al*. Quercetin decreases the expression of ErbB2 and ErbB3 proteins in HT-29 human colon cancer cells. J Nutr Biochem 2005; 16: 155-62.

[257] Lee YK, Park SY, Kim YM, Lee WS, Park OJ. AMP kinase/cyclooxygenase-2 pathway regulates proliferation and apoptosis of cancer cells treated with quercetin. Exp Mol Med 2009; 41: 201-7.

[258] Kim HJ, Kim SK, Kim BS, *et al*. Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signaling pathway. J Agric Food Chem 2010; 58: 8643-50.

[259] Mutoh M, Takahashi M, Fukuda K, *et al*. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. Carcinogenesis 2000; 21: 959-63.

[260] Shan BE, Wang MX, Li RQ. Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/beta-catenin signaling pathway. Cancer Invest 2009; 27: 604-12.

[261] Chondrogianni N, Kapeta S, Chinou I, Vassilatou K, Papassideri I, Gonos ES. Anti-ageing and rejuvenating effects of quercetin. Exp Gerontol 2010; 45: 763-71.

[262] Duthie SJ, Dobson VL. Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr 1999; 38: 28-34.

[263] Undeger U, Aydin S, Basaran AA, Basaran N. The modulating effects of quercetin and rutin on the mitomycin C induced DNA damage. Toxicology Letters 2004; 151: 143-9.

[264] Kurzawa-Zegota M, Najafzadeh M, Baumgartner A, Anderson D. The protective effect of the flavonoids on food-mutagen-induced DNA damage in peripheral blood lymphocytes from colon cancer patients. Food Chem Toxicol. Food Chem Toxicol. 2012; 50: 124-9.

[265] Yuan ZP, Chen LJ, Fan LY, *et al*. Liposomal quercetin efficiently suppresses growth of solid tumors in murine models. Clin Cancer Res 2006; 12: 3193-9.

[266] Deschner EE, Ruperto J, Wong G, Newmark HL. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 1991; 12: 1193-6.

[267] Matsukawa Y, Nishino H, Okuyama Y, *et al*. Effects of quercetin and/or restraint stress on formation of aberrant crypt foci induced by azoxymethane in rat colons. Oncology 1997; 54: 118-21.

[268] Yang K, Lamprecht SA, Liu Y, *et al*. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 2000; 21: 1655-60.

[269] Dihal AA, de Boer VC, van der Woude H, *et al*. Quercetin, but not its glycosidated conjugate rutin, inhibits azoxymethane-induced colorectal carcinogenesis in F344 rats. J Nutr 2006; 136: 2862-7.

[270] Volate SR, Davenport DM, Muga SJ, Wargovich MJ. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis 2005; 26: 1450-6.

[271] Choi SY, Park JH, Kim JS, Kim MK, Aruoma OI, Sung MK. Effects of quercetin and beta-carotene supplementation on azoxymethane-induced colon carcinogenesis and inflammatory responses in rats fed with high-fat diet rich in omega-6 fatty acids. Biofactors 2006; 27: 137-46.

[272] Warren CA, Paulhill KJ, Davidson LA, *et al*. Quercetin may suppress rat aberrant crypt foci formation by suppressing inflammatory mediators that influence proliferation and apoptosis. J Nutr 2009; 139: 101-5.

[273] Murphy EA, Davis JM, McClellan JL, Carmichael MD. Quercetin's effects on intestinal polyp multiplicity and macrophage number in the Apc(Min/+) mouse. Nutr Cancer 2011; 63: 421-6.

[274] Bobe G, Albert PS, Sansbury LB, *et al*. Interleukin-6 as a potential indicator for prevention of high-risk adenoma recurrence by dietary flavonols in the polyp prevention trial. Cancer Prev Res (Phila) 2010; 3: 764-75.

[275] Klein CB, King AA. Genistein genotoxicity: Critical considerations of in vitro exposure dose. Toxicol Appl Pharmacol 2007; 224: 1-11.

[276] Kousidou OCh, Tzanakakis GN, Karamanos NK. Effects of the natural isoflavonoid genistein on growth, signaling pathways and gene expression of matrix macromolecules by breast cancer cells. Mini Rev Med Chem 2006; 6: 331-7.

[277] Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr 1999; 129: 758-767.

[278] Ariazi EA, Jordan VC. Estrogen-related receptors as emerging targets in cancer and metabolic disorders. Curr Top Med Chem 2006; 6: 203-15.

[279] Si H, Liu D. Phytochemical genistein in the regulation of vascular function: new insights. Curr Med Chem 2007; 14: 2581-9.

[280] Marini H, Minutoli L, Polito F, *et al*. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. Ann Intern Med 2007; 146: 839-847.

[281] Szkudelska K, Nogowski L. Genistein--a dietary compound inducing hormonal and metabolic changes. J Steroid Biochem Mol Biol 2007; 105: 37-45.

[282] Seibel J, Molzberger AF, Hertrampf T, Laudenbach-Leschowski U, Diel P. Oral treatment with genistein reduces the expression of molecular and biochemical markers of inflammation in a rat model of chronic TNBS-induced colitis. Eur J Nutr 2009; 48: 213-20.

[283] Booth C, Hargreaves DF, Hadfield JA, McGown AT, Potten CS. Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis in vitro. Br J Cancer 1999; 80: 1550-7.

[284] Zhu Q, Meisinger J, Van Thiel DH, Zhang Y, Mobarhan S. Effects of soybean extract on morphology and survival of Caco-2, SW620, and HT-29 cells. Nutr Cancer 2002; 42: 131-40.

[285] Kim EJ, Shin HK, Park JH. Genistein inhibits insulin-like growth factor-I receptor signaling in HT-29 human colon cancer cells: a possible mechanism of the growth inhibitory effect of Genistein. J Med Food 2005; 8: 431-8.

[286] Yu Z, Tang Y, Hu D, Li J. Inhibitory effect of genistein on mouse colon cancer MC-26 cells involved TGF-beta1/Smad pathway. Biochem Biophys Res Commun 2005; 333: 827-32.

[287] Glick AB. TGFbeta1, back to the future: revisiting its role as a transforming growth factor. Cancer Biol Ther 2004; 3: 276-83.

[288] Yu Z, Li W, Liu F. Inhibition of proliferation and induction of apoptosis by genistein in colon cancer HT-29 cells. Cancer Lett 2004; 215: 159-66.

[289] Kameoka S, Leavitt P, Chang C, Kuo SM. Expression of antioxidant proteins in human intestinal Caco-2 cells treated with dietary flavonoids. Cancer Lett 1999; 146: 161-7.

[290] Ogasawara M, Matsunaga T, Suzuki H. Differential effects of antioxidants on the in vitro invasion, growth and lung metastasis of murine colon cancer cells. Biol Pharm Bull 2007; 30: 200-4.

[291] Li Q, Chen H. Epigenetic modifications of metastasis suppressor genes in colon cancer metastasis. Epigenetics 2011; 6: 849-52.

[292] Majid S, Dar AA, Ahmad AE, *et al*. BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer. Carcinogenesis 2009; 30: 662-70.

[293] King-Batoon A, Leszczynska JM, Klein CB. Modulation of gene methylation by genistein or lycopene in breast cancer cells. Environ Mol Mutagen 2008; 49: 36-45.

[294] Wang Z, Chen H. Genistein increases gene expression by demethylation of WNT5a promoter in colon cancer cell line SW1116. Anticancer Res 2010; 30: 4537-45.

[295] Bielecki A, Roberts J, Mehta R, Raju J. Estrogen receptor-β mediates the inhibition of DLD-1 human colon adenocarcinoma cells by soy isoflavones. Nutr Cancer 2011; 63: 139-50.

[296] Di Leo A, Barone M, Maiorano E, *et al*. ER-beta expression in large bowel adenomas: implications in colon carcinogenesis. Dig Liver Dis 2008; 40: 260-6.

[297] Hwang JT, Ha J, Park OJ. Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemo-resistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. Biochem Biophys Res Commun 2005; 332: 433-40.

[298] Nakamura Y, Yogosawa S, Izutani Y, Watanabe H, Otsuji E, Sakai TA. Combination of indol-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting Akt phosphorylation and progression of autophagy. Mol Cancer 2009; 8: 100.

[299] Min WK, Sung HY, Choi YS. Suppression of colonic aberrant crypt foci by soy isoflavones is dose-independent in dimethylhydrazine-treated rats. J Med Food 2010; 13: 495-502.

[300] Sørensen IK, Kristiansen E, Mortensen A, *et al*. The effect of soy isoflavones on the development of intestinal neoplasia in ApcMin mouse. Cancer Lett 1998; 130: 217-25.

[301] Guo JY, Li X, Browning JD Jr, *et al*. Dietary soy isoflavones and estrone protect ovariectomized ERalphaKO and wild-type mice from carcinogen-induced colon cancer. J Nutr 2004; 134: 179-82.

[302] Lambert JD, Kwon SJ, Ju J, *et al*. Effect of genistein on the bioavailability and intestinal cancer chemopreventive activity of (-)-epigallocatechin-3-gallate. Carcinogenesis 2008; 29: 2019-24.

[303] Kennedy AR. The evidence for soybean products as cancer preventive agents. J Nutr 1995; 125: 733-743.

[304] Adams KF, Lampe PD, Newton KM, *et al*. Soy protein containing isoflavones does not decrease colorectal epithelial cell proliferation in a randomized controlled trial. Am J Clin Nutr 2005; 82: 620-6.

[305] Kono S, Imanishi K, Shinchi K, Yanai F. Relationship of diet to small and large adenomas of the sigmoid colon. Jpn J Cancer Res 1993; 84: 13-9.

[306] Witte JS, Longnecker MP, Bird CL, Lee ER, Frankl HD, Haile RW. Relation of vegetable, fruit, and grain consumption to colorectal adenomatous polyps. Am J Epidemiol 1996; 144: 1015-25.

[307] Proksch P, Edrada RA, Ebel R. Drugs from the seas - current status and microbiological implications. Appl Microbiol Biotechnol 2002; 59: 125-134.

[308] Sithranga Boopathy N, Kathiresan K. Anticancer drugs from marine flora: an overview. J Oncol. 2010; 2010: 214186.

[309] Villa FA, Gerwick L. Marine natural product drug discovery: Leads for treatment of inflammation, cancer, infections, and neurological disorders. Immunopharmacol Immunotoxicol 2010; 32: 228-37.

[310] Cragg GM, Newman DJ. Chemical diversity: a function of biodiversity. Trends Pharmacol Sci 2002; 23: 404-5.

[311] Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. Chem Rev 2009; 109: 3012-43.

[312] Johnson EA, Schroeder WA. Microbial carotenoids. Adv Biochem Eng Biotechnol 1996; 53: 119-78.

[313] Pasquet V, Morisset P, Ihammouine S, *et al*. Antiproliferative activity of violaxanthin isolated from bioguided fractionation of Dunaliella tertiolecta extracts. Mar Drugs 2011; 9: 819-31.

[314] Palozza P, Torelli C, Boninsegna A, *et al*. Growth-inhibitory effects of the astaxanthin-rich alga Haematococcus pluvialis in human colon cancer cells. Cancer Lett 2009; 283: 108-17.

[315] Ramos AL, Torello CO, Queiroz ML. Chlorella vulgaris modulates immunomyelopoietic activity and enhances the resistance of tumor-bearing mice. Nutr Cancer 2010; 62: 1170-80.

[316] Guedes AC, Amaro HM, Malcata FX. Microalgae as sources of carotenoids. Mar Drugs 2011; 9: 625-44.

[317] Plaza M, Herrero M, Cifuentes A, Ibanez E. Innovative natural functional ingredients from microalgae. J Agric Food Chem 2009; 57: 7159-70.

[318] Del Campo JA, Garcia-Gonzalez M, Guerrero MG. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl Microbiol Biotechnol 2007; 74: 1163-74.

[319] Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.

[320] Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 1996; 10: 690-701.

[321] Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 1996; 96: 1027-39.

[322] Palozza P, Serini S, Maggiano N, *et al*. beta-Carotene downregulates the steady-state and heregulin-alpha-induced COX-2 pathways in colon cancer cells. J Nutr 2005; 135: 129-36.

[323] Smith W, Saba N. Retinoids as chemoprevention for head and neck cancer: where do we go from here? Crit Rev Oncol Hematol 2005; 55:143-52.

[324] Sun SY, Lotan R. Retinoids and their receptors in cancer development and chemoprevention. Crit Rev Oncol Hematol 2002; 41: 41-55.

[325] Lotan R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy. J Natl Cancer Inst 1995; 87: 1655-7.

[326] Cha KH, Koo SY, Lee DU. Antiproliferative effects of carotenoids extracted from Chlorella ellipsoidea and Chlorella vulgaris on human colon cancer cells. J Agric Food Chem 2008; 56: 10521-6.

[327] Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003; 189: 41-54.

[328] Cheeseman KH, Slater TF. An introduction to free radical biochemistry. Br Med Bull 1993; 49: 481-93.

[329] Ku HH, Brunk UT, Sohal RS. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. Free Radic Biol Med 1993; 15: 621-7.

[330] Lucchi L, Bergamini S, Iannone A, *et al*. Erythrocyte susceptibility to oxidative stress in chronic renal failure patients under different substitutive treatments. Artif Organs 2005; 29: 67-72.

[331] Waters DJ, Shen S, Xu H, *et al*. Noninvasive prediction of prostatic DNA damage by oxidative stress challenge of peripheral blood lymphocytes. Cancer Epidemiol Biomarkers Prev 2007; 16: 1906-10.

[332] Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med (Berl) 1996; 74: 297-312.
[333] Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol. 2004; 44: 239-67.
[334] Khan SK, Malinski T, Mason RP, *et al*. Novel astaxanthin prodrug (CDX-085) attenuates thrombosis in a mouse model. Thromb Res 2010; 126: 299-305.
[335] Haines DD, Varga B, Bak I, *et al*. Summative interaction between astaxanthin, Ginkgo biloba extract (EGb761) and vitamin C in suppression of respiratory inflammation: a comparison with ibuprofen. Phytother Res 2011; 25: 128-36.
[336] Chew BP, Mathison BD, Hayek MG, Massimino S, Reinhart GA, Park JS. Dietary astaxanthin enhances immune response in dogs. Vet Immunol Immunopathol 2011; 140: 199-206.
[337] Fassett RG, Coombes JS. Astaxanthin: a potential therapeutic agent in cardiovascular disease. Mar Drugs 2011; 9: 447-65.
[338] Guerin M, Huntley ME, Olaizola M. Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol. 2003; 21: 210-6.
[339] Hu F, Wang Yi B, Zhang W, *et al*. Carotenoids and breast cancer risk: a meta-analysis and meta-regression. Breast Cancer Res Treat. 2012; 131: 239-53.
[340] Biel RK, Csizmadi I, Cook LS, Courneya KS, Magliocco AM, Friedenreich CM. Risk of endometrial cancer in relation to individual nutrients from diet and supplements. Public Health Nutr 2011; 14: 1-13.
[341] Yasui Y, Hosokawa M, Mikami N, Miyashita K, Tanaka T. Dietary astaxanthin inhibits colitis and colitis-associated colon carcinogenesis in mice via modulation of the inflammatory cytokines. Chem Biol Interact 2011; 193: 79-87.
[342] Nagendraprabhu P, Sudhandiran G. Astaxanthin inhibits tumor invasion by decreasing extracellular matrix production and induces apoptosis in experimental rat colon carcinogenesis by modulating the expressions of ERK-2, NFkB and COX-2. Invest New Drugs 2011; 29: 207-24.
[343] Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab (Lond) 2010; 5: 7-18.
[344] Khachik F, Spangler CJ, Smith JC, Canfield LM, Steck A, Pfander H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 1997; 69: 1873-81.
[345] Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br J Ophthalmol 1998; 82: 907-10.
[346] Krinsky NI .Carotenoid protection against oxidation. Pure Appl Chem 1979; 51: 649–60.
[347] Sánchez F, Fernández JM, Acien FG, Rueda A, Pérez-Parra J, Molina E. Influence of culture conditions on the productivity and lutein content of the new strain Scenedesmus almeriensis. Process Biochem 2008; 43: 398-405.
[348] Olmedilla B, Granado F, Blanco I, Vaquero M. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. Nutrition 2003; 19: 21-4.
[349] Dwyer JH, Navab M, Dwyer KM, *et al*. Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. Circulation 2001; 103: 2922-7.
[350] Barnes PJ. Nuclear factor-kappa B. Int J Biochem Cell Biol 1997; 29: 867-70.
[351] Krishnaswamy R, Devaraj SN, Padma VV. Lutein protects HT-29 cells against Deoxynivalenol-induced oxidative stress and apoptosis: prevention of NF-kappaB nuclear localization and down regulation of NF-kappaB and Cyclo-Oxygenase-2 expression. Free Radic Biol Med 2010; 49: 50-60.
[352] Serpeloni JM, Barcelos GR, Friedmann Angeli JP, Mercadante AZ, de Lourdes Pires Bianchi M, Greggi Antunes LM. Dietary carotenoid lutein protects against DNA damage and alterations of the redox status induced by cisplatin in human derived HepG2 cells. Toxicol In Vitro 2012; 26: 288-94.
[353] Kim Y, Seo JH, Kim H. beta-Carotene and lutein inhibit hydrogen peroxide-induced activation of NF-kappaB and IL-8 expression in gastric epithelial AGS cells. J Nutr Sci Vitaminol (Tokyo) 2011; 57: 216-23.
[354] Selvaraj RK, Shanmugasundaram R, Klasing KC. Effects of dietary lutein and PUFA on PPAR and RXR isomer expression in chickens during an inflammatory response. Comp Biochem Physiol A Mol Integr Physiol 2010; 157: 198-203.
[355] Murtaugh MA, Ma KN, Caan BJ, *et al*. Interactions of peroxisome proliferator-activated receptor {gamma} and diet in etiology of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 1224-9.
[356] Almushatat AS, Talwar D, McArdle PA, *et al*. Vitamin antioxidants, lipid peroxidation and the systemic inflammatory response in patients with prostate cancer. Int J Cancer 2006; 118: 1051-3.
[357] Reynoso-Camacho R, Gonzalez-Jasso E, Ferriz-Martinez R, *et al*. Dietary supplementation of lutein reduces colon carcinogenesis in DMH-treated rats by modulating K-ras, PKB, and beta-catenin proteins. Nutr Cancer 2011; 63: 39-45.
[358] Raju J, Swamy MV, Cooma I, *et al*. Low doses of beta-carotene and lutein inhibit AOM-induced rat colonic ACF formation but high doses augment ACF incidence. Int J Cancer 2005; 113: 798-802.
[359] Fernandez-Sevilla JM, Acien Fernandez FG, Molina Grima E. Biotechnological production of lutein and its applications. Appl Microbiol Biotechnol 2010; 86: 27-40.
[360] Cordero BF, Couso I, Leon R, Rodriguez H, Vargas MA. Enhancement of carotenoids biosynthesis in Chlamydomonas reinhardtii by nuclear transformation using a phytoene synthase gene isolated from Chlorella zofingiensis. Appl Microbiol Biotechnol 2011; 91: 341-51.
[361] Geresh S, Mamontov A, Weinstein J. Sulfation of extracellular polysaccharides of red microalgae: preparation, characterization and properties. J Biochem Biophys Methods 2002; 50: 179-87.
[362] Fabregas J, García D, Fernandez-Alonso M, *et al*. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antiviral Res 1999; 44: 67-73.
[363] Smelcerovic A, Knezevic-Jugovic Z, Petronijevic Z. Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. Curr Pharm Des 2008;14: 3168-95.
[364] Levy-Ontman O, Arad SM, Harvey DJ, Parsons TB, Fairbanks A, Tekoah Y. Unique N-glycan moieties of the 66-kDa cell wall glycoprotein from the red microalga Porphyridium sp. J Biol Chem 2011; 286: 21340-52.
[365] Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from Spirulina platensis, aphanizomenon flos-aquae and Chlorella pyrenoidosa. Planta Med 2001; 67: 737-42.
[366] Balachandran P, Pugh ND, Ma G, Pasco DS. Toll-like receptor 2-dependent activation of monocytes by Spirulina polysaccharide and its immune enhancing action in mice. Int Immunopharmacol 2006; 6: 1808-14.
[367] Rasala BA, Muto M, Lee PA, *et al*. Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of Chlamydomonas reinhardtii. Plant Biotechnol J 2010; 8: 719-33.
[368] Specht E, Miyake-Stoner S, Mayfield S. Micro-algae come of age as a platform for recombinant protein production. Biotechnol Lett 2010; 32: 1373-83.
[369] Niu YF, Zhang MH, Xie WH, *et al*. A new inducible expression system in a transformed green alga, Chlorella vulgaris. Genet Mol Res 2011; DOI: 10.4238/2011.October.21.1.
[370] Chiaiese P, Palomba F, Tatino F, *et al*. Engineered tobacco and microalgae secreting the fungal laccase POXA1b reduce phenol content in olive oil mill wastewater. Enzyme Microb Technol 2011; 49: 540-6.
[371] Augustsson K, Michaud DS, Rimm EB, *et al*. A prospective study of intake of fish and marine fatty acids and prostate cancer. Cancer Epidemiol Biomarkers Prev 2003; 12: 64-7.
[372] Cheng J, Ogawa K, Kuriki K, *et al*. Increased intake of n-3 polyunsaturated fatty acids elevates the level of apoptosis in the normal sigmoid colon of patients polypectomized for adenomas/tumors. Cancer Lett 2003; 193: 17-24.
[373] Dommels YE, Haring MM, Keestra NG, Alink GM, van Bladeren PJ, van Ommen B. The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE(2) synthesis and cytotoxicity in human colorectal carcinoma cell lines. Carcinogenesis 2003; 24: 385-92.
[374] Feagan BG, Sandborn WJ, Mittmann U, *et al*. Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC Randomized Controlled Trials. JAMA 2008; 299: 1690-97.

[375] Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med 1996; 334: 1557-60.

[376] Sijtsma L, de Swaaf ME. Biotechnological production and applications of the omega-3 polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol 2004; 64: 146-53.

[377] Boeckaert C, Vlaeminck B, Dijkstra J, *et al*. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J Dairy Sci 2008; 91: 4714-27.

[378] van Beelen VA, Roeleveld J, Mooibroek H, *et al*. A comparative study on the effect of algal and fish oil on viability and cell proliferation of Caco-2 cells. Food Chem Toxicol. 2007; 45: 716-24.

[379] Hassan A, Ibrahim A, Mbodji K, *et al*. An alpha-linolenic acid-rich formula reduces oxidative stress and inflammation by regulating NF-kappaB in rats with TNBS-induced colitis. J Nutr 2010; 140: 1714-21.

[380] Piette M, Saugier J. Effect of the intravenous infusion of a lipid emulsion on blood leukocytes in the rabbit. Ann Pharm Fr 1970; 28: 529-34.

[381] Kostadinova R, Wahli W, Michalik L. PPARs in diseases: control mechanisms of inflammation. Curr Med Chem 2005;12: 2995-3009.

[382] Desreumaux P, Dubuquoy L, Nutten S, *et al*. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPAR-gamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001; 193: 827-38.

[383] Göttlicher M, Demoz A, Svensson D, Tollet P, Berge RK, Gustafsson JA. Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor. Biochem Pharmacol 1993; 46: 2177-84.

[384] Ávila-Román FJ, Talero E, Gómez-Hurtado M, Domínguez C, de los Reyes C, Ortega MJ, Zubía E, García-Mauriño S, Motilva V. Potent anti-inflammatory activity of oxylipins from microalgae. Abstracts of the 14th Congress of the European Shock Society. Taormina, Italy, Aug 31-Sept 2, Shock. 2011; 36 (Suppl 1): 8.

[385] Hontecillas R, Wannemeulher MJ, Zimmerman DR, *et al*. Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid. J Nutr 2002; 132: 2019-27.

[386] Ohtsuka Y, Okada K, Yamakawa Y, *et al*. Omega-3 Fatty Acids Attenuate Mucosal Inflammation in Premature Rat Pups. J Pediatr Surg 2011; 46: 489-95.

[387] Cooney JM, Barnett MP, Brewster D, *et al*. Proteomic Analysis of Colon Tissue from Interleukin-10 Gene-Deficient Mice Fed Polyunsaturated Fatty Acids with Comparison to Transcriptomic Analysis. J Proteome Res 2011; 11: 1065-77.

[388] Camuesco D, Galvez J, Nieto A, *et al*. Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. J Nutr 2005; 135: 687-94.

[389] Camuesco D, Comalada M, Concha A, *et al*. Intestinal anti-inflammatory activity of combined quercitrin and dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, in rats with DSS-induced colitis. Clin Nutr 2006; 25: 466-76.

[390] Cho JY, Chi SG, Chun HS. Oral administration of docosahexaenoic acid attenuates colitis induced by dextran sulfate sodium in mice. Mol Nutr Food Res 2011; 55: 239-46.

[391] Varnalidis I, Ioannidis O, Karamanavi E, *et al*. Omega 3 fatty acids supplementation has an ameliorative effect in experimental ulcerative colitis despite increased colonic neutrophil infiltration. Rev Esp Enferm Dig 2011; 103: 511-18.

[392] John S, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. Eur J Gastroenterol Hepatol 2010; 22: 602-6.

[393] Hossain Z, Hosokawa M, Takahashi K. Growth inhibition and induction of apoptosis of colon cancer cell lines by applying marine phospholipid. Nutr Cancer 2009; 61: 123-30.

[394] Calviello G, Di Nicuolo F, Gragnoli S, *et al*. n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. Carcinogenesis 2004; 25: 2303-10.

[395] Tang FY, Cho HJ, Pai MH, Chen YH. Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells. J Nutr Biochem 2009; 20: 426-34.

[396] Allred CD, Talbert DR, Southard RC, Wang X, Kilgore MW. PPARgamma1 as a molecular target of eicosapentaenoic acid in human colon cancer (HT-29) cells. J Nutr 2008; 138: 250-6.

[397] Kato T, Kolenic N, Pardini RS. Docosahexaenoic acid (DHA), a primary tumor suppressive omega-3 fatty acid, inhibits growth of colorectal cancer independent of p53 mutational status. Nutr Cancer 2007; 58: 178-87.

[398] Takahashi M, Fukutake M, Isoi T, *et al*. Suppression of azoxymethane-induced rat colon carcinoma development by a fish oil component, docosahexaenoic acid (DHA). Carcinogenesis. 1997; 18: 1337-42.

[399] Latham P, Lund EK, Johnson IT. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis 1999; 20: 645-50.

[400] Fukunaga K, Hossain Z, Takahashi K. Marine phosphatidylcholine suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by inducing apoptosis. Nutr Res 2008; 28: 635-40.

[401] van Beelen VA, Spenkelink B, Mooibroek H, *et al*. An n-3 PUFA-rich microalgal oil diet protects to a similar extent as a fish oil-rich diet against AOM-induced colonic aberrant crypt foci in F344 rats. Food Chem Toxicol 2009; 47: 316-20.

[402] Fini L, Piazzi G, Ceccarelli C, *et al*. Highly purified eicosapentaenoic acid as free fatty acids strongly suppresses polyps in Apc(Min/+) mice. Clin Cancer Res 2010; 16: 5703-11.

[403] West NJ, Clark SK, Phillips RK, *et al*. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. Gut 2010; 59: 918-25.

[404] Courtney ED, Matthews S, Finlayson C, *et al*. Eicosapentaenoic acid (EPA) reduces crypt cell proliferation and increases apoptosis in normal colonic mucosa in subjects with a history of colorectal adenomas. Int J Colorectal Dis 2007; 22: 765-76.

[405] Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palabra AF. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg Chim Acta 2003; 356: 328-34.

[406] Nobre B, Marcelo F, Passos R, Beiraó L, Palabra A, Gouveia L, Mendes R. Supercritical carbon dioxide extraction of astaxanthin and other carotenoids from the microalga Haematococcus pluvialis. Eur Food Technol 2006; 223: 787-90.

[407] Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Mol Nutr Food Res 2011; 55: 150-65.

[408] Mercurio F. Manning AM. NFkB as a primary regulador of the stress response.Oncogen 1999; 18: 6163-71.

[409] de la Vega M, Díaz E, Vila M, León R. Isolation of a new strain of Picochlorum sp and characterization of its potential biotechnological applications. Biotechnol Prog. 2011; 27: 1535-43.

[410] Mendes RL, Fernandes HL, Coelho JP, *et al*. Supercritical CO2 extraction of carotenoids and other lipids from Chlorella vulgaris. Food Chem 1995; 53: 99-103.

[411] Cerón MC, García-Malea MC, Rivas J, *et al*. Antioxidant activity of Haematococcus pluvialis cells grown in continuous culture as a function of their carotenoid and fatty acid content..Appl Microbiol Biotechnol. 2007; 74: 1112-9.

[412] Herrero M, Ibáñez E, Cifuentes A, Reglero G, Santoyo S. Dunaliella salina microalga pressurized extracts as potencial antimicrobials. J Food Prot 2006; 69: 2471-7.

[413] Mendiola JA, Jaime L, Santoyo S, *et al*. Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. Food Chem 2007; 102: 1357-67.

[414] Xue C, Hu Y, Saito H, *et al*. Molecular species composition of glycolipids from Sprirulina platensis. Food Chem 2002; 77: 9-13.

[415] Sanghvi AM, Lo YM. Present and potential industrial applications of macro- and microalgae. Recent Pat Food Nutr Agric 2010; 2: 187-94.

[416] Tonon T, Harvey D, Larson TR, Graham IA. Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. Phytochemistry 2002; 61: 15-24.

[417] Guil-Guerrero JL, Belarbi EH, Rebolloso-Fuentes MM. Eicosapentaenoic and arachidonic acids purification from the red microalga Porphyridium cruentum Bioseparation 2000; 9: 299-306.

[418] Piñero Estrada JE, Bermejo Bescós P, Villar del Fresno AM. Antioxidant activity of different fractions of Spirulina platensis protean extract. Farmaco 2001; 56: 497-500.

[419] Shibata S, Hayakawa K, Egashira Y, Sanada H. Hypocholesterolemic mechanism of Chlorella: Chlorella and its indigestible fraction enhance hepatic cholesterol catabolism through up-regulation of cholesterol 7alpha-hydroxylase in rats. Biosci Biotechnol Biochem 2007; 71: 916-25.

[420] Mendiola JA, García-Martínez D, Ruperez FJ, *et al*. Enrichment of vitamin E from Spirulina platensis microalga by SFE. J Supercrit Fluid 2008; 43: 484-9.

Received: March 12, 2012 **Accepted: March 29, 2012**

[421] Abd El-Baky HH, El-Baz FK, El Baroty GS. Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. J Sci Food Agric 2010; 90: 299-303.

[422] Cha TS, Chen CF, Yee W, Aziz A, Loh SH. Cinnamic acid, coumarin and vanillin: Alternative phenolic compounds for efficient Agrobacterium-mediated transformation of the unicellular green alga, Nannochloropsis sp. J Microbiol Methods 2011; 84: 430-4.

[423] Plaza M, Santoyo S, Jaime L, *et al*. Screening for bioactive compounds from algae. J Pharm Biomed Anal 2010; 51: 450-5.