Pharmacological and dietary agents for colorectal cancer chemoprevention: Effects on polyamine metabolism (Review)

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Abstract. Chemoprevention is the long-term use of different chemical agents, both synthetic and natural, to prevent or delay the onset of disease. Since colorectal cancer has a significant environmental component, it is an ideal disease in which to evaluate the potential benefits of chemopreventive agents. The polyamines, spermine, spermidine and putrescine have been involved in almost all the steps of colorectal tumorigenesis. Consequently, polyamine biosynthesis and catabolism can be considered as promising targets for cancer chemoprevention. A variety of drug formulations have been tested for their efficacy in affecting polyamines in a strategy of colorectal cancer prevention. Different molecules, such as biosynthesis inhibitors and catabolism inducers, have been proposed alone or in combination with other drugs proved to diminish the colorectal cancer risk. Interestingly, also diet can play a role in cancer prevention by affecting polyamines. Several dietary components, such as probiotics or flavonoids, have been shown to affect the polyamine metabolic pathway in colorectal neoplastic tissue. On the other hand, the polyamines ingested with diet might contrast the above cited effects shown by both drugs and nutritional factors. It is, therefore, fundamental to acquire more data also on these aspects in view of an innovative approach to colorectal oncology. This review summarizes data on the role of polyamine metabolism in neoplastic transformation of colorectal mucosa and as possible target for colorectal cancer chemoprevention. Attention will be focused on the influence of drugs and nutritional factors on polyamine metabolism, as well as the role played by dietary polyamines.

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1. Introduction

Colorectal cancer (CRC) is one of the main causes of death in Western countries. Many colon cancer treatment options are available, including surgery, chemotherapy and radiation, but chemoprevention is a fundamental approach to reduce cancer risk. Chemoprevention is the long-term use of different chemical agents, both synthetic and natural, by healthy individuals to prevent or delay the onset of disease. Since CRC has a significant environmental component, it is an ideal disease in which to evaluate the potential benefits of chemopreventive agents. Different CRC chemoprevention strategies are under investigation. They include prevention of radical formation and DNA hypomethylation, prevention or suppression of mutations, inhibition of cell proliferation and induction of tumor cell differentiation (1).

Among the several biochemical alterations found in cancer cells, one of the most noticeable is a change in the intracellular polyamine content. Polyamines are polycationic compounds that play a key role in almost all the steps of colorectal tumorigenesis. In CRC tissue, the polyamine content as well as the activities of two important enzymes in their biosynthesis such as ornithine decarboxylase (ODC)

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Abbreviations: CRC, colorectal cancer; ODC, ornithine decarboxylase; SAM-DC, S-adenosylmethionine decarboxylase; SSAT, spermidine-spermine-N1-acetyl transferase; PAO, polyamine oxidase; AZ, antizyme; AZI, antizyme inhibitor; DFMO, difluoromethylornithine; APC, adenomatous polyposis coli; GI, gastrointestinal; NSAIDs, non-steroidal anti-inflammatory drugs; SNP, single nucleotide polymorphism; AOM, azoxymethane; L.GG, *Lactobacillus rhamnosus* strain GG; ERs, estrogen receptors; ACF, abberrant crypt foci; PPARγ peroxisome proliferator-activated receptor γ; EGCG, (-)-epigallocatechin-3-gallate; CRA, colorectal adenoma

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and S-adenosylmethionine decarboxylase (SAM-DC), are increased 3-4 fold over that found in the equivalent normal colonic tissue. Besides, polyamines have been considered as possible markers of neoplastic proliferation in the colon. Noticeably, it is not only polyamine synthesis, but also their uptake that is enhanced in rapidly proliferating neoplastic cells in the colon. In contrast to all other cell systems in the body, colon cancer cells are exposed to high concentration of polyamines. These derive from the cells of the gut mucosa, which release their polyamine content into the lumen during the process of cell extrusion and death, but also from the gut bacteria and food. Therefore, also the introduction of polyamines by exogenous environment with diet may be of importance in a strategy of cancer prevention (2).

This review summarizes data on the role of polyamine metabolism in neoplastic transformation of colorectal mucosa and as possible target for CRC chemoprevention. Attention will be focused on the influence of drugs and nutritional factors on polyamine metabolism, as well as the role played by dietary polyamines.

2. Polyamine metabolism and colorectal cancer

Polyamines (putrescine, spermidine and spermine) are organic cations present in every living cell. They have pleiotropic effects on cell physiology and play a relevant role in cell proliferation and differentiation (3). These molecules are positively charged at the primary and secondary amino groups at physiological pH. Thus, polyamines may act as ligands at multiple sites on DNA, RNA, proteins, phospholipids and nucleotide triphosphates (4). Accordingly, biological functions of polyamines are mainly involved in the regulation of gene expression by altering DNA structure and modulating signal transduction pathways (5,6).

The polyamine functions and their metabolic pathway have been extensively studied (7,8). Fig. 1 shows a schematic representation of the polyamine metabolic pathway. Briefly, biosynthesis is mediated by the key enzyme ODC which converts the amino acid ornithine into putrescine. This is then sequentially converted into spermidine and spermine, through the action of the enzyme SAM-DC and spermidine/spermine synthase. The central enzyme in the polyamine catabolic pathway is the spermidine-spermine-N1-acetyl transferase (SSAT), which adds acetyl groups to terminal amine groups in spermidine and spermine. These acetylated polyamines are then substrates for the enzyme polyamine oxidase (PAO) which retro-converts these acetylated derivatives into lower chain amines.

Intracellular levels of polyamines are tightly controlled and this occurs in addition to the multi-level control of synthesis and catabolism, also by uptake and efflux (9).

The protein ornithine decarboxylase antizyme (AZ) can effectively control polyamine levels not only by inactivating ODC and inducing its degradation, but also by increasing polyamine efflux and decreasing polyamine uptake. However, an antizyme inhibitor (AZI) has been characterized and the overexpression of this compound in certain forms of cancer has been reported (10).

Many biochemical alterations have been found in cancer cells, but one of the most consistent is a change in the intracellular polyamine content. Polyamine concentrations increase during carcinogenesis and an increase in ODC activity accompanies neoplastic transformation (11). As with other tumors, polyamine content of CRC is higher when compared to the adjacent mucosa and equivalent normal tissue (12,13). The increase is due to the loss in polyamine homeostasis occurring during the dysregulation of cell proliferation (2). This is also proven by evidence of an upregulation of polyamine biosynthesis (14), a decrease in their catabolism (15,16) and an increased uptake (17). Since polyamine metabolism is an integral component of the mechanism of the carcinogenesis in colorectal tissue, polyamine levels and ODC activity have been considered even as specific markers for neoplastic proliferation in the colon (18). Particularly, ODC has been shown to be critical in cell transformation and it has been suggested to be a proto-oncogene (19).

A possible role of polyamines in regulating oncogene expression and function through transcriptional and post-ranscriptional processes has also been suggested (20). On the contrary, two of the most commonly mutated genes in colon cancer, such as the adenomatous polyposis coli (APC) tumor suppressor gene and K-ras oncogene, have been shown to regulate the expression of several polyamine metabolic genes (e.g., ODC and SAT) (15,21). In this context, it has also been observed that polyamine biosynthesis is involved in human colorectal carcinogenesis in a manner that is K-ras-dependent and p53-independent; besides, K-ras mutation and polyamine biosynthesis seem to be preferentially associated with polypoid tumors rather than flat colorectal tumors of the colon (22,23). Given that polyamines and cancers seem to be tightly connected, modulations of the polyamine metabolic pathway such as polyamine uptake and efflux, have received much attention in cancer drug development for chemoprevention of human CRC (24).

3. Influence of drugs on polyamine metabolism for CRC chemoprevention

ODC, being the rate limiting enzyme in polyamine biosynthesis, has represented the first target in the polyamine pathway for cancer therapy. Several studies have focused on the use of difluoromethylornithine (DFMO), an ODC inhibitor, as chemopreventive and chemotherapeutic agent (25,26). Although DFMO has been proven to inhibit the growth of tumor cells *in vitro*, it has not been so convincing in its anti-neoplastic properties, being cytostatic rather than cytotoxic, at least *in vivo* (27). Besides, prohibitively high doses of DFMO were usually required to inhibit malignant tumor growth in early chemotherapeutic trials (28,29).

The lack of efficacy by DFMO against established tumors has been put in relation to the availability of extracellular polyamines, derived from the diet, the retroconversion pathway as well as the polyamine content supplied by the gastrointestinal (GI) microbiota (30). However, it has also been observed that very low, non-toxic doses of DFMO were able to inhibit stimulation of proliferation by various carcinogens and this evidence has led to investigation of DFMO as a cancer chemopreventive agent. Probably, the greatest potential for DFMO action in chemoprevention may be exerted against CRC, where the decrease in ODC activity and polyamine

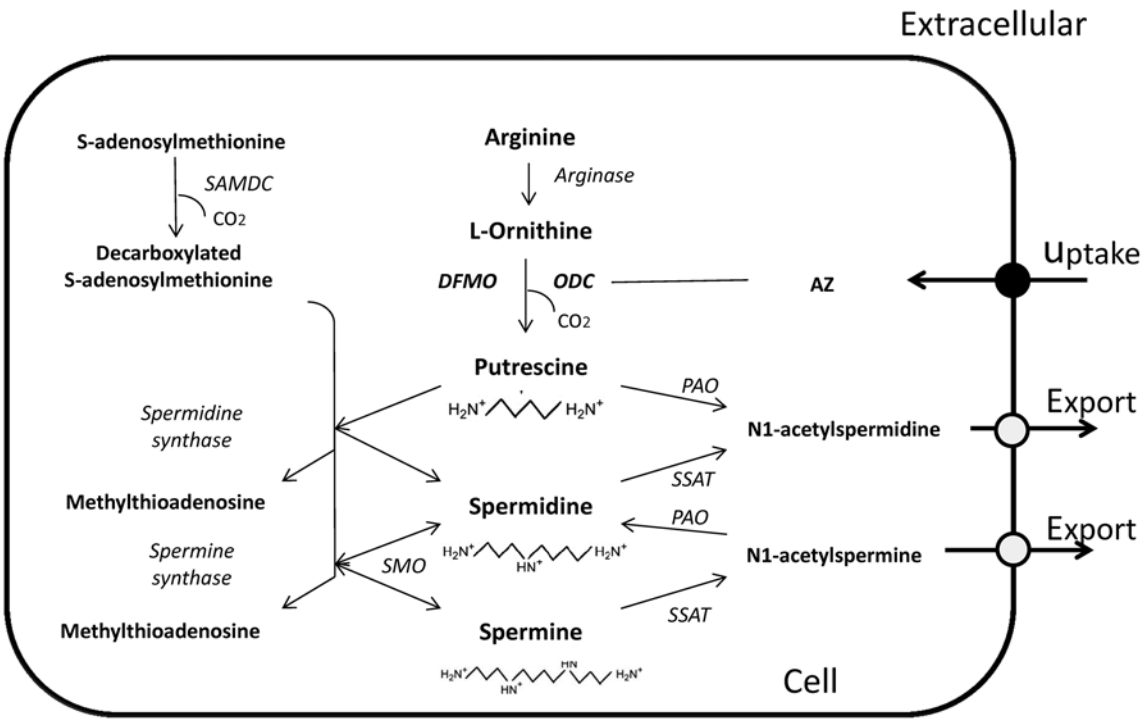

Figure 1. Polyamine metabolic pathway. ODC, ornithine decarboxylase; SAM-DC, S-adenosylmethionine decarboxylase; SSAT, spermidine-spermine-N1-acetyl transferase; PAO, polyamine oxidase; AZ, antizyme; DFMO, difluoromethylornithine; SMO, spermine oxidase.

content have been proven to limit significantly the formation of tumors (31).

A series of problem must be solved before conclusive evidence might be obtained from chemopreventive trials. Firstly, the putative chemopreventive agents have to be administered over long periods of time. Therefore, a major problem to be resolved concerns not only efficacy, but also the presence of minor toxicity and unpleasant side effects. Secondly, appropriate intermediate and endpoint biomarkers which allow the monitoring of the effectiveness of the drugs must be identified before the start of the research (32). In this connection, the early prevention clinical trials of DFMO have been conducted including pilot phase IIa and IIb studies aimed at evaluating the safety of treatment as well as the effective reduction in the colorectal tissue polyamine content (33).

It has been observed that an oral dose of 0.2 g/m^2/day can be both effective in reducing colorectal mucosal polyamine contents and safe, given that no relevant signs of toxicity were observed in the treatment group, compared to the placebo group (34).

It is known that combining different molecules in a strategy of chemoprevention could offer the prospect of reduced toxicity by lowering doses of individual agents. Therefore, a variety of combined formulations in order to decrease intracellular polyamine levels have been proposed and studied for their efficacy in cancer prevention. As an example, one successful strategy is based on the administration of DFMO along with non-steroidal anti-inflammatory drugs (NSAIDs). As demonstrated by epidemiological reports, a regular use of NSAIDs is associated with a decreased risk for CRC (35), whereas experimental studies have shown that DFMO acts, at least additively, with a number of NSAIDs (36).

This drug combination proved to decrease polyamine synthesis by inhibiting ODC (action due to DFMO), while contemporary increasing catabolism and cellular export of polyamines by activating SAT (induced by NSAIDs) (37). This combination produced an additive reduction of colon carcinogenesis also in laboratory animals (38) and it was observed that sulindac and celecoxib are both potent stimulators of SAT and inhibitors of intestinal carcinogenesis in Apc Min/+ mouse (39).

More recently, a 3-year trial (40) strongly validated a significant effect of the combined administration of low doses of DFMO (500 mg/die) plus sulindac (150 mg/die) on the polyamine production in rectal mucosa. Although no relationship between changes in polyamine levels and response were observed, the baseline polyamine levels affected the DFMO plus sulindac effects for colorectal adenoma (CRA) prevention.

By combining DFMO with NSAIDs, or other proven chemopreventive agents, it is also expected that fewer side effects occur during treatment. In this connection, it has been reported that DFMO plus sulindac administration is safe and effective in chemoprevention of CRA in people with prior colon polyps (41). However, other phase III trials involving adenoma patients, showed that the same combination of DFMO plus sulindac is actually effective, but confers a modest risk of ototoxicity and the potential risk of cardiovascular toxicity (42).

The usual side-effect ototoxicity and differential treatment outcomes related to the DFMO plus sulindac treatment could be associated with the specific germline single nucleotide polymorphism (SNP) in the ODC-1 promoter region. SNP has been investigated as a marker for colorectal adenoma (43). In this connection, it has been observed that aspirin is able to decrease the risk of CRA recurrence especially in individuals homozygous for the ODC1 minor A allele compared with

those with the major G allele (44). This has been confirmed and corroborated by other studies (45). These findings suggest that genetic features of the polyamine metabolism may be markers for both treatment benefit and toxicity.

Some data also indicate a potential link between obesity and polyamine inhibition in humans (46); besides, an association between obesity and risk of CRA has been reported (47). In spite of these observations, in a recent study it has been shown that obesity does not substantially modify the CRA risk reduction ascribed to DFMO plus sulindac versus placebo (48). This evidence strongly supports the need for chemopreventive clinical trials to refine the risk:benefit as well as the risk:risk profiles of the putative chemopreventive agents.

Based on the above, it is evident that the colorectal chemoprevention strategy with DFMO plus NSAIDs provides important proofs of principle that targeting polyamine metabolism can really be an effective strategy for reducing those risk factors closely associated with the development of colon cancer in humans.

Apart from ODC, the other polyamine biosynthesis enzymes, SAM-DC and spermidine/spermine synthase, have also received considerable attention as target for polyamine level reduction. Most SAM-DC inhibitors rapidly depleted spermidine and spermine concentrations in the cells, but their effects disappeared when *in vivo* models were tested. Probably, this happened because the accumulation of putrescine might compensate for spermidine in cells. Inhibitors of spermidine/spermine synthase have not been shown to be efficacious in either *in vitro* or *in vivo* system (49). PAO inhibitors have also been developed, since this enzyme converts spermine to spermidine, in order to overcome cell growth inhibition by polyamine depletion. These inhibitors are potent killers of cancer cells *in vitro* and showed promise when used with DFMO in carcinogenesis model *in vivo* (25,50).

As above mentioned, in addition to inhibition of polyamine biosynthesis, induction of polyamine catabolism has also been a major pharmacological goal. The enzyme SSAT can be induced by a variety of compounds like NSAIDs and polyamine analogues (51). These latter molecules need a special mention, due to their pleiotropic effects on cancer cells. Polyamine analogues are able to reduce the cell polyamine content by both upregulating catabolism (inducing SSAT), decreasing biosynthesis by negative feedback inhibition and by competing with exogenous polyamines for uptake. Besides, polyamine analogues can inhibit cell growth by acting like endogenous polyamines and bind to intracellular polyamine binding sites, thus rendering them 'non-functional' (52). *In vitro* studies have clearly demonstrated a role for polyamine analogues as multi-site inhibitors of the polyamine pathway, although the efficacy of each type of analogue in cancer chemoprevention and therapy remains to be fully characterized *in vivo* (53).

Other aspects of the polyamine metabolism pathway including polyamine uptake and efflux have also been targeted for cancer drug development. Uptake inhibitors have shown promise *in vitro* and have increased the efficacy of DFMO as an anticancer agent (17,54).

Aside from specific inhibitor/inducer drugs for the polyamine pathway and transport, also several nutritional components thought to be useful in colon chemoprevention have been shown to affect the polyamine metabolic pathway as well as to impair the tissue polyamine content in colorectal neoplastic tissue.

4. Influence of nutritional factors on polyamine metabolism for CRC chemoprevention

The GI tract is constantly connected with the external environment, therefore all the possible modifications in daily diet might significantly modify the exposure to different carcinogenetic factors. In this framework, the identification of those components in diet that may display at different degree some antitumor activity as well as the understanding of their mechanisms of action, may lead to significant advances in human cancer prevention. Several diet components have been demonstrated as having some anti-neoplastic activity. From the early 1970s, many studies following different designs (e.g., correlation studies, case-control, cohort studies) have hypothesized that dietary fibers may show protective effects against diverse neoplasms, including GI neoplasms (55). Their positive action in the GI environment has been essentially put in relation with the bacterial strains resident in the GI lumen (56).

The intestinal microbiota have been considered as a potential target for an active anticancer strategy. In view of its fundamental role in human health, manipulation of the intestinal microbiota by microrganisms shown to positively affect the GI tract (e.g., probiotics) or other compounds found in plants, has been considered as a logical approach to prevent or inhibit the neoplastic transformation of GI mucosa.

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (57). Together with these positive bacterial strains, other substances, mainly found in plants have been deeply investigated for their chemopreventive and chemotherapeutic properties against human cancers. These substances include flavonoids such as genistein (found in soy), quercetin (onions), apigenin (celery, parsley), green tea (polyphenols), etc. On these bases this chapter tries to review data on those nutritional components useful for CRC chemoprevention in relation to their potentiality in affecting the polyamine metabolism.

Probiotics. At present, the most commonly used microorganisms for manipulating the GI environment belong to *Lactobacillus* and *Bifidobacterium genera* (58). These two genera contain several species and strains of which many are being used as probiotic strains and most of them are categorized as lactic acid-producing bacteria (59). Usually, probiotics are consumed in the form of yogurt, fermented milks or other fermented foods, even if more recent studies have proposed their administration by using vegetables (e.g., artichokes, olives) as carriers (60-62). Their end products are mainly organic acids (lactic and acetic acids) that tend to lower the pH of the intestinal content, creating a less favorable environment for harmful bacteria.

Several health-promoting effects have been attributed to the probiotic lactic acid bacteria, but the most interesting and controversial is their anticancer activity against different neoplasms, including CRC (63).

These bacteria have been shown to possess antimutagenic and anticarcinogenic properties and data from epidemio-

Table I. Evidence for antineoplastic mechanisms of action of probiotics.

a. Reduction in the activity of fecal enzymes (betaglucuronidase, azoreductase, nitroreductase and 7-alphadehydrogenase) considered as having a role in colon cancer.
b. Reduction in the incidence of chemically induced tumors in rats.
c. *In vitro* prevention of damage to DNA in colon cancer cell lines.
d. *In vitro* binding of mutagens by different components of probiotic bacteria.
e. Improvement of immune system defense.
f. Reduction in the polyamine content in colon cancer cell lines and in laboratory animals.

logical and experimental studies clearly indicate that ingestion of lactobacilli and bifidobacteria, or their fermented dairy products, reduce the risk of certain types of cancer and inhibit tumor growth (64,65). The postulated mechanisms involve alterations of luminal pH in the gut, production of antimicrobial compounds, competition for pathogen binding and receptor sites, competition for available nutrients and growth factors, stimulation of immunomodulatory cells, and production of lactase (66). Table I reports some of the evidence for antineoplastic actions by probiotics.

Some studies have emphasized a relationship between polyamine biosynthesis and probiotic action in carcinogenesis and tumor growth. In studies performed in mice (67), the administration of *Bifidobacterum longum* cultures significantly suppressed the rate of cellular proliferation, the expression and activity of ODC, as well as mutated ras-p21 in a manner strongly correlating with inhibition of tumor induction by azoxymethane (AOM). *Lactobacillus brevis* strain showed pro-apoptotic effects in Jurkat cells and it was hypothesized that this ability in inducing apoptosis could be associated with polyamine synthesis (68). Besides, a peculiar *Lactobacillus brevis* strain (CD2 strain) demonstrated anti-proliferative biochemical features, essentially related to the activity of arginine deiminase (69). This enzyme is able to catalyse the catabolism of arginine and to affect the biosynthesis of polyamines (70). Di Marzio *et al* (68) advanced the hypothesis of an involvement of arginine deiminase in a study performed on the human T leukemia Jurkat cell line. The authors demonstrated that lyophilized and sonicated preparations of *L. brevis* (CD2) were able not only to cause arginine-dependent polyamine synthesis inhibition, but also to induce consequently a relevant apoptotic effect.

Previously, Orlando *et al* (71) performed a study aimed at investigating the effects of increasing concentrations of *Lactobacillus rhamnosus* strain GG (*L.GG*) homogenate on cell growth and proliferation in neoplasms originating from different GI tracts, such as gastric HGC-27 and colonic DLD-1 cells, focusing attention in their polyamine profile and biosynthesis. Additionally, in order to verify which bacterial fraction was involved in the anti-proliferative effects, the cytoplasm and cell wall extracts were tested separately. Both cell lines proved to be sensitive to the growth inhibition by the highest concentrations of bacterial homogenate with a significant reduction in their polyamine concentrations. Interestingly, either HGC-27 or DLD-1 cells were resistant to the bacterial cell wall fractions, whereas increasing cytoplasm fraction concentrations induced an evident anti-proliferative effect. These data suggested that cytoplasm extracts could be responsible for *L.GG* action on proliferation of these two cell lines from gastric and colonic neoplasms. Another probiotic, *Saccharomyces boulardii* (*S. boulardii*) usually prescribed in a lyophilized form, demonstrated not only to act as a carrier able to release different active metabolic compounds such as enzymes and trophic factors during its intestinal transit, but also to secrete its polyamine content (mainly spermine and spermidine), thus directly affecting gene expression and protein synthesis (72).

Also the effects of a probiotic mixture of 8 different bacterial strains (VSL#3) on polyamine biosynthesis, Ki-67 levels and apoptosis in the normal colon of rats have been evaluated (73). It has been postulated that probiotic mixtures may have a higher efficacy than single strains due to an additive or even synergistic effects when put together with other probiotic strains. The combined use of these probiotic strains (*S. thermophilus*, *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. casei*, *L. bulgaricus*) caused a significant decrease in colonic polyamine levels, ODC activity and Ki-67 compared to controls along with a significant increase in the apoptotic index. In this framework, the results of this study suggested that probiotics could also reduce proliferation rates in a normal condition not affected by hyperproliferative or neoplastic growth.

In humans, the effects on fecal probiotic metabolites (polyamines, lactate and acetate) and mutagenicity following administration of a yogurt containing *Bifidobacterium lactis* LKM512 were investigated in healthy adults (74). Consumption of LKM512 yogurt increased fecal spermidine levels, but not fecal lactate and acetate contents and significantly reduced the mutagenicity level to 79.2%. These results allowed the authors to hypothesize that increased gut spermidine level by LKM512 yogurt was responsible for the reduction of mutagenicity in the human. In this connection, the link between cell proliferation, polyamines and probiotics could be regulated not only by the peculiar metabolic features of the microbial strains, but also by the period of administration or the proliferative behavior of different segments of GI mucosa. On this basis, and in view of the potential offered by probiotics in affecting the proliferative activity of GI mucosa, the possible implications in humans still deserve further deeper investigations.

Flavonoids. Other substances with postulated anti-neoplastic properties naturally present in foods are flavonoids. This group include phytoestrogens, plant compounds with estrogen-like activities. The main varieties are isoflavones such as genistein and daidzein found predominantly in soy products (75), and lignans found in whole grains, vegetables, fruits and flax seed (76).

Epidemiologic reports suggest that phytoestrogens contained in soy are causally related to protection against hormone-dependent cancers, probably by competing with estradiol for estrogen receptors (77) and data from literature

provide evidence of an association between high consumption of phytoestrogen-rich foods and a low incidence of breast and prostate cancer within populations of Asian countries (78). There are also data regarding these substances and colonic neoplastic transformation with a significant reduction in CRC risk (79). The evidence is important, because dietary intake is modifiable. Therefore, identifying dietary phytoestrogens with antitumor activity and investigating their mechanisms of action may lead to significant advances in the prevention of human cancer. Also cell-culture studies and animal experiments have shown that flavonoids are tumor-inhibitory for CRC (80). It has been observed that ODC activity and polyamine concentrations were significantly lower in the mammary epithelium of rats treated with soy protein than in controls (81). Previous studies clearly demonstrated that, although the GI tract is not a classical sex hormonal target, sex steroid hormones can affect cell proliferation and turnover, playing an important role also in the neoplastic transformation in the colon (82,83). This evidence suggests that estrogens as well as phytoestrogens introduced with diet may exert a protective role against CRC also in humans regardless of gender (84).

Among flavonoids, phytoestrogen genistein has drawn attention in recent years due to a variety of biological activities that may account for its cancer-preventive effects. *In vitro* experiments demonstrated that genistein influences proliferation, differentiation and apoptosis in different tumor cell types (85). The anticancer effects of genistein involve inhibition of angiogenesis, topoisomerase, tyrosine kinase activity and antioxidant processes (86).

Genistein possesses noticeable structural similarity with estrogens and effects resembling estrogenicity. This phytoestrogen is able to bind both the estrogen receptors (ERs) α and β. As cited above, both *in vivo* and *in vitro* studies clearly established that estrogens can exert an inhibitory effect on GI cell proliferation by interacting with growth factors, apoptotic processes and polyamine metabolism (82,83). In this connection, it has been demonstrated that genistein inhibited cell proliferation in ER-positive (MCF-7) and ER-negative (MDA-MB-231) human breast carcinoma cell lines. In addition, ODC activity was reduced to 53.8% of the control after 6-h treatment with 50 mM genistein in MCF-7 breast cancer cells (87).

Research by our group investigated the effects on the polyamine biosynthesis and cell growth following administration of increasing concentrations of genistein (from 0.01 μM up to 100 μM) in the DLD-1 human colon cancer cells (88). This cell line has the peculiarity of being ER-positive. Starting from 1 μM, genistein administration reduced significantly ODC activity compared to untreated cells. Regarding the polyamine profile, the administration of increasing concentrations of genistein (namely, from 0.01 to 100 μM) decreased the single and total polyamine contents. Besides, the polyamine content was inversely correlated to genistein concentrations. When DLD-1 cell line was depleted by culture of the estrogenic content and exposed to increasing genistein concentrations, it also showed an evident pro-apoptotic (increase in Bax mRNA expression) and anti-proliferative action. It is conceivable that mechanisms by which genistein affects growth of DLD-1 cells may include either induction of apoptosis or a decrease in cell proliferation rate by involving a lessening in ODC activity and the polyamine levels. Of note, these results were obtained *in vitro* using a wide range of genistein concentrations with values falling within the physiological blood levels in human, further supporting indications for a diet rich in this isoflavone in terms of CRC prevention.

The modulating effects of dietary feeding of two flavonoids, diosmin and hesperidin were investigated in male F344 rats during the initiation and post-initiation phases on colon carcinogenesis initiated with AOM (89). In that study, the incidence and number of neoplasms in the gut of F344 rats together with, or followed by, a diet containing diosmin or hesperidin were significantly smaller than those of rats receiving AOM alone. Besides, administration of diosmin and hesperidin, alone or in combination, was able to significantly inhibit the development of aberrant crypt foci, the ODC activity in colonic mucosa as well as to reduce polyamine levels in the blood. As a consequence, it is conceivable that the significant anticancer properties shown by these flavonoids may be partly ascribed to their antiproliferative effects through the suppression of ODC activity and polyamine biosynthesis. In this framework, the polyamine content in both blood and tissue, may be one of the intermediate biomarkers.

The dietary flavonoid apigenin found in many fruits and vegetables, with parsley, celery and chamomile tea showing the highest amount, has been demonstrated to significantly inhibit at 10 and 30 μM the ODC activity of Caco-2 cells. Besides, ODC activity in the colon mucosa of CF-1 mice was reduced with 0.1% dietary apigenin by 42% compared with the control. Aberrant crypt foci (ACF) formation was also reduced by 50% with 0.1 % dietary apigenin in AOM-induced CF-1 mice (90).

Another study (91) demonstrated that quercetin, found in fruits and vegetables such as citrus fruits, apples, onions, parsley, sage, tea and red wine, can affect proliferation, differentiation and apoptosis of DLD-1 cells by both decreasing polyamine biosynthesis and inducing apoptosis. At concentrations ≥50 μM, quercetin significantly reduced ODC activity, putrescine and spermidine levels compared to controls cells. Higher quercetin concentrations (≥70 μM) caused a significant reduction in the conversion of MTT tetrazolium salt and [^{3}H]-thymidine incorporation. The same concentrations were needed to induce the apoptosis.

The apoptotic effects of apple procyanidins, oligomeric compounds formed from catechin and epicatechin molecules, were also established to involve the inhibition of polyamine catabolism (92). Procyanidins caused an activation of the intrinsic apoptotic pathway through enhanced polyamine catabolism and mitochondrial membrane depolarization. Besides, they caused a profound intracellular depletion of polyamines in SW620 cells. Apple procyanidins diminished the activities of ODC and SAM-DC, key enzymes of polyamine biosynthesis; the latter induced spermidine/spermine N1-acetyltransferase, which, in turn, started polyamines retroconversions. As a consequence of the enzymatic changes, polyamine concentrations diminished, and N(1)-acetyl-polyamines accumulated in SW620 cells. The observation that apple procyanidins enhance polyamine catabolism and reduce polyamine biosynthesis activity similar to known inducers of SSAT, without sharing their toxicity, let the author hypothesized that apple procyanidins could be useful for chemopreventive and therapeutic interventions.

More recently (93), a metabolomic study on the anti-proliferative effect of dietary polyphenols on human colon cancer cells was conducted by using different methodological approaches. CE, RP/UPLC and HILIC/UPLC all coupled to TOF MS were combined to achieve a global metabolomic examination of the effect of dietary polyphenols on HT-29 colon cancer cells. Diverse metabolites, showing different expression after the treatment with polyphenols, were identified in colon cancer cells. Significant alterations in polyamine content along with changes in glutathione metabolism with more reduced glutathione/oxidized glutathione (GSH/GSSG) ratio were observed after the treatment with polyphenols in polyphenols-treated cells. These results from metabolomics further support the chemopreventive effect of the tested dietary polyphenols on colon cancer.

In conclusion, a plethora of plants and plant products contains metabolically active substances with anti-neoplastic properties, thus the possibility to regard flavonoids as representative phytochemical functional foods has made them attractive for *in vivo* studies on cancer risk.

Resveratrol. Resveratrol is a polyphenol classified as a phytoalexin, contained in grapes, wine and peanuts. Resveratrol seems to be, at least in part, responsible for the positive effects of a moderate red wine consumption on the development of cardiovascular diseases (94). Additionally, it has been reported that the resveratrol and its analogues have a potent chemopreventive effect in multiple carcinogenesis model in either *in vivo* or *in vitro* studies (95,96). It is likely that the anticancer and the chemopreventive activities of resveratrol and its analogues could also be explained by the influence on polyamine metabolism. Different evidence was derived from *in vitro* studies. Schneider *et al* (97) reported that Caco-2 colorectal adenocarcinoma cells administered with 25 μmol/l resveratrol accumulated at the S/G2 phase transition of the cell cycle, causing a 70% growth inhibition. Resveratrol produced a significant decrease of ODC activity with a concomitant reduction of the intracellular putrescine and spermidine content. Moreover, 24-h treatment with the resveratrol analogue cis-3,5,4' trimethoxystilbene decreased ODC and SAM-DC activities at a concentration of 0.3 μmol/l associated with a reduction of the putrescine content (98).

In Caco-2 cells, resveratrol was also proved to induce modification of polyamine metabolism (99) by inhibiting ODC activity and mRNA levels. c-Myc protein that controls the ODC promoter diminished by resveratrol treatment, demonstrating that decreased expression of the ODC gene is responsible for the inhibition of ODC activity. SAM-DC was also inhibited when high concentrations (>50 μmol/l) were used. In addition, resveratrol upregulated SSAT activity, inducing polyamine degradation. The SSAT gene is a target for the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) and Ulrich *et al* (100) postulated that p38MAPK and transcription factor PPARγ can be considered as molecular targets of resveratrol in the regulation of cell proliferation and SSAT activity, respectively.

In conclusion, the potential inhibitory effect of resveratrol on polyamine metabolism in carcinoma cells could be mediated by two different pathways: inhibition of polyamine synthesis and increased polyamine catabolism (101).

Green tea and (-)-epigallocatechin-3-gallate (EGCG). Green tea, widely consumed in Far East countries, contains polyphenolic compounds which account for 30% of the dry weight of the leaves. Most of the polyphenols are flavanols, and EGCG is the most abundant and representative for its putative antineoplastic effect. Green tea plays an important role in reducing cancer risk and in delaying cancer outbreak and recurrence (102) and epidemiological studies have revealed that the incidence of stomach and prostate cancers are low among populations that introduce regularly green tea in their diet. Various experimental studies performed *in vivo* and *in vitro* have confirmed the anticancer effects by green tea and/or EGCG (103).

It has been shown that green tea and its active components interfere with signal transduction pathways and mRNA expression in human colon cancer cells (104) and data are available on the possible relationship between green tea and/or EGCG and polyamine metabolism (105). Earlier studies demonstrated that in rodents with skin tumor induced by carcinogens, the application of EGCG was able to prevent the neoplasm onset and contemporary inhibited the ODC expression (106). These results suggest that EGCG may be an effective chemopreventive agent in individuals with early, pre-neoplastic stages of cancer.

The ODC/Ras double transgenic mouse model that develops spontaneous skin tumors due to overexpression of ODC and a v-Ha-ras transgene was used as a model to test the administration of EGCG in the drinking water. EGCG significantly decreased both tumor number and total tumor burden compared with untreated ODC/Ras mice without decreasing the elevated polyamine levels present in the ODC/Ras mice. EGCG selectively decreased both proliferation and survival of primary cultures of ODC overexpressing transgenic keratinocytes, but not keratinocytes from normal littermates nor ras-infected keratinocytes. This decreased survival was due to EGCG-induced apoptosis and not terminal differentiation. Moreover, in skin from EGCG-treated ODC transgenic mice, caspase 3 was detected only in epidermal cells that possess very high levels of ODC protein (107).

Other studies have demonstrated that ECGC inhibited MAPK activity as well as the syntheses of Jun and Fos, in this way acting in a manner similar to DFMO (108). Moreover, it has been observed that feeding mice with green tea polyphenols for 7 days abolished the typical over-production of ODC in prostate cancers, also in this case showing effects similar to those by DFMO (109).

On these bases, it could be hypothesized that both EGCG and DFMO may share some anticancer and chemopreventive activities by inhibiting ODC with the undoubted advantage that, unlike DFMO, green tea and/or EGCG are natural products, which can be consumed at large quantities without any harmful side-effects (106).

5. Influence of dietary polyamines on CRC chemoprevention

In addition to endogenous polyamines, dietary polyamines and their metabolites by intestinal microorganisms have been shown to be major determinants of the total body polyamine pool. The colonic lumen contains polyamines from both the diet or exported by enteric bacteria and these polyamines are transported via mechanisms not yet well described on the apical cell surface (110). As far as diet is concerned, poly-

amine content is high in several food products including fruits, cheeses and meat. Again, high concentrations of putrescine can be found in common diets, particularly in orange and grapefruit juice (111).

The early studies have shown that intestinal and dietary polyamines can enhance colonic tumorigenesis, and can minimize the effects of ODC inhibitors (112). In tumor-bearing animal models, it has been demonstrated that a polyamine deficient diet significantly enhances the antitumoral effect of DFMO plus neomycin (113). Overall, the use of polyamine transport inhibitors, alone or in combination with DFMO, provides a method to target cancers with high polyamine requirements (114).

It has been observed that the primary effect of dietary putrescine was to increase tumor grade; besides the effectiveness of sulindac to suppress intestinal carcinogenesis was partially abrogated by dietary putrescine in a murine FAP model (115).

Polyamine metabolism is also dependent on levels of the precursor amino acids arginine and ornithine. In a study by Yerushalmi *et al* (116), Apc Min/+ mice were fed with arginine concentrations corresponding to the higher range of arginine consumed by humans eating a western style diet. Authors reported that large amounts of arginine in diet increase colonic polyamine levels as well as carcinogenesis. This finding strongly supports the concept that dietary arginine could be a risk factor for colon carcinogenesis also in humans (117,118). In this connection, it has been found that patients with a family history of CRC and reporting meat consumption in the highest quartile had a statistically significantly decreased overall survival and increased risk of death, compared with those in the lower quartiles (119).

Recently, it has also been demonstrated that an association between high dietary polyamine intake and CRA risk in humans can be modified by sex and ODC genotype. Particularly for women, the risk of CRC development showed a positive trend with increasing quartiles of polyamine intake, and participants with higher polyamine intakes and the ODC GG genotype had significantly higher odds of CRC compared with those subjects showing the same genotype but lower polyamine intake (120). Therefore, a diet low in polyamines could represent an adjunctive strategy to therapeutic prevention using polyamine-inhibitory agents.

Raj *et al* (121) have observed a significant interaction between treatment with DFMO plus sulindac and dietary polyamine intake on the risk of recurrent adenomas in a CRA prevention phase III clinical trial. Patients in the highest quartile of dietary polyamine intake have been found to have no significant metachronous adenoma risk reduction after treatment with DFMO plus sulindac, in contrast to a significant 81% risk reduction observed for patients in the lowest quartile of dietary polyamine intake.

Collectively, the above evidence suggests that dietary polyamines may be involved in human colon carcinogenesis; therefore, approaches to limit dietary polyamines may represent additional strategies for CRC prevention. In this context, special diets low in polyamines have already been developed for other type of cancers and it has been observed that the polyamine-lowering regimen is associated with improved pain control and survival characteristics (122). However, due to the abundance of polyamines in the food supply and the strong preclinical data that relate polyamine exposure to tumor growth, these findings still support the need for additional investigation of dietary polyamines in human health.

6. Conclusions

Polyamines and their enzymes are strongly related to neoplastic proliferation in the GI tract. Therefore, all the strategies of effective chemotherapeutic and chemopreventive interventions targeting polyamines, will certainly require a combinatorial approach directed towards all the multiple features shown by their metabolic pathway.

Several agents in diet, thought to be useful in CRC chemoprevention, have been shown to affect the polyamine metabolic pathways. A combined chemopreventive intervention using protocols based on the use of these agents, along with polyamine inhibitors and/or inducers, would enhance their properties representing a suitable alternative option for the management of CRC patients. Moreover, also dietary polyamines may be involved in human colon carcinogenesis, conditioning the effects of drugs and nutritional components. It is, therefore, fundamental to acquire more data on this aspect that could represent an innovative and interesting approach to colorectal oncology. Future studies will determine whether strategies targeting the polyamine pathways will contribute to prevention of colon cancer.

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