Impact of dietary amino acids and polyamines on intestinal carcinogenesis and chemoprevention in mouse models

E.W. Gerner

Department of Cell Biology and Anatomy, The University of Arizona, Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ 85724, U.S.A.

Abstract

Colon cancer in humans is influenced by both genetic and dietary risk factors. The majority of colon cancers have somatic mutations in the APC (adenomatous polyposis coli) tumour-suppressor gene. Dietary arginine enhances the risk of APC-dependent colon carcinogenesis in mouse models by a mechanism involving NOS2 (nitric oxide synthase 2), as elimination of NOS2 alleles suppresses this phenotype. DFMO (difluoromethylornithine), a specific inhibitor of polyamine synthesis, also inhibits dietary arginine-induced colon carcinogenesis in C57BL/6J-Apc<sup>Min</sup>/J mice. The primary consequence of dietary arginine is to increase the adenoma grade in these mice. Either loss of NOS2 alleles or inhibition of polyamine synthesis suppresses the arginine-induced increase in adenoma grade. In addition to promoting intestinal carcinogenesis, polyamines can also reduce the efficacy of certain intestinal cancer chemopreventive agents. The NSAID (non-steroidal anti-inflammatory drug) sulindac is a potent inhibitor of intestinal carcinogenesis in the C57BL/6J-Apc<sup>Min</sup>/J mouse model and is used to treat humans with FAP (familial adenomatous polyposis). Dietary putrescine reduces the ability of sulindac to suppress intestinal tumorigenesis in the mouse model. These data suggest that reducing polyamine metabolism and dietary polyamine levels may enhance strategies for colon cancer chemoprevention.

Introduction

The rationale for interest in the ubiquitous polyamines in intestinal and colon carcinogenesis stems from experimental evidence in human cells and genetically altered mouse models indicating that the gene encoding ODC (ornithine decarboxylase), the first enzyme in polyamine synthesis, is regulated by the product of the APC (adenomatous polyposis coli) tumour-suppressor gene [1,2]. APC is a component of the Wnt signalling pathway, which is crucial for normal intestinal development, and is mutated in FAP (familial adenomatous polyposis), an inherited syndrome associated with increased risk of colon cancer in humans [3]. APC acts to regulate ODC gene expression via a mechanism involving the c-Myc oncogene protein product, which is reviewed elsewhere [4,5], as depicted in Figure 1. The majority of non-FAP colon cancers contain somatic mutations in the APC gene [6], indicating the relevance of this pathway in human colon carcinogenesis. Details of this pathway are further supported by evidence indicating that either conditional knock-out of the c-myc gene in intestinal epithelial cells [7], or treatment with a specific ODC inhibitor [1], reduces intestinal tumorigenesis in the Apc<sup>Min</sup>/+ mouse model of FAP. As indicated in Figure 1, tissue polyamines are derived from metabolism via ODC and transport from extracellular sources. Polyamine metabolism is dependent on levels of the precursor amino acids arginine and ornithine, while transport in polarized colonic epithelial cells is dependent on two potentially unique sources of polyamines. The colonic lumen contains polyamines found in the diet or exported by enteric bacteria, and these polyamines are transported via mechanisms not yet well described on the apical surface [8,9]. Polyamines circulating in the blood can also find their way into colonic epithelial cells via basolateral transporters, which are also not well described to date. Arginine transport into colonic epithelial cells occurs presumably via basolateral transporters, as the major flux of this amino acid is from the intestines, in the fed state, or muscles to the liver and kidney, during starvation [10]. This mini-review focuses on recent studies implicating colonic polyamines in colon carcinogenesis induced by dietary arginine or dietary sources of the polyamines themselves.

Dietary arginine and colon carcinogenesis

Arginine is catabolized by arginase for production of ornithine, which serves as a substrate for polyamines. Arginine is also a substrate for NOS (nitric oxide synthase), resulting in NO production. These relationships are depicted in Figure 2. We have evaluated the role of dietary arginine and the NOS2 gene in intestinal and colon carcinogenesis in the Apc<sup>Min</sup>/+ mouse model. We chose a range of dietary arginine levels for our studies in mice that corresponded to human diets ranging from low to high in arginine amounts. We
The APC tumour-suppressor gene plays a central role in Wnt signalling, in part by regulating the degradation of β-catenin (β-CAT). Wnt signals influence the stability of a protein complex containing β-catenin, conductin and GSK3 (glycogen synthase kinase 3). In the absence of Wnt or the presence of wild-type APC protein, β-catenin is degraded by the 26 S proteasome. In the presence of Wnt, or the absence of APC (as occurs in many colon cancers), β-catenin target genes including c-myc are expressed. Myc expression, in turn, leads to the expression of ODC, the first enzyme in polyamine metabolism. ODC decarboxylates ornithine, which is derived from arginine. Polyamine levels in colonic epithelial cells are also influenced by apical and basolateral polyamine transporters. TCF-4, T-cell factor 4.

Dietary polyamines and colon carcinogenesis
Several studies have shown that intestinal and dietary polyamines can influence tumorigenesis at distant sites [14,15], and in some cases, minimize the effects of ODC inhibitors [16]. Dietary polyamines have been found to enhance intestinal and colonic tumorigenesis [17]. Recent studies from our group indicate that the primary effect of dietary putrescine is to increase tumour grade [18]. Our mouse studies used dietary putrescine levels (1% in the drinking water) that are equivalent to humans drinking 1–2 cups per day of grapefruit juice [19]. Attenuation of tumour growth by depletion of tissue polyamines may require inhibition of both polyamine synthesis, via ODC, and polyamine uptake. One group has already reported evidence supporting this hypothesis [20].

Influence of dietary polyamines on responses to colon cancer preventive agents
The NSAID (non-steroidal anti-inflammatory drug) sulindac displays chemopreventive activity in patients with FAP [21].

found that dietary arginine enhanced colon tumour incidence and grade in the ApcMin/+ model. Loss of NOS2 alleles blocked the arginine-dependent increases in colon tumorigenesis in this model [11]. Treatment of ApcMin/+ mice with DFMO (difluoromethylornithine) suppressed the arginine-dependent increase in both colon tumour burden and grade [12]. These data provide evidence that dietary arginine is a luminal risk factor for colon carcinogenesis in the ApcMin/+ mouse model, and that NOS2 and ODC mediate this risk. It is useful here to note that inhibition of ODC enzyme activity with the inhibitor DFMO had little effect on colon tumorigenesis in ApcMin/+ mice fed diets not supplemented with arginine [1,13]. Thus DFMO may affect colon tumorigenesis in this model only when colon tumorigenesis is induced, as in the case of supplemental arginine. Arginine increases not only the number of colonic tumours in these mice, but also the tumour grade as assessed by both size and morphological parameters. The major effect of DFMO is to reduce the grade of arginine-induced colon tumours. We speculate that arginine-induced colon tumorigenesis might represent a risk factor for human colon tumorigenesis, and that DFMO may exert its anti-colon carcinogenic effects on high grade adenomas. However, both these points remain to be established.
Figure 2 | Metabolic pathways involving arginine and colon tumorigenesis

Tissue arginine derives from either dietary sources or metabolism as described in the text. Arginine is converted into ornithine, via the urea cycle enzyme arginase (ARG). Ornithine can be decarboxylated by ODC to form the diamine putrescine. Putrescine is the substrate for the longer-chain amines spermidine and spermine, which can be acetylated by SSAT and exported. DFMO is a specific inhibitor of ODC, while several NSAIDs have been shown to activate SSAT. Arginine is also metabolized by NOSs, including NOS2. Both NOS2 and ODC appear to be pro-tumorigenic factors in mouse models of colon carcinogenesis.

Sulindac metabolites induce apoptosis in colon tumour cells, in part, by a polyamine-dependent mechanism which can be suppressed with exogenous putrescine [22]. To determine the relevance of this mechanism in animals, we treated ApcMin/+ mice, a model of human FAP, with sulindac alone or in combination with dietary putrescine (1% in the drinking water). Sulindac increased steady-state RNA levels and enzymatic combination with dietary putrescine (1% in the drinking water). Sulindac increased steady-state RNA levels and enzymatic activity of the polyamine catabolic enzyme SSAT (spermidine/spermine N\textsuperscript{1}-acyltransferase), and intestinal levels of monoacetylspermidine, spermidine and spermine, in the small intestine of mice [18]. Dietary putrescine increased intestinal putrescine contents while the combination of dietary putrescine and sulindac yielded the highest levels of intestinal putrescine. Sulindac reduced intestinal tumour number by nearly 90%. The effectiveness of sulindac to suppress intestinal carcinogenesis was partially abrogated by dietary putrescine. These data suggest that sulindac exerts at least some of its anti-carcinogenic effects in mice via a polyamine-dependent mechanism. While these results were reassuring, in that they supported our interpretation that sulindac was acting in part by a mechanism involving polyamines, they raise another concern. High concentrations of putrescine can be found in common diets. For example, mice consuming 1% putrescine in drinking water take in a similar amount of putrescine as a human consuming 1–2 cups of grapefruit juice per day [19]. Sulindac is prescribed for a number of clinical indications thought to involve inflammation, it may be advantageous to restrict dietary putrescine consumption in patients undergoing treatment with this NSAID. It is unknown whether this effect of putrescine is limited to sulindac or if dietary putrescine might adversely affect the efficacy of other NSAIDs. We have found that several NSAIDs, including sulindac, as discussed earlier, aspirin [23,24] and celecoxib [25] influence polyamine metabolism in part by cyclo-oxygenase-independent mechanisms inducing SSAT. If these NSAIDs act to suppress tissue polyamine contents, then sufficient dietary putrescine would be expected to reduce the efficacy of their actions related to polyamine-dependent processes. On the other hand, effects such as those mediated by cyclo-oxygenases may be unaffected. Alternatively, dietary polyamines could be affecting metabolism of NSAIDs. There is evidence that putrescine can decrease the activity of cytochrome P450 3A4 levels in a rat liver model [26]. This cytochrome has been implicated in the metabolism of certain NSAIDs under specific conditions [27].

Relevance of animal studies to humans

The dibasic amino acid arginine is a conditionally essential amino acid known to be involved in several important physiological functions, but its role in cancer is poorly understood [28]. In our mouse studies discussed above, we found that dietary arginine could enhance colon tumorigenesis in the ApcMin/+ mouse model of FAP. We fed our mice arginine concentrations ranging from 1 to 4 g of arginine per kg of body weight per day [11]. These concentrations correspond to the higher range of arginine consumed by humans eating a Western style diet [29], when corrected for the approx. 7.5-fold higher metabolic rate in mice, compared with humans [30]. Consequently, dietary arginine could be a risk factor for colon carcinogenesis in humans.

In order to test the potential significance of our mouse studies, which have suggested important roles for dietary arginine in colon carcinogenesis, we evaluated self-reported meat consumption (as a surrogate for arginine intake) in a group of patients from a gene-environment study [25]. We found that patients with a family history of colorectal cancer 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 [25]. These results suggest that overall meat consumption may have a significant role in human colon carcinogenesis. Since meat is the major source of dietary arginine, together with our animal studies, these findings suggest that dietary arginine may be involved in human colon carcinogenesis.

Our observations that dietary putrescine also enhanced colon tumorigenesis in ApcMin/+ mice, especially in terms of tumour grade, and that dietary putrescine can suppress the chemopreventive action of sulindac, may also be relevant to humans. Putrescine is found in high amounts in orange and grapefruit juice [19], and consequently can be consumed by humans in levels approaching those used in our mouse studies [18].

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Approaches to limit dietary arginine and dietary polyamines may be useful strategies for colon cancer prevention. Arginine and polyamines derived from either dietary or intestinal luminal sources, such as enteric bacteria, may promote colon tumorigenesis in adult humans, while the polyamines may act to reduce the efficacy of colon cancer prevention strategies such as NSAID treatment. Others have already developed special diets low in polyamines for cancer patients [31]. In the future, our group will be prospectively assessing the roles of dietary arginine and putrescine contents in human cancer chemoprevention trials to determine the relevance of these factors in human colon cancer prevention and treatment.

References

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