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Cancer pharmacoprevention: Targeting polyamine metabolism to manage risk factors for colon cancer

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Eugene W. Gerner[‡§1], **Elizabeth Bruckheimer**[‡], **and Alfred Cohen**[‡]

From [‡]*Cancer Prevention Pharmaceuticals, Tucson, Arizona 85718 and the* [§]*Department of Cell and Molecular Medicine, University of Arizona, Tucson, Arizona 85711*

Edited by Eric R. Fearon

Cancer is a set of diseases characterized by uncontrolled cell growth. In certain cancers of the gastrointestinal tract, the adenomatous polyposis coli (APC) tumor suppressor gene is altered in either germline or somatic cells and causes formation of risk factors, such as benign colonic or intestinal neoplasia, which can progress to invasive cancer. APC is a key component of the WNT pathway, contributing to normal GI tract development, and APC alteration results in dysregulation of the pathway for production of polyamines, which are ubiquitous cations essential for cell growth. Studies with mice have identified nonsteroidal anti-inflammatory drugs (NSAIDs) and difluoromethylornithine (DFMO), an inhibitor of polyamine synthesis, as potent inhibitors of colon carcinogenesis. Moreover, gene expression profiling has uncovered that NSAIDs activate polyamine catabolism and export. Several DFMO–NSAID combination strategies are effective and safe methods for reducing risk factors in clinical trials with patients having genetic or sporadic risk of colon cancer. These strategies affect cancer stem cells, inflammation, immune surveillance, and the microbiome. Pharmacotherapies consisting of drug combinations targeting the polyamine pathway provide a complementary approach to surgery and cytotoxic cancer treatments for treating patients with cancer risk factors. In this Minireview, we discuss the role of polyamines in colon cancer and highlight the mechanisms of select pharmacoprevention agents to delay or prevent carcinogenesis in humans.

Background

Polyamines were described as early as the late 17th century with their discovery credited to Van Leeuwenhoek as discussed in Ref. 1. Their importance as targets for cancer treatment has only become apparent since the 1960s, as highlighted in the timeline shown in Fig. 1. In several seminal papers, Dykstra and Herbst (2) and Raina *et al.* (3) reported strong associations between concentrations of specific polyamines and tissue growth in rodents. Russell and Snyder (4) extended these original findings to other species and tumor models. In addition, they demonstrated that the activity of ornithine decarboxylase 1 (ODC1) was rapidly induced by growth stimuli. They also found that the enzyme had an exceedingly short half-life (~10 min), suggesting that ODC1 was under strict regulatory control. By the mid-1970s, O'Brien *et al.* (5) showed that a variety of tumor promoters of different classes had similar abilities to induce both ODC1 enzyme activity and skin tumor formation. These reports were all important original findings but did not provide evidence of cause–effect relationships between polyamines and growth.

Herbert Tabor, for whom this issue of minireviews is dedicated on the anniversary of his 100th birth year, and colleagues at the National Institutes of Health used genetic methods to address the question of causality. They established that polyamines were not essential for growth of bacteria in general (6, 7), but the lack of enzymes to produce polyamines did compromise bacterial growth under conditions that suggested polyamines were acting by a mechanism affecting protein translation (8). In collaboration with his wife Celia, Herbert Tabor showed that polyamines were essential for growth in specific strains of yeast (9, 10).

In this same time frame, Metcalf *et al.* (11) at the Merrell Research Institute in Strasbourg, France (known by its French name Centre de Recherche Merrell International-CRMI), reported the synthesis of difluoromethylornithine (DFMO),[2] a highly targeted drug whose mechanism involved enzyme activation and irreversible inhibition of ODC1, in 1978. Scientists at CRMI quickly reported the growth inhibitory and anti-tumor effects of DFMO and other ODC1 inhibitors (12–14). These early investigations found that the profound growth inhibitory effects of DFMO were not accompanied by cytotoxicity. Slaga and co-workers (15) used DFMO to show that inhibition of polyamine synthesis could inhibit skin carcinogenesis in mouse models and that the effect of the drug was on specific features of tumor promotion. Kingsnorth *et al.* (16) were the first group to

This article is part of a series on "Polyamines," written in honor of Dr. Herbert Tabor's 100th birthday. E. W. G. is a co-founder, board member, and Chief Scientific Officer (CSO) of Cancer Prevention Pharmaceuticals (CPP), Inc. E. B. is Vice President for Drug Development, and A. C. is Chief Medical Officer at Cancer Prevention Pharmaceuticals.

[1] To whom correspondence should be addressed: Professor Emeritus (retired), Cancer Prevention Pharmaceuticals, 1760 E. River Rd., Tucson, AZ 85716. Tel.: 520-908-7774; E-mail: egerner@canprevent.com.

[2] The abbreviations used are: DFMO, difluoromethylornithine; APC, adenomatous polyposis coli; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug; FAP, familial adenomatous polyposis; IEN, intraepithelial neoplasia; CRC, colorectal cancer; CRA, colorectal adenoma; OAZ, ornithine decarboxylase inhibitory protein antizyme; TGFβ, transforming growth factor β; PPARγ, peroxisomal proliferator-activated receptor γ; PTI, polyamine transport inhibitor; ESC, embryonic stem cell; MTAP, 5′-methylthioadenosine phosphorylase; NET, neutrophil extracellular trap; ODC, ornithine decarboxylase.

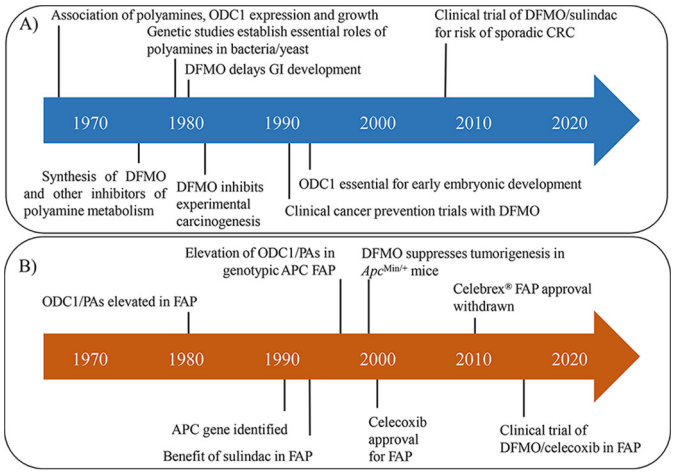

Figure 1. Timeline of research findings linking the polyamine pathway to cancer development. *A,* key observations and accomplishments (discussed in text) that established the polyamine pathway as an integral aspect of carcinogenesis and a target for treating cancer risk factors. *B,* specific findings and clinical trials in patients with FAP that supported the rationale for developing a combination drug product consisting of DFMO and an NSAID.

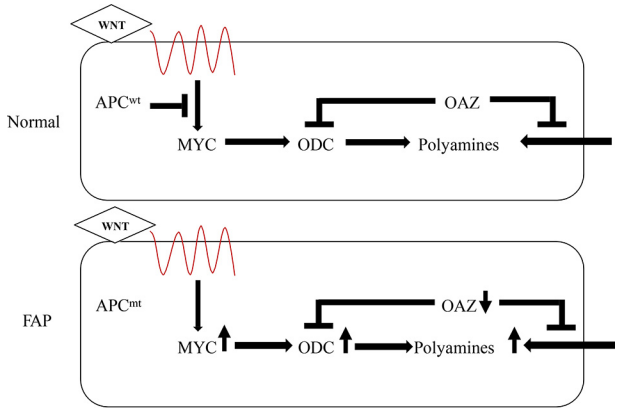

Figure 2. Role of the APC tumor suppressor gene in signaling expression of genes regulating the polyamine pathway. *Top* (*Normal*), in individuals with normal APC (APC^{WT}), the canonical WNT pathway controls MYC expression (in part regulated by APC) and MYC target genes, including ODC and other polyamine metabolic genes. *Bottom* (*FAP*), in genotypic patients with FAP, ODC activity and polyamine contents in apparently normal mucosa are elevated, compared with nongenotypic family members. In the $Apc^{Min/+}$ mouse model, mutant APC is associated with an increase in ODC, a decrease in antizyme (OAZ) RNA, and a consequent increase in intestinal tumor and normal tissue polyamines. OAZ interacts with ODC 3A2 to initiate ODC degradation and inhibits polyamine transport.

show DFMO inhibited colon carcinogenesis in a rodent model of colon carcinogenesis, the dimethylhydrazine-treated rat.

Clinical trials of high-dose intravenous DFMO as a therapy for advanced cancers were conducted in the 1980s and were generally negative from both a safety and efficacy perspective (17). Clinical trials in patients with conditions other than cancer were also conducted, and these trials led to regulatory approval of high-dose DFMO administered intravenously for treatment of patients with a form of African Sleeping Sickness in 1990 (18) and a topical form of DFMO for female hirsutism in 2000 (19). Achieving regulatory approval of an oral dosage form of DFMO has never been accomplished for any medical indication and is a current major challenge for development of this and related drugs to treat cancer risk factors.

A major motivation for the work described in this Minireview was identification of risk factors for leading causes of disease and death in the United States and the understanding that some of these factors are associated with the risk of cancer (20). Many common risk factors, such as diet, tobacco use, high body-mass index, air pollution, and low physical activity, are not amenable to interventions with pharmacotherapies. However, some cancer-specific risk factors can be managed by pharmacotherapies to reduce risk of disease and mortality analogous to targeting high cholesterol in patients with a risk of cardiovascular disease using pharmacoprevention strategies (*e.g.* statins) (21). Cancer-specific risk factors that could be targets for pharmacoprevention strategies include intraepithelial neoplasia (IEN) (22). Colorectal adenomas (CRA) are an example of an IEN risk factor for colorectal cancer (CRC). Failure to remove CRAs is associated with an increase in CRC in humans (23). More recent studies indicate that screening for CRAs, which is associated with the removal of large/advanced CRAs, is strongly associated with a reduction in mortality (24). A significant challenge in the field of oncology is to determine whether managing cancer risk factors, such as CRAs, with pharmacotherapies can prevent or delay cancer and reduce cancer disease burden and deaths.

The major goal of the work summarized in this Minireview was to understand the mechanistic basis of pharmacoprevention agents and determine whether intervening in the polyamine pathway could be used successfully to delay/prevent carcinogenesis in humans.

Polyamines as mediators of colon carcinogenesis

Following the earlier observations of the association of polyamines and growth, Luk *et al.* (25) took advantage of the new inhibitor of this pathway, DFMO, to address the importance of polyamines in gut development in rodent models. They reported that DFMO could delay gut development in fetal rats and recovery from chemotherapy-induced gut injury in adult rats (25). This finding led Luk *et al.* (25) to ask whether the expression of ODC1 and polyamines, which appeared to be important in normal gut mucosal development, was altered in the apparently normal gut mucosa of patients with familial adenomatous polyposis (FAP), a genetic syndrome associated with near 100% risk of development of colon cancer. They discovered that both ODC1 and polyamines are elevated in the apparently normal colonic mucosa of FAP patients and appeared to identify genotypic individuals (26). Following the identification of the *APC* gene in humans (27), the elevation of ODC1 enzyme activity and polyamine contents in apparently normal colonic mucosa of genotypic FAP patients was established (28).

Fig. 2 depicts the signaling of ODC1 and the polyamine pathway in patients with FAP and in normal individuals. This depiction is based on studies in humans and mouse models. Multiple intestinal neoplasia in the $Apc^{Min/+}$ mouse model of FAP is caused by a mutation in the murine homolog of the human *APC* gene (29). Expression of *ODC1* and other genes in the polyamine pathway, including the gene encoding the ornithine decarboxylase inhibitory protein antizyme (OAZ), are influenced by the mutant APC-encoding gene in the mouse model (30).

ODC1 RNA levels were increased in both intestinal and colonic mucosa, whereas *OAZ* RNA levels are decreased especially in the intestinal mucosa of these mice.

Mechanistic studies in human cells support the pathway depiction shown in Fig. 2. He *et al.* (31) showed that c-MYC is transcriptionally activated by the APC signaling pathway, and ODC1 was known to be a transcriptional target of c-MYC (32). Conditional expression of WT APC in human colon cancer cells containing mutant APC demonstrated that WT APC was restrictive for ODC1 expression (33). This suppression depended on the presence of canonical MYC-binding sites in the ODC1 promoter. Tissue-specific knockdown of c-MYC in $Apc^{Min/+}$ mice established that MYC was involved in APC-dependent intestinal and colonic carcinogenesis (34) and that treatment of $Apc^{Min/+}$ mice with DFMO reduced both intestinal and colonic carcinogenesis (30, 35).

Polyamines may be involved in colon carcinogenesis due to disruption in pathways other than the APC/MYC pathway. Green and Hudson (36) have reviewed the roles of several signaling pathways implicated in the development of colon cancers. KRAS-dependent tumorigenesis is inhibited by DFMO in human Caco-2 xenografts (37), and colon carcinogenesis in transforming growth factor β (TGFβ)–deficient mice is associated with changes in the polyamine and other metabolic pathways in the gut microbiome of these mice (38). The role of the microbiome will be discussed further in a subsequent section of this Minireview.

Drug combinations targeting the polyamine pathway to inhibit carcinogenesis

DFMO was an effective but incomplete inhibitor of experimental carcinogenesis in the $Apc^{Min/+}$ mouse (30) and other models (39). Sporn (40) was an early advocate for using combinations of agents as a means to increase efficacy and reduce toxicity of treatments to suppress carcinogenesis. The nonsteroidal anti-inflammatory drug (NSAID) sulindac reduced colonic and rectal polyps in patients with FAP in a statistically significant but incomplete manner in a randomized placebo-controlled trial (41). The efficacy of combinations of DFMO with other NSAIDs, including paroxysm (42), indomethacin (43), and aspirin (44), was reported for several models of experimental carcinogenesis.

The precise mechanism by which NSAIDs inhibit carcinogenesis remains elusive. Whereas NSAIDs are generally considered to work via their effects on cyclooxygenases and prostaglandin metabolism, noncyclooxygenase mechanisms have been reported (45). Indomethacin suppresses the tumor-promoter induction of ODC1 in experimental skin carcinogenesis (46). To understand potential mechanisms of action of sulindac, patterns of gene expression resulting from treatment with sulindac sulfone, a sulindac metabolite lacking cyclooxygenase inhibitory activity, were measured in human colon tumor-derived cells (47). Sulindac sulfone inhibited cell growth and induced apoptosis and the expression of the spermidine/spermine *N*-acetyltransferase (SAT1), a polyamine catabolic gene product implicated in polyamine export (48). The sulindac sulfone induction of *SAT1* gene expression was shown to occur via the cyclooxygenase-independent transcriptional activation

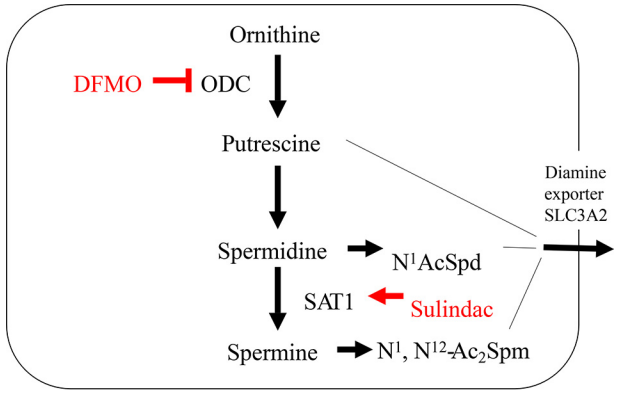

Figure 3. DFMO and sulindac reduce polyamines via dual mechanisms of action. DFMO is an enzyme-activated irreversible inhibitor of ODC. As discussed in the text, DFMO has been reported to have some activity against arginase. Sulindac and other NSAIDs can activate SAT1 by specific transcriptional mechanisms. The diamine putrescine and the SAT1 products N^1-acetylspermidine and N^1,N^{12}-diacetylspermine are substrates for polyamine export mediated by the solute carrier transporter (SLC3A2). Thus, DFMO acts with sulindac and other NSAIDS in complementary ways to inhibit polyamine biosynthesis (DFMO) and activate polyamine export (NSAIDS).

of *SAT1* by a peroxisomal proliferator-activated receptor γ (PPARγ)–dependent mechanism acting at a specific PPARγ-responsive element in the *SAT1* gene. Treatment of cells with sulindac sulfone induces *SAT1* and stimulates polyamine export. Other NSAIDs also induce *SAT1*, and presumably polyamine export, but by unique mechanisms (49).

These results led us to hypothesize that combinations of DFMO and NSAIDs, such as sulindac, might be working via complementary mechanisms (depicted in Fig. 3) to suppress dysregulated and high levels of polyamines in neoplasia by inhibiting both polyamine synthesis and stimulating polyamine catabolism and export (50). Experimental studies in the $Apc^{Min/+}$ mouse supported this hypothesis (DFMO and sulindac combination in cancer chemoprevention; United States patent no. 6,258,845, 2001) (51). A clinical trial of DFMO and sulindac in patients with sporadic risk of colorectal cancer showed dramatic efficacy to reduce both metachronous colorectal adenomas (52) and rectal mucosal polyamine but not prostaglandin E2 contents (53). Results from an international clinical trial of DFMO and the NSAID celecoxib in patients with FAP, and based on earlier preclinical data in the $Apc^{Min/+}$ mouse (51), were reported in 2016 (54). This clinical trial found that the combined DFMO-NSAID treatment had a highly statistically significant 80% reduction on global measures of tumor burden from baseline, compared with the 30% reduction from baseline for the NSAID alone. This trial reported a difference in total polyp number from baseline in the two groups, but the difference was not statistically significant. A trial of DFMO combined with sulindac in patients with FAP is in progress and uses a composite primary end point closely related to global tumor burden (55).

Other drug combinations with DFMO are being investigated in a number of preclinical cancer models. Several groups have synthesized polyamine transport inhibitors (PTIs) (56–58) that suppress polyamine uptake as depicted in Fig. 2. PTIs appear to work best in concert with a polyamine synthesis inhibitor, like DFMO, to act as polyamine-blocking therapy (56, 59–61). PTIs

are expected to begin clinical trial evaluations in the near future.

Relevance of polyamines to the extracolonic sequelae of APC mutations

The high penetrance of *APC* mutations in FAP results in nearly 100% of patients developing colon and rectal cancer if they retain their colons and rectums. Consequently, the standard of care for these patients in 2018 is colectomy with either proctectomy or rectum-preserving surgery, followed by close monitoring of any retained colonic/rectal tissue. Nonetheless, these patients still develop several types of extracolonic sequelae, including intestinal polyposis, especially in the duodenum, and other neoplasia, including desmoid tumors.

$Apc^{Min/+}$ mice express increased levels of ODC1 RNA and polyamines in intestinal tissues, compared with normal littermates. Administration of DFMO alone is effective in suppressing carcinogenesis in the small intestines in these mice (30). Combinations of DFMO and NSAIDS are potent inhibitors of carcinogenesis in both the large and small intestines in this model of FAP (51, 62). Isobologram analysis of drug–drug interactions in human colon cancer-derived cells in culture indicates that DFMO and sulindac, or its metabolites, interact in at least an additive manner (63), supporting the conclusion depicted in Fig. 3 that the two agents are acting by complementary mechanisms. The grade of intestinal polyps is polyamine-dependent, and the anti-intestinal carcinogenic effects of sulindac in $Apc^{Min/+}$ mice can be rescued by dietary putrescine (64). Together, these findings support a role for polyamines in intestinal carcinogenesis in $Apc^{Min/+}$ mice and the rationale for combination DFMO and sulindac in therapy for this end point. A current clinical trial evaluating the DFMO sulindac combination for control of intestinal polyposis is in progress (55)

$Apc^{Min/+}$ mice crossed with $p53^{-/-}$ mice show enhanced formation of desmoid tumors, which are another example of the extracolonic sequelae of loss of normal APC in germline. The combination of DFMO and the NSAID piroxicam exerted a moderate effect on development of desmoids in this model. This combination reduced formation of desmoids by nearly 50% (62), which was significant but less substantial than the effect this same combination exerted on intestinal carcinogenesis. These experimental findings suggest that the mechanisms depicted in Figs. 2 and 3 are likely operational in the colon, small intestine, fibroblasts (the source of desmoid tumors), and other tissues. A major unanswered question is whether DFMO alone or in combination with NSAIDs or other agents can be used effectively to manage desmoids in a clinically significant manner.

Mechanisms of cancer pharmacoprevention using drugs targeting the polyamine pathway

DFMO is a targeted therapy acting in a very selective manner to irreversibly inhibit a single enzyme (ODC1). There are limited data suggesting DFMO may have a modest effect on arginase (65). Sulindac is a much less selective drug, acting by both cyclooxygenase-dependent and -independent mechanisms. The anti-growth effects of DFMO are reversed by adding an extracellular source of polyamines, which can replete cellular pools via the transport processes depicted in Fig. 2. The anti-intestinal carcinogenic effects of sulindac can also be reversed by providing $Apc^{Min/+}$ mice with putrescine in the drinking water (64), supporting the conclusion that sulindac suppresses tumor formation in these mice by a polyamine-dependent mechanism.

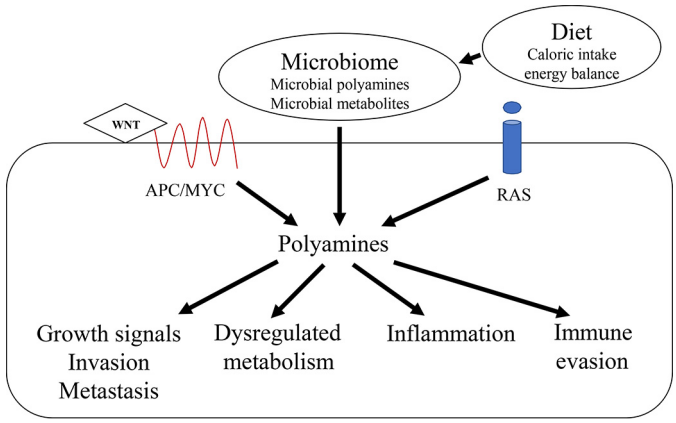

Figure 4. Role of polyamines in Hallmarks of Cancer. Polyamine metabolism is signaled by a number of pathways, including the APC-dependent mechanisms, as in FAP, dietary factors acting via RAS, and other pathways and the microbiome. Dysregulation of polyamine metabolism occurs when features of these pathways are altered (*e.g.* mutations in APC and RAS). The polyamines exert effects on a range of cell phenotypes, including Hallmarks of Cancer such as growth signals, invasion and metastasis, broad aspects of cell metabolism, inflammation, and immune responses.

Although DFMO and NSAIDs appear to affect the levels of cell and tissue polyamines by influencing the activity and/or expression of specific polyamine metabolic proteins, the mechanisms by which polyamines affect carcinogenesis are more complicated. Evidence supporting some of these mechanisms are further discussed in terms of the Hallmarks of Cancer, as proposed by Hanahan and Weinberg (66, 67) and summarized in Fig. 4.

Roles of polyamines in growth signaling, self-renewal, invasion, and metastasis

The original Hallmarks of Cancer proposed six capabilities that were general features of cancer. As seen in Figs. 2 and 4, unregulated expression of genes like MYC or RAS lead to the simple analogy of the "accelerator pedal stuck on," where the polyamines are the accelerant. Mutation/deletion of genes like APC is loss of a "brake" on growth mediated by the polyamines.

Additionally, the polyamine metabolic genes *ODC1* and adenosylmethionine decarboxylase (*AMD1*) have been implicated in the self-renewal of embryonic stem cells (ESCs) (68). AMD1 has been shown to be essential for differentiation of ESCs to neural precursor cells (69). Forced expression of either *ODC1* or *AMD1* is able to maintain patterns of ESC gene expression in the absence of inducers. Studies have not yet demonstrated a role for polyamines or polyamine metabolic genes in specific cancer stem cells. MYC is known to control the balance of self-renewal and differentiation in hematopoietic stem cells (70). Knockdown of c-MYC in the intestinal tract reduces intestinal carcinogenesis due to mutant APC (34), but intestinal homeostasis appears to occur in a MYC-independent manner (71). It remains an unanswered question whether

known MYC transcriptional targets like the polyamine metabolic genes play any role in putative intestinal or colonic cancer stem cells.

Polyamines mediate other cancer hallmarks, including invasion (72) and metastasis (73). Dietary putrescine increases tissue polyamine contents and intestinal tumor grade when administered to $Apc^{Min/+}$ mice (64). DFMO inhibits cell motility and migration and suppresses tumor-forming ability in colon tumor cells expressing an activated KRAS (37).

The distinction between "regulated" and "dysregulated" is an important nuance in interpreting the consequences of the polyamine pathway on cell/tissue phenotypes. The polyamines are associated with optimal growth in single and multicellular organisms, but their regulation is equally important. The polyamines are critical to good health in humans, and some diseases may be associated with age-related down-regulation of these molecules (74). In a different context, loss of regulation (or dysregulation) of this pathway, as depicted in Fig. 2 for patients with FAP, leads to severe pathological consequences, which are largely reversible by agents targeting the polyamine pathway in preclinical models (30, 51) and in initial clinical trials in humans (54). The distinction has significant consequences for possible therapeutic interventions. In the case of dysregulated metabolism, such as occurs in cancer, therapies will aim to reduce abnormally high polyamine levels. In the case of polyamine deficiencies associated with regulated metabolism, therapies will aim to increase abnormally low polyamines.

Roles of polyamines in deregulated metabolism

Deregulated cancer metabolism was added to the list of Hallmarks of Cancer by Hanahan and Weinberg in 2011 (67). Cancer metabolism has many facets (75), and polyamines appear to participate in several of these processes. Activation of SAT1 and polyamine acetylation suppresses tumor growth in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model of prostate carcinogenesis (76), modulates polyamine metabolic flux, and leads to broad metabolic consequences in cell and rodent models (77, 78). Polyamine pools can affect the production of 5′-methylthioadenosine, via the *S*-adenosylmethionine salvage enzyme 5′-methylthioadenosine phosphorylase (MTAP) (79). MTAP has been implicated in several cancers, both as a tumor promoter (79) and suppressor (80). Inhibitors of MTAP suppress growth and metastasis of at least one model of human lung cancer (81).

Another facet of altered metabolism involving the polyamines is the LIN28/*let-7* pathway, which is thought to play a key role in the regulation of self-renewal of both normal and cancer stem cells. LIN28 controls developmental timing in *Caenorhabditis elegans* (82) and glucose metabolism in mice (83). It cooperates with the WNT signaling pathway to promote invasive intestinal and colonic carcinogenesis (84). An unbiased screen of noncoding RNAs discovered that DFMO increased the steady-state levels of a number of microRNAs, including *let-7*, in human colon cancer–derived cells (85). The mechanism of this increase was due to DFMO-dependent decreases in cellular putrescine and spermidine pools and depletion of cellular amounts of hypusine-modified eukaryotic translation initiation factor 5A (eIF-5A). The hypusine modification uses spermidine as a substrate (see Minireview by Park and Wolff for details (108)). Genetic knockdown experiments indicated that eIF-5A was associated with expression of LIN28 protein, and depletion of eIF-5A isoforms was associated with decreases in LIN28 and increases in *let-7* RNA. In neuroblastoma, *let-7* is regulated by multiple mechanisms, including the MYC family member MYCN and LIN28. An unanswered question in this field is as follows: can low levels of *let-7* RNA be increased using a small molecule like DFMO, or other drugs directed at the polyamine pathway, in clinically significant settings in humans?

Role of polyamines in inflammation

Inflammation is a process that may be essential for tumorigenesis (86). The polyamines have been implicated in inflammation in cancer in several ways. One mechanism involves polyamine oxidation with the generation of aldehydes and reactive oxygen species (ROS) (87–89). This topic is dealt with extensively in the Minireview by Casero and co-workers (109).

Ornithine, putrescine, and the polyamines are downstream products of arginine metabolism, which has been widely implicated in cancer and other inflammation-associated diseases (90). Arginases convert arginine to ornithine, and specific arginases have been implicated in *H. pylori*–associated gastric carcinogenesis by affecting host polyamine metabolism (91). DFMO inhibits arginine- and NOS2-dependent carcinogenesis in the $Apc^{Min/+}$ mouse model of FAP (35).

A novel mechanism of polyamine action in inflammation involves the formation of neutrophil extracellular traps (NETs), which have been implicated in tumor cell migration and metastasis (92). NETs are composed of extracellular DNA, extruded by an active process, that coats pancreatic and other tumor cells. The tumor cells also produce and secrete the chemokine CXCL8. NETs capture, as a consequence of CXCL8, circulating neutrophils that also produce this chemokine and matrix metalloproteinases such as stromelysin. Together, these factors promote tumor invasion and metastasis. Polyamines are involved in the extrusion of DNA. Elevated polyamine levels lead to the formation of nuclear aggregates of polyamines, which are subsequently exported (93).

Treatment with the DNase of pancreatic cancer cells producing NETs suppresses measures of cell migration and motility. Similarly, treatment of mice injected with xenografts of pancreatic cancer cells producing NETs reduces the growth of the xenografts (92). It remains to be determined whether treatment of tumor xenografts in mouse models, which is inhibited by DFMO (37), does so by reducing the formation of NETs.

Roles of polyamines in anti-tumor immune responses

Polyamines are elevated in tumors, and inhibitors of polyamine metabolism exert their anti-tumor effects by acting on both tumor cells (intrinsic cancer cell mechanisms) and the tumor microenvironment (extrinsic mechanisms) (94). DFMO alone or in combination with the statin rosuvastatin reduced polyamines and enhanced natural killer cell activity associated with cancer prevention in the azoxymethane-induced model of colon carcinogenesis (95). Combination therapy with DFMO and a polyamine transport inhibitor reduced tumor cell polyamines and relieved immunosuppression in the microenviron-

ment allowing activation of anti-tumor T-cells to reduce tumor growth in another mouse model of colon cancer (60). Polyamines have been broadly implicated in the function of normal immune cells (96). The effects of inhibitors of polyamine metabolism on tumor immunity and immune suppression may involve both cancer cell intrinsic and extrinsic mechanisms, as immune evasion may be tumor-induced in certain circumstances (97)

Role of the polyamines on the impact of diet and the microbiome on carcinogenesis

Microorganisms in the human GI tract (referred to here as the microbiome) are well established to impact colon carcinogenesis, and its role in other cancer types and diseases continues to be investigated (98). Of note, the microbiome is a rich source of exogenous polyamines and can contribute to the overall polyamine content in an organism in normal and disease settings. Through the microbiome and host sources, the GI is a rich source of polyamines to support tumor growth (99). For instance, the role of the microbiome in colon carcinogenesis has been shown in germ-free mice that fail to develop colon cancer in a TGFβ-deficient mouse model that is predisposed for cancer (100). Metabolomic analyses have identified increased levels of N^1,N^{12}-Ac_2Spm in both cancer and normal host tissues of patients with bacterial biofilms (101). Treatment of patients with antibiotics indicates that levels of N^1,N^{12}-Ac_2Spm are contributed by both the host and bacterial biofilm. Biofilms containing tumorigenic bacteria appear to affect colonic neoplasia at an early stage of carcinogenesis, based on studies of apparently normal colonic mucosa from patients with FAP (102). Urinary levels of N^1,N^{12}-Ac_2Spm are highly statistically and significantly associated with occurrence of colon and breast cancer in humans (103).

Polyamines present in certain foods in the human diet have been estimated (104), and these estimates have been associated with risk of colon carcinogenesis in humans (105) and resistance to the DFMO-sulindac therapy for reducing metachronous colorectal adenomas in human clinical trials (106). It is unlikely, however, that polyamines in the diet act as sources for host tissue polyamine pools. Rather, the diet provides a source of a number of elements (*e.g.* energy, red meat) that impact the host via both direct metabolic mechanisms and indirect mechanisms mediated by the microbiome. The impact of diet on host polyamine metabolism, assessed by measuring urinary polyamine levels, indicated that these other factors, rather than the estimated dietary polyamine content, were more closely associated with urinary polyamine contents (107).

Conclusions

The polyamine pathway is an important contributor to both normal growth and development and specific pathologies, including neoplasia. Dysregulation of the polyamine pathway, as a consequence of tumor suppressor gene inactivation (*e.g.* APC in colon carcinogenesis) or oncogene activation (*e.g.* MYC in colon carcinogenesis or neuroblastoma), is causatively associated with carcinogenesis. Mechanisms of this association include the role of polyamines in normal and cancer stem cells, inflammation, immunity/immune evasion, the microbiome, and diet and affect both tumor and host. The relative importance of each of these mechanisms linking dysregulation of the polyamine pathway and carcinogenesis remains to be established in specific types of cancer. Clinical evidence indicates that such therapies can successfully reduce risk factors for certain cancers (*e.g.* colonic and intestinal polyposis for sporadic or genetic forms of colon cancer). A major challenge for the future is to determine whether these therapies can also reduce those specific cancers and decrease cancer-related deaths.

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