Nutritional factors and polyamine metabolism in colorectal cancer

Michele Linsalata, Sc.D.*, and Francesco Russo, M.D.

Laboratory of Experimental Biochemistry, National Institute for Digestive Diseases, I.R.C.C.S. Saverio de Bellis, Castellana Grotte (BA), Italy

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Abstract

Colorectal cancers are a significant cause of mortality in Western societies. The progression of the disease from normal epithelium to the acquisition of the malignant phenotype is accompanied by several biochemical and genetic alterations. Compelling experimental and epidemiologic evidences indicate that diet and nutrition are key factors in modulating colon cancer onset and progression. Therefore, identifying dietary constituents with antitumor activity and investigating their mechanisms of action may lead to significant advances in the prevention of these neoplasms. Moreover, it seems that the potential protection against colorectal cancers of some nutritional factors could be associated with modifications in cellular proliferation and growth. The naturally occurring polyamines, spermine, spermidine, and putrescine, play a key role in hyperproliferation and cell migration and are involved in almost all steps of colorectal tumorgenesis. Mucosal polyamine levels, as a measurement of dysregulated hyperproliferation, have been demonstrated to be significantly associated with cancer risk and have been considered even a specific marker for neoplastic proliferation. Consequently, polyamine metabolism can be considered an attractive target for cancer chemoprevention and chemotherapy. This review summarizes the findings on the possible mechanisms of action of some nutritional components such as flavonoids, polyphenols, and probiotics in colorectal cancers, focusing attention on polyamine metabolism as a possible target. Acquiring more data on this aspect could represent an innovative and interesting approach for new therapeutic and chemopreventive strategies in the management of patients with colorectal neoplasms. © 2008 Elsevier Inc. All rights reserved.

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Introduction

Cancer is one of the main causes of death in Western countries. Cancer is not a single disease but an accumulation of several events, genetic and epigenetic, arising in a single cell over a long interval. Among factors that contribute to its appearance, diet has a fundamental role, particularly in relation to the increase in the incidence of breast cancer, prostate cancer, and colorectal cancer (CRC). It has been estimated that 30–40% of all kinds of cancer can be prevented by living a healthy lifestyle, including regular physical activity, avoiding obesity, and eating primarily a plant-based diet [1].

With respect to CRC, this tumor can strike men and women of all ages, but occurs most frequently in older adults. The number of CRC cases begins to increase in people starting at around age 40 y and peaks after age 70 y. A family history of cancer or previous CRC or adenomatous polyps increases the risk, as does the age at which the risk begins. Other bowel conditions, such as ulcerative colitis and Crohn’s disease, increase the likelihood of CRC. Additionally, a diet high in calories and fat and high intake of red and processed meat may increase CRC risk [2]. This hypothesis is also supported by the fact that CRC is not nearly as common in Asia and in other parts of the world where diets tend to be low in animal fat and high in fiber, although race appears to have no influence [3]. Despite these suggestions, there is currently no persuasive evidence from randomized clinical trials to assert that increased dietary fiber intake will reduce the incidence or recurrence of neoplastic conditions [4]. Notwithstanding, the gastrointes-
tinal tract works in a constant link with the external environment, and modifications in the dietary intake might modify the exposition to different carcinogenic factors. Thus, identifying dietary components with antitumor activity and investigating their mechanisms of action may lead to significant advances in CRC prevention.

Among substances naturally found in foods, evidence suggests that dietary flavonoids may have anticancer properties. In the group of flavonoids, phytoestrogens are plant compounds with estrogen-like activities. There are two principal varieties of phytoestrogens, namely isoflavones and lignans. Isoflavones genistein and daidzein are found predominantly in soy products [5], and lignans are found in the fiber from whole grains, berries, fruits, vegetables, and flax seed [6].

Epidemiologic studies have suggested that dietary phytoestrogens are causally related to protection against hormone-dependent cancers (e.g., breast), probably by competing with estradiol for estrogen receptors [7]. Other evidences connect these substances to CRC risk [8,9]. Although the gastrointestinal tract cannot be considered a classic sex hormonal target, previous studies have clearly demonstrated that sex steroid hormones can affect cell proliferation and turnover, playing an important role also in the colonic neoplastic transformation [10–12]. These evidences suggest that estrogen and, conceivably, nutritional phytoestrogens may exert a protective role against CRC in humans, regardless of sex [13].

In vivo and in vitro studies have reported that the plant polyphenol resveratrol and its analogs show a potent chemoprotective effect in multiple carcinogenesis models [14,15]. Resveratrol-containing foods include grapes, wine, and peanuts. It has been proposed that resveratrol is at least in part responsible for the beneficial effects of moderate red wine consumption on the development of cardiovascular diseases [16]. Additionally, the inhibitory potency of resveratrol in various stages of tumor development has attracted much attention for its proposed ability to suppress proliferation of a wide variety of tumor cells, including cancers of the stomach and colon. Resveratrol has been shown to suppress the activation of several transcription factors (e.g., nuclear factor-κB [NF-κB]), to inhibit protein kinases (e.g., mitogen-activated protein kinase), and to down-regulate products of genes such as cyclo-oxygenase-2 or vascular endothelial growth factor, interleukin-1, and interleukin-6. Resveratrol has also been shown to strengthen the apoptotic effects of cytokines, chemotherapeutic agents, and γ-radiation [17].

Other substances with intriguing potentialities in modulating cell proliferation are certain lactic acid–producing enterobacterial food supplements, the so-called probiotics. Probiotics have been claimed to prevent chronic non-malignant diseases and cancer of the gastrointestinal tract [18,19]. Usually, they are ingested with yogurt, fermented milks, or other fermented foods. The mechanisms by which probiotics exert their effects are largely unknown but may involve modification of gut pH, antagonism of pathogens through production of antimicrobial compounds, competition for pathogen-binding and receptor sites and for available nutrients and growth factors, stimulation of immunomodulatory cells, and production of lactate [20].

Generally, nutritional factors that prevent colon carcinogenesis are also growth inhibitors and inducers of apoptosis [21]. Because the patterns and rates of mucosal cell proliferation have been suggested as reliable measurements of CRC risk, the modulation of cellular proliferation by agents known to prevent cancer formation might serve as an intermediate endpoint in cancer prevention trials.

On these bases, this review summarizes data on possible mechanisms of action of the above-mentioned nutritional components in CRC, focusing attention on polyamine metabolism as possible target. This can represent an interesting matter of study because polyamines are organic cations with multiple functions in cell proliferation and differentiation, derived from amino acids, and found in all organisms. These polycationic compounds also play a key role in almost all steps of colorectal tumorigenesis [22].

**Polyamines and CRCs**

Putrescine, spermidine, and spermine are the main polyamines found in prokaryotes and eukaryotes [23]. Clues to polyamine functions can be seen from their chemical structure. These molecules are positively charged at the primary and secondary amino groups at physiologic pH. Thus polyamines may act as ligands at multiple sites on DNA, RNA, proteins, phospholipids, and nucleotide triphosphates [24]. Biological functions of polyamines are mainly in the regulation of gene expression by altering DNA structure and by modulating signal transduction pathways [25,26]. For optimal functioning of the cell, intracellular polyamine content needs to be strictly controlled and this occurs at the levels of biosynthesis, catabolism, uptake, and efflux. The polyamine functions and their metabolic pathway have been extensively studied [27–30]. Figure 1 shows a schematic representation of the polyamine metabolic pathway.

Among the several biochemical alterations in cancer cells, one of the most consistent is the change in the intracellular polyamine content. As with other tumors, CRC shows increased polyamine content compared with the adjacent mucosa and with equivalent normal tissue [11,24,31]. The increase is due to the loss in polyamine homeostasis occurring during the dysregulation of cell proliferation [32–34]. This is also proven by evidences of an upregulation of polyamine biosynthesis [35,36], a decrease in their catabolism [37–39], and an increased uptake [40].

A possible role for polyamines in regulating oncogene expression and function, through transcriptional and post-transcriptional processes, has also been suggested [41]. Recent studies have shown that polyamine biosynthesis is involved in human colorectal carcinogenesis in a manner
that is largely K-ras dependent and p-53 independent [42] and that K-ras mutations and polyamine biosynthesis are preferentially associated with polypoid tumors rather than flat colorectal tumors of the colon [43].

From a clinical point of view, polyamine levels seem to have little diagnostic significance, but they can represent a reliable biomarker of neoplastic growth [44]. In this connection, it has been observed that tissue spermine levels represent a significant prognostic factor for disease recurrence in patients with CRC [45]. In addition, the detection of circulating or urinary polyamines can assume some clinical importance during follow-up of patients with cancer for the assessment of chemotherapy and the detection of remission and relapse [46–49].

Pharmacologic targeting of polyamine metabolism for treatment of CRC may include different ways of intervention. First, this can be performed by using polyamine biosynthesis inhibitors. Difluoromethylornithine (DFMO), a suicide inhibitor that permanently inactivates ornithine decarboxylase (ODC), has been shown to significantly inhibit the growth of different types of tumor cells, although clinical trials have failed to confirm this in vitro finding [50]. The failure of DFMO monotherapy in humans is probably due to a compensatory increase in polyamine uptake induced by polyamine depletion. This could be overcome by removing the major exogenous polyamine sources (e.g., by decontamination of the gut with antibiotics and/or provision of a low-polyamine diet) [51,52].

Second, an alternative approach to inhibition of enzymes in the polyamine biosynthetic pathway may hypothesize the use of polyamine analogs. These substances show three lines of attack on polyamine content: 1) competition for uptake, 2) inhibition of biosynthesis, and 3) stimulation of breakdown, making them potentially more efficient in the depletion of intracellular polyamine content. Interested readers are referred to a recent review by Wallace and Niiranen [53].

Unlike analogs, whose application in chemoprevention needs further studies, inhibitors such as DFMO has been used with some success as cancer chemopreventatives. Notwithstanding, early clinical cancer therapeutic trials with high doses (>3 g · m⁻² · d⁻¹) of DFMO were disappointing because several side effects occurred [54]. Subsequently, results of a randomized placebo-controlled phase IIb trial in patients with adenomatous colon polyps indicated that an oral dose of 0.2 g/m² of DFMO per day was effective in reducing colorectal polyamine contents and safe [55]. Additionally, experimental studies have shown that DFMO acts, at least additively, with a number of non-steroidal anti-inflammatory drugs that have been shown a protective effect against CRC [56,57]. It has been demonstrated that non-steroidal anti-inflammatory drugs such as sulindac can lead to an increase in spermidine/spermine N1-acetyltransferase (SSAT) enzyme transcription by activating the nuclear peroxisome proliferator-activated receptor-γ [58]. Others, such as aspirin, seem to induce SSAT through activation of NF-κB and NF-κB response elements in the region of the SSAT gene [59]. Thus, chemopreventive intervention using a combination of DFMO and an agent that induces catabolism such as a non-steroidal anti-inflammatory drug should be able to stop the disease from progressing beyond the adenoma phase. This rationale has provided the basis for a phase IIb/III clinical trial based on a combination of DFMO (500 mg/d) and sulin-
dac (150 mg/d) administered to decrease the rate of recurrence of adenomatous polyps in the colon. The study is based on the administration of these drugs for 36 mo and it will be completed by the end of 2008 [60].

Aside from specific inhibitor/inducer substances for the polyamine pathway, several nutritional components thought to be useful in CRC chemoprevention have been shown to affect the polyamine metabolic pathway and to impair tissue polyamine content in gastrointestinal neoplastic tissue.

**Effects of nutritional components on polyamine metabolism in CRC**

**Flavonoids**

Experimental studies have indicated the presence of CRC-protective agents in fruits and vegetables; prominent on this list are flavonoids [61]. The modulating effects of dietary feeding of two flavonoids, diosmin and hesperidin, during the initiation and postinitiation phases on colon carcinogenesis have been investigated in male F344 rats [62]. In this study, the incidence and multiplicity of neoplasms in the large intestine of rats initiated by azoxymethane (AOM) together with, or followed by, a diet containing diosmin or hesperidin were significantly lower than in rats given AOM alone. In addition, feeding of diosmin and hesperidin, alone and in combination, significantly inhibited the development of aberrant crypt foci, colonic mucosal ODC activity, and polyamine levels in the blood. Hence, it is possible that the significant anticancer properties of these compounds may be partly due to their antiproliferative effects through the suppression of ODC activity and polyamine biosynthesis on carcinogen-exposed crypts.

It has been shown that apigenin, a common dietary flavonoid abundantly present in fruits and vegetables, at 10- and 30-μM concentrations, significantly inhibited the ODC activity of Caco-2 cells by 26% and 57%, respectively [63]. In the same study, colonic ODC activity in CF-1 mice was reduced by almost 54% compared with control cells. Interestingly, 6-h treatment with 50 μM of genistein reduced ODC activity by 42% compared with control cells.

In vitro experiments have demonstrated that genistein influences proliferation, differentiation, and apoptosis in a variety of intestinal epithelial cells [67].

Several possible mechanisms for the anticancer effects of genistein have been proposed, including inhibition of angiogenesis, topoisomerase, tyrosine kinase activity, and antioxidant properties [68]. Moreover, 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 [69].

Genistein is structurally similar to estrogens and produces effects suggestive for estrogenicity, being capable of binding to the two subtypes of estrogen receptors (ERs), ER-α and ER-β. In this connection, in vivo and in vitro studies have shown that estrogens can exert an inhibitory effect on gastrointestinal cell proliferation by interacting with growth factors, apoptotic processes, and polyamine metabolism [10,11]. Shon et al. [70] reported that genistein inhibits cell proliferation in ER-positive (MCF-7) and ER-negative (MDA-MB-231) human breast carcinoma cell lines. Interestingly, 6-h treatment with 50 μM of genistein reduced ODC activity by almost 54% compared with control cells.

**Table 1**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Total polyamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.06 ± 0.02a</td>
<td>0.06 ± 0.02a</td>
<td>6.70 ± 2.05a</td>
<td>9.51 ± 2.91a</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>0.04 ± 0.01bc</td>
<td>1.70 ± 0.32bc</td>
<td>5.30 ± 1.43bc</td>
<td>7.04 ± 1.73bc</td>
</tr>
<tr>
<td>10 μM</td>
<td>0.03 ± 0.01bc</td>
<td>1.54 ± 0.44bc</td>
<td>5.50 ± 0.56bc</td>
<td>7.07 ± 0.92bc</td>
</tr>
<tr>
<td>20 μM</td>
<td>0.03 ± 0.005b</td>
<td>1.25 ± 0.27b</td>
<td>5.47 ± 1.05b</td>
<td>6.74 ± 1.2ab</td>
</tr>
<tr>
<td>30 μM</td>
<td>0.03 ± 0.005b</td>
<td>1.14 ± 0.079b</td>
<td>5.30 ± 0.86b</td>
<td>6.46 ± 0.85ab</td>
</tr>
<tr>
<td>100 μM</td>
<td>0.03 ± 0.005b</td>
<td>1.26 ± 0.14b</td>
<td>4.40 ± 1.12b</td>
<td>5.69 ± 1.2b</td>
</tr>
</tbody>
</table>

*For each concentration, mean values not sharing a common superscript (a, b, c, d) differ significantly (P < 0.05, Wilcoxon Mann-Whitney test). All data represent the mean ± SD of four consecutive experiments. Polyamines are expressed as nanomoles per milligram of protein.*
Starting from 1 DLD-1 human colon cancer cell line have been investigated. Biosynthesis and cell growth processes in the ER-positive diseases[16]. Additionally, the inhibitory potency of resveratrol consumption on the development of cardiovascular diseases suggests that a diet rich in this isoflavone can be potentially effective in reducing ODC activity and polyamine levels. These properties may represent another antiproliferative mechanism by genistein in the protection against CRC.

More than 300 plants and plant products contain phytoestrogens, and it has been hypothesized that they may have an effect in the modification of cancer risk by diet; thus, the possibility to regard genistein as a representative phytochemical functional food has made it attractive for in vivo studies on cancer risk. Actually, the results obtained in vitro with a wide range of genistein concentrations and with values decreasing within the human physiologic blood levels strongly suggest that a diet rich in this isoflavone can be potentially effective in a chemopreventive strategy against CRC.

Resveratrol

Resveratrol is a polyphenol contained in grapes, wine, and peanuts. It has been suggested that resveratrol is at least in part responsible for the beneficial effects of moderate red wine consumption on the development of cardiovascular diseases [16]. Additionally, the inhibitory potency of resveratrol in various stages of tumor development has attracted much attention [15]. In vitro and in vivo studies have shown that resveratrol and its analogs exhibit potent chemopreventive efficacy in multiple carcinogenesis models [72,73]. The anticancer and chemopreventive activities of resveratrol and its analogs may also be explained by the possible positive influence on polyamine metabolism. Schneider et al. [74] demonstrated that treatment of Caco-2 colorectal adenocarcinoma cells with 25 μmol/L of resveratrol causes a 70% cell growth inhibition. The cells accumulate at the S/G2-phase transition of the cell cycle. Moreover, resveratrol causes a significant decrease in ODC activity with the reduction of the intracellular putrescine and spermidine contents.

In an animal model of human familial adenomatous polyposis, 24-h treatment with the resveratrol analog, cis-3,5,4’-trimethoxystilbene, decreased ODC and S-adenosylmethionine decarboxylase activities at a concentration of 0.3 μmol/L with a concomitant reduction of putrescine levels [75]. These evidences suggest that polyamines can represent one of several targets involved in the antiproliferative effects of these substances.

Previously, it has been proved that resveratrol inhibition of ODC activity in Caco-2 cells can be attributable to attenuated ODC protein and mRNA levels. Moreover, c-Myc protein that controls the ODC promoter decreases after resveratrol treatment, demonstrating that decreased expression of the ODC gene can be responsible for the inhibition of ODC activity. S-adenosylmethionine decarboxylase is also inhibited when high resveratrol concentrations (≥50 μmol/L) are used. In addition, resveratrol potently upregulates SSAT activity, inducing polyamine degradation [76]. The induction of SSAT can also produce an efficient system to generate locally high concentrations of hydrogen peroxide that may play a critical role in the signaling pathway, ultimately leading to cell death.

The SSAT gene is a target for the transcription factor peroxisome proliferator-activated receptor-γ. In a cell culture model of colon cancer, Ulrich et al. [77] observed that the p38 mitogen-activated protein kinase and the transcription factor peroxisome proliferator-activated receptor-γ can be considered molecular targets of resveratrol in the regulation of cell proliferation and SSAT activity, respectively. As regards the latter, results obtained from studies with novel polyamine analogs have suggested that induction of SSAT can represent a promising approach to chemoprevention [78].

In summary, the inhibitory effect of resveratrol on polyamine metabolism in carcinoma cells may presumably be mediated through two different pathways: inhibition of polyamine synthesis and increased polyamine catabolism [79].

Probiotics

Primary prevention, early detection, and secondary prevention have been suggested as the most promising ap-
proaches for reducing CRC morbidity and mortality [80]. In this framework, the beneficial effects of certain lactic acid–producing enterobacterial food supplements, the so-called probiotics, are assuming increasing importance in a preventive strategy for chronic non-malignant diseases and cancer of the gastrointestinal tract [18,19].

These lactic cultures, which are primarily used for fermentation of milk and other dairy products, have been shown to possess antimutagenic and anticancinogenic properties. Data from epidemiologic and experimental studies have indicated that ingestion of certain lactic cultures, such as lactobacilli and bifidobacteria, or their fermented dairy products reduce the risk of certain types of cancer and inhibit tumor growth [81,82].

The precise mechanisms by which these lactic cultures exert their antitumorigenic influence are not fully elucidated [83]. Some studies have emphasized a relation between polyamine biosynthesis and probiotic action in carcinogenesis and tumor growth. Evidence has been provided that *Lactobacillus brevis* strain induces apoptosis of Jurkat cells and it has been hypothesized that the apoptotic death–inducing ability of these bacterial samples could be associated with polyamine synthesis [84]. Moreover, a close relation between lactobacilli and polyamine metabolism has been observed in the preneoplastic condition and cancer of the stomach [85,86].

As regards CRC, it has been observed that dietary administration of *Bifidobacterium longum* cultures causes a significant suppression of colon tumor incidence and tumor multiplicity and a reduction of tumor size in AOM-treated rats. In the same animals, this probiotic strain also inhibited cell proliferation rate, ODC activity, and the expression of total and mutated ras–p21. Thus, it is likely that changes in these intermediate biomarkers that are associated with colon tumorigenesis would also be involved in colon tumor inhibition exerted by lactic cultures [87].

In addition, the ability of probiotics to affect cell proliferation and polyamine metabolism has further been supported by a recent study [88] where it has been observed that 4-wk administration of high doses of a cocktail of different bacterial strains (VSL#3) induced a significant decrease in colonic levels of polyamines, ODC activity, and Ki-67 compared with controls. Paradoxically, this could be considered an undesirable effect, especially in a mucosa with an intense cellular turnover such as that in the colon, but it supports once more the need for deeper investigations on the metabolic changes induced by these bacteria in the colonic environment.

Notwithstanding, all these results strengthen the supposition of an antiproliferative effect of probiotics on the gastrointestinal mucosa by affecting polyamine biosynthesis. The link among cell proliferation, polyamines, and probiotics could be regulated by different factors such as 1) the peculiar metabolic features of the administered probiotic strains, 2) different survival times in the lumen, 3) the period of administration, and 4) the proliferative behavior of different segments of gastrointestinal mucosa. On these bases, and in view of the potential offered by probiotics in affecting the proliferative activity of the gastrointestinal mucosa, the possible implications in human surely deserve further investigations.

**Conclusion**

Polyamines and their metabolizing enzymes are tightly linked to neoplastic proliferation in the gastrointestinal tract. Consequently, polyamine deprivation, modulations of the polyamine metabolic pathway, and impairment of polyamine uptake into neoplastic cells can be a logical way of CRC chemoprevention and chemotherapy.

Polyamine pool sizes and flux are regulated by a number of processes in a cell- and tissue-specific manner. In this context, strategies of effective chemotherapeutic and chemopreventive interventions targeting polyamines will necessarily require a combinatorial approach toward 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 also the polyamine metabolic pathway in cancer cells. The available evidence suggests that nutritional components such as flavonoids, resveratrol, or probiotics can actually modulate the polyamine content in intestinal cancer cells, thus affecting their proliferation.

A combined chemopreventive and therapeutic intervention using protocols based on the use of these agents and polyamine inhibitors and/or analogs would enhance their properties, representing a suitable alternative option for the management of patients with cancers of the digestive tract. It is therefore fundamental to acquire more data on this aspect that could represent an innovative and interesting approach to gastrointestinal oncology.

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