Polyamines and colon cancer

V. Milovic1 and L. Turchanowa
2nd Department of Medicine and Department of General Pharmacology, J.W. Goethe University, Theodor Stern Kai 7, D-60590 Frankfurt, Germany

Abstract
In colon cancer, the activities of polyamine-synthesizing enzymes and polyamine content are increased 3–4-fold over that found in the equivalent normal colonic mucosa, and polyamines have even been attributed as markers of neoplastic proliferation in the colon. Furthermore, and in contrast with all other cell systems in the body, normal and neoplastic cells in the colon are exposed to high concentrations of putrescine from the lumen, synthesized by colonic microflora. While such a high polyamine supply may be of benefit in non-neoplastic colonic mucosal growth, the role of luminal polyamines in colon cancer is a clear concern. Luminal polyamines are readily taken up by neoplastic colonocytes, they are utilized in full to support neoplastic growth, and their uptake is strongly up-regulated by the mitogens known to play an important role in colonic carcinogenesis. Inhibition of polyamine synthesis and their uptake, impaired utilization of exogenous polyamines, and enhanced catabolism of polyamines in neoplastic colonocytes are therefore logical approaches in the chemoprevention of colorectal cancer.

Introduction
Colorectal cancer is the fourth most common form of cancer worldwide. It affects men and women almost equally, with about 400 000 new cases in men and 380 000 in women annually. Almost 400 000 deaths from colorectal cancer still occur worldwide every year; in spite of improved early detection and chemotherapy, colon cancer is the second most common cause of death from neoplasia in men in the European Union [1].

In colorectal cancer tissue, the activities of ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (‘AdoMetDC’) and polyamine content are increased 3–4-fold over that found in the equivalent normal colonic tissue, and polyamines have even been attributed as markers of neoplastic proliferation in the colon [2–7]. Furthermore and in contrast to all other cell systems in the body, colon cancer cells are exposed to high concentrations of putrescine from the lumen; the faecal putrescine concentration is well within the millimolar range, while spermidine and spermine concentrations are approx. 50–60 µM [8]. Luminal polyamines in the colon originate from colonic microflora: bacterial species normally present in the human colon are able to synthesize large amounts of polyamines [9–12]. It is not only polyamine synthesis, but also their uptake that is enhanced in rapidly proliferating neoplastic cells in the colon. Polyamine uptake is additionally up-regulated by mitogens such as epidermal growth factor [13] and tumour promoters such as deoxycholate [14]. Once in the cell, exogenous putrescine is rapidly converted into spermidine and spermine; rapidly proliferating colon cancer cells are able to utilize the entire available polyamine pool [15].

Polyamines and colorectal tumorigenesis
Colorectal cancers, both sporadic or hereditary, are caused by a defined sequence of molecular events. There are at least two different pathogenetic pathways in colorectal tumorigenesis: the chromosomal instability pathway and the microsatellite instability pathway; the two major inherited syndromes, familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (‘HNPCC’), being the examples of these two mechanisms [16,17]. These different pathways, however, converge on common pathological entities that have crucial functions in the regulation of normal homoeostasis in colonic crypts (Figure 1).

Colorectal tumours are a result of the mutational activation of oncogenes, coupled with the inactivation of tumour-suppressor genes, when the latter changes predominate. On the other hand, the steady state of cell growth in normal colonic epithelium is maintained by a dynamic balance between continuous cell renewal in the crypts, migration along the crypt–villus axis, and shedding of cells at the villus tip. Transformed colonic epithelial cells do not repress DNA synthesis during migration along the crypt–villus axis; they then develop additional abnormal properties, enabling them to be retained in the mucosa and to develop into various types of adenomatous lesion [18]. Mutations in at least four to five genes are necessary for the formation of a malignant tumour; fewer changes result in benign tumorigenesis [19].

Polyamines are involved in almost all steps of colonic tumorigenesis, their roles in hyperproliferation [20–22] and cell migration [23] being investigated and reviewed in detail. There is also recent evidence suggesting that ODC modifies signalling dependent on the expression of the adenomatous...
polyamines and colorectal tumorigenesis

(A) Adenoma-carcinoma sequence, adapted from [17] and [22]. Changes in polyamine-metabolizing enzymes and polyamine content occur during both the early and late stages of colonic tumorigenesis: while ODC activity and polyamine synthesis are enhanced whenever hyperproliferation occurs, down-regulation of the polyamine-catabolizing enzymes spermidine/spermine-N1-acetyltransferase (SSAT) and polyamine oxidase (PAO) is likely to occur during the late stages. APC, adenomatous polyposis coli; COX, cyclo-oxygenase. (B) Polyamines are involved in many steps of epithelial cell migration along the crypt-villus axis, both in the small intestine and in the colon (reviewed in [23]).

Polyamines as markers of neoplastic growth, and targets in colon cancer chemoprevention

The polyamine metabolic pathway was found to be influenced and polyamine content in colon cancer cells was impaired by several agents thought to be useful in colon cancer chemoprevention. Although clinical studies with these potentially chemopreventive agents are still missing, the experimental data resulting from studies done in colon cancer cells in culture suggest that resveratrol (a natural component of grapes and wine), geraniol (a component of plant essential oils), and flavanols and procyanidins (compounds present in cocoa and chocolate), *in vitro*, inhibit growth and deplete polyamine content in colon cancer cells [25–27]. These studies suggest that a number of potential chemopreventive agents down-regulate ODC expression/activity and subsequently deplete polyamine content in colon cancer cells, but that they also activate cellular catabolism of polyamines and thereby enhance their efflux from the cell [26]. Although the effect of potential chemopreventive agents is likely to represent a secondary phenomenon and is not to be due to any specific action on the polyamine metabolic pathway, such studies suggest that polyamines and their metabolizing enzymes could be, above all, reliable intermediate markers of neoplastic proliferation in the colon, and clinically relevant parameters of follow-up in colon cancer chemoprevention trials.

Specific inhibition of polyamine-synthesizing enzymes, and ODC in particular, has been long considered as a desired goal in chemoprevention and chemotherapy for hyperproliferative diseases, including cancer. Recent chemoprevention trials indicate that α-difluoromethylornithine (DFMO), a suicide ODC inhibitor, can be given over long periods of time at low doses that suppress polyamine contents in gastrointestinal and other epithelial tissues but cause no
detectable side effects [28]. Current trials are investigating the efficacy of DFMO to suppress surrogate end-point biomarkers (e.g. colon polyp recurrence) in patients with an elevated risk of the development of specific epithelial cancers, mainly those of gastrointestinal origin, such as colon, oesophagus and stomach [29]. Polyamine levels in rectal mucosa were continuously suppressed by daily oral doses of 0.2 g/m² DFMO that had few or no side effects [30]. Another possibility, to combine DFMO with non-steroidal anti-inflammatory drugs (agents of proven benefit in colon cancer chemoprevention) is at present under clinical testing; preliminary results showed little or no side effects when the dosage recommended in humans was used [31].

The studies evaluating DFMO and non-steroidal anti-inflammatory drugs, such as celecoxib and sulindac, as chemopreventive agents in patients with sporadic colonic cancers, mainly those of gastrointestinal origin, such as colon, oesophagus and stomach [29]. Polyamine levels in rectal mucosa were continuously suppressed by daily oral doses of 0.2 g/m² DFMO that had few or no side effects [30]. Another possibility, to combine DFMO with non-steroidal anti-inflammatory drugs (agents of proven benefit in colon cancer chemoprevention) is at present under clinical testing; preliminary results showed little or no side effects when the dosage recommended in humans was used [31].

The studies evaluating DFMO and non-steroidal anti-inflammatory drugs, such as celecoxib and sulindac, as chemopreventive agents in patients with sporadic colonic polyyps and Barrett’s oesophagus are carried out at present in different centres [32]. The results of these trials are eagerly awaited, and will be most helpful in estimating a true value of the concept that depletion of colonic mucosal polyamines can prevent the development of colorectal cancer.

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References

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