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Polyamines and cancer: Implications for chemoprevention and chemotherapy

Shannon L. Nowotarski[1], **Patrick M. Woster**[2], and **Robert A. Casero Jr.**[1,*]

[1]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland 21287

[2]Department of Drug Discovery and Biomedical Sciences The Medical University of South Carolina Charleston, South Carolina 29425

Abstract

Polyamines are small organic cations that are essential for normal cell growth and development in eukaryotes. Under normal physiological conditions, intracellular polyamine concentrations are tightly regulated through a dynamic network of biosynthetic and catabolic enzymes and a poorly characterized transport system. This precise regulation ensures that the intracellular concentration of polyamines is maintained within strictly controlled limits. It has frequently been observed that the metabolism of, and the requirement for, polyamines in tumours is frequently dysregulated. Elevated levels of polyamines have been associated with breast, colon, lung, prostate, and skin cancers, and altered levels of the rate limiting enzymes in both biosynthesis and catabolism have been observed. Based on these observations and the absolute requirement for polyamines in tumour growth, the polyamine pathway is a rational target for chemoprevention and chemotherapeutics. Here we describe the recent advances made in the polyamine field and focus on the roles of polyamines and polyamine metabolism in neoplasia through a discussion of the current animal models for the polyamine pathway, chemotherapeutic strategies that target the polyamine pathway, chemotherapeutic clinical trials for polyamine pathway specific drugs, and ongoing clinical trials targeting polyamine biosynthesis.

Keywords

polyamine; cancer; chemoprevention; chemotherapy; ODC; SSAT; SMO

INTRODUCTION

Polyamine biosynthesis

Polyamines are small, naturally occurring, polycationic alkylamines that are essential for normal cell growth and development in eukaryotes (Refs 1, 2). Both their charge distribution and flexibility allow polyamines to bind to various anionic macromolecules including DNA, RNA, proteins, and acidic phospholipids (Refs 3, 4). The vital importance of polyamines for

*Robert A. Casero Jr., Ph.D. The Johns Hopkins University School of Medicine, Bunting/Blaustein Cancer Research Building 1 1650 Orleans Street - Room 551 Baltimore, MD 21287 rcasero@jhmi.edu.

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normal cellular processes is further highlighted by their roles in maintaining chromatin structure, regulating ion-channels, maintaining membrane stability, and scavenging free radicals (Refs 4, 5, 6, 7, 8).

Under normal physiological conditions, polyamines are tightly regulated by a complex network of biosynthetic, catabolic, and poorly understood transport mechanisms (Refs 9, 10, 11). Such controls aid in the maintenance of a tightly regulated concentration of polyamines in the cell, although the actual amount of free polyamines is likely very low. It has been well established that an increase in intracellular polyamine concentration, primarily through an upregulation of the polyamine biosynthetic enzymes, correlates with increased cell proliferation as well as tumourigenesis (Refs 1, 2, 9, 12). For example, elevated levels of polyamines have been associated with breast, colon, prostate, and skin cancers (Refs 13, 14, 15, 16). Moreover, data indicate that the polyamine pathway is a downstream target of numerous oncogenes, such as *Myc* and *Ras* (Refs 17, 18, 19, 20). Together, these data validate the polyamine pathway as a chemopreventive and chemotherapeutic target.

In mammals, the amino acid ornithine, a product of the urea cycle, is converted to the diamine putrescine by the rate-limiting enzyme ornithine decarboxylase (ODC) (Figure 1). ODC expression is tightly regulated by mechanisms including transcription, post-transcriptional processing, changes in translational efficiency, and altered stability of the protein (Refs 21, 22, 23). The extensive regulatory systems controlling ODC underscore its importance as a critical enzyme essential for normal cell growth and development. The vital necessity of ODC is further verified by data demonstrating that the homozygous deletion of ODC in mice is lethal at 3.5 days post-fertilization (Ref. 24).

ODC is highly regulated at the transcriptional level by various factors, including growth factors, hormones, and tumour-promoting agents (Refs 25, 26). The promoter region of the *Odc* gene contains numerous sequences that are homologous to known transcription factor binding sites (Refs 22, 27, 28). For example, ODC was the first direct target to be identified for the *Myc* oncogene, a mediator of proliferation, differentiation, and apoptosis (Refs 29, 30).

The ODC enzyme, which is active as a homodimer, has a short half-life, ranging from 10-30 minutes (Ref. 9). The ODC degradation process is unique in that it is ubiquitin independent (Ref. 31). For degradation, monomeric ODC non-covalently associates with the ODC antizyme protein (AZ), thus inactivating it. Subsequently, AZ directs ODC to the 26S proteasome for degradation (Ref. 22). The AZ family consists of at least three differently distributed proteins, all of which function as ODC inhibitors (Ref. 22). The best-characterized AZ family member is AZ1. AZ1 is synthesized in a polyamine-dependent manner and is translationally regulated by a +1 frameshift event that occurs when cellular polyamine content is high (Ref. 32). To date, the exact mechanism of this polyamine-specific event remains elusive; however, it appears to involve a psuedoknot structure that is integral to the process (Ref. 33).

The second rate-limiting step in the polyamine biosynthetic pathway is catalysed by S-adenosylmethionine decarboxylase (AdoMetDC), a pyruvoyl-containing decarboxylase

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(Ref. 34). The decarboxylation of S-adenosylmethionine (SAM) by AdoMetDC creates decarboxylated SAM (dcSAM), which donates its propyl amines to form spermidine and spermine from putrescine via the aminopropyl transferases spermidine synthase and spermine synthase, respectively (Refs 35, 36, 37). A dcSAM molecule can only be used in the synthesis of polyamines. This is potentially very significant in that SAM is also the methyl source for many transmethylation reactions, including histone and DNA methylation processes that are further elaborated upon later in this review.

Polyamine catabolism

A highly regulated catabolic pathway further controls the intracellular polyamine pools. Spermidine/spermine N^1-acetyltransferase (SSAT) is a propylamine acetyltransferase that catalyses the formation of N^1-acetylspermine or N^1-acetylspermidine by transferring the acetyl group from acetyl-coenzyme A to the N^1 position of spermine or spermidine, respectively (Refs 38, 39). These acetylated polyamines can be exported or can serve as substrates for the flavin-dependent polyamine oxidase (APAO) that successively produces spermidine or putrescine, as well as the byproducts 3-aceto-aminopropanal and hydrogen peroxide (H_2O_2) (Refs 40, 41, 42).

In the late 1990s, a novel polyamine catabolic enzyme was discovered serendipitously. During an attempt to clone APAO, an enzyme was identified that possessed many of APAO's structural properties (Refs 43, 44). However, the newly discovered enzyme was highly inducible and oxidized unsubstituted spermine. The protein, spermine oxidase (SMO), is a FAD-dependent enzyme that oxidizes spermine to produce spermidine, 3-aminopropanal, and H_2O_2 (Ref. 45). SMO induction has recently been demonstrated in inflammation-associated cancers of the colon and lung (Refs 46, 47). It is hypothesized that SMO induction in response to tumour necrosis factor alpha (TNFα) or other cytokines results in the production of reactive oxygen species (ROS) and subsequently causes DNA damage. This sequence of events is key for both the initiation and progression steps of inflammation-associated neoplastic development and suggests that SMO may be an attractive target for chemoprevention.

Polyamine transport

As mentioned above, intracellular polyamine pools are further regulated by a poorly characterized polyamine transport system (Figure 2). Although mammalian polyamine transport is not well characterized, it is clear that it plays a critical role in maintaining intracellular polyamine concentrations within specific ranges. Because humans consume a polyamine-rich diet (polyamines are found in high quantities in cheese and red meat (Ref. 48)) and because the intestinal flora are able to excrete polyamines, a better understanding of the polyamine transport system is necessary to fully exploit the polyamine metabolic pathway for therapeutic advantage (Ref. 11).

Observations by several groups have shown a correlation between the polyamine transport system and ODC enzyme activity, and have linked inhibition of the two to AZ (Refs 49, 50). In mammals, all three AZ family members are able to repress both ODC activity and the polyamine transport system (Ref. 49). Treatment with DFMO and other ODC enzymatic

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inhibitors has been shown to deplete cellular ODC enzyme activity while inducing polyamine transport activity via an unknown mechanism (Ref. 50). It has been hypothesized, however, that this coordination between ODC enzyme activity and the polyamine transport system is not through alterations in AZ levels, but rather, through changes in the abundance of the unidentified polyamine permease (Refs 50, 51). Interestingly, treating cells with polyamine analogues can cause an induction of AZ therein limiting their own accumulation within the cell (Ref. 52). However, the exact mechanism of how the polyamine transport system is inhibited by AZ is an area of contention. Moreover, antizyme inhibitors (AZI), catalytically inactive relatives of ODC, are able to activate both the polyamine transport system and ODC enzyme activity (Ref. 49). These findings demonstrate the self-regulating capacity of polyamines via a poorly understood mechanism.

Furthermore, in mammals, an energy dependent and selective polyamine transport system has been proposed and biochemically characterized. Currently, there are three proposed models for the polyamine transport system. One model suggests that polyamines are transported into cells via a yet to be identified transporter that is powered by membrane potential. Polyamines enter the cell via a plasma membrane polyamine permease. Accumulated polyamines are then localized to polyamine-sequestering vesicles (PSVs), which closely resemble the acidic vesicles of late endocytosis, in a process that is dependent on a vacuolar ATPase pH gradient and proton exchange (Ref. 53). In this model, PSVs are not directly associated with the membrane components of the polyamine transporter. However, this model accounts for the fact that overall polyamine content in the cell is high despite a low level of free cellular polyamines. Moreover, this model suggests a system in which the polyamine levels in the cell can rapidly change through the release of sequestered polyamines (Ref. 50). A second model describes a mechanism by which heparan sulphate and glypican 1 (GPC1) work together to transport spermine. In this model, spermine binds to the heparan sulfate groups in GPC1 on the cell surface and is subsequently internalized. Once inside the cell, the spermine is freed through a NO-oxidation-mediated process (Ref. 54). A third mechanism for mammalian polyamine transport hypothesizes a caveolin-1-dependent internalization of polyamines that are bound to an unknown polyamine membrane receptor (Ref. 55). In this model, only putrescine is released from the vesicles through a SLC3A2 exporter, and a nitric oxide synthase 2 (NOS2)-dependent reaction destabilizes the spermine-receptor complexes (Ref. 56). Although there has been much recent progress in identifying the potential players in polyamine transport, there is still considerable work needed. Numerous mechanisms have been proposed to explain this process, however, none have identified all of the molecular components involved that are consistent with the observed biochemical data. The identification of the polyamine transporter components is crucial to enable the full exploitation of the transporter to both deliver drugs targeting polyamine metabolism and to use polyamine-like structures to selectively deliver drugs to targets other than polyamine metabolic enzymes.

Polyamines and cancer

There is a vast body of literature describing the relationship between increased levels of polyamines and cancer, most of which suggest an association of induced polyamine biosynthesis with neoplasia (Refs 2, 9, 57). In the 1960s, Russell and Snyder first

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demonstrated high levels of ODC enzyme activity in human cancer (Ref. 12). ODC activity and polyamine levels were subsequently found to be increased in familial adenomatous polyposis, a genetic predisposition to colon cancer due to an adenomatous polyposis coli (APC) mutation (Ref. 58). In addition, a correlation between a single-nucleotide polymorphism (SNP) in the *Odc* intron 1 and human colon cancer risk was assessed. These studies revealed that individuals taking aspirin who were homozygous G or heterozygous G/A at the (G315A) SNP site were more likely to develop colon polyps than homozygous A individuals taking aspirin. The A-allele was further shown to reduce the risk of colon cancer because the c-Myc antagonist, MAD1, selectively repressed *Odc* expression in an A-allele-specific manner (Ref. 59). Pioneering work in the field of polyamines and skin carcinogenesis established that ODC is both necessary and sufficient for the onset of tumours in mice (Refs 10, 15). ODC has been shown to be elevated in human non-melanoma skin cancer (Ref. 60). Moreover, ODC induction and increased levels of polyamines have been associated with breast cancer (Refs 13, 61) and prostate cancer (Ref. 14). Other metabolic enzymes like spermidine synthase and spermine synthase have yet to be convincingly coordinated with tumourigenesis in humans.

Recently, polyamine catabolic enzymes have been linked to carcinogenesis. For example, increased SMO expression has been demonstrated in inflammatory-associated cancers. *Helicobacter pylori* (*H. pylori*) infection and subsequent inflammation has been associated with gastric cancer (Ref. 62). Xu et al. demonstrated that the infection of gastric epithelial cells with *H. pylori* upregulates SMO expression and results in increased DNA damage and apoptosis. Moreover, they showed that an eradication of *H. pylori* correlated with a reduction in SMO expression. The *H. pylori*-induced DNA damage and H_2O_2 production was attenuated upon treatment with the SMO inhibitor MDL 72,527 or by knockdown of SMO via RNAi (Ref. 63). These studies established SMO as a downstream target of *H. pylori* infection and, consequently, a contributor to the development of gastric cancer. Furthermore, SMO expression was shown to be elevated in the tissue samples of patients with prostatic intraepithelial neoplasia (PIN) and prostate cancer when compared to normal prostate tissue samples (Ref. 64). SMO has also recently been shown to be increased in ulcerative colitis, another inflammatory condition leading to a high incidence of colon cancer (Ref. 65).

Animal models useful in the study of polyamine metabolism and disease

Biosynthetic enzyme animal models—Transgenic animals for many of the major enzymes in the polyamine metabolic and catabolic pathways now exist. The first mouse model developed to study the polyamine pathway overexpressed ODC. Interestingly, the ubiquitous overexpression of ODC via its own promoter did not result in increased tumourigenesis in these mice (Ref. 66). However, a targeted overexpression of ODC in the skin, using keratin-specific promoters, resulted in increased tumour formation (Ref. 67). Other studies have shown that ODC haploinsufficiency dramatically lowers the tumour yield in mice. Guo et al. demonstrated that $ODC^{+/-}$ mice were phenotypically normal but displayed lower levels of tumour multiplicity and tumour incidence in the skin when compared to $ODC^{+/+}$ mice (Ref. 68). Moreover, Nilsson et al. showed that reducing the ODC gene dosage by half decreased lymphomagenesis in the Eu-Myc model (Ref. 69).

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These data underscore the complexity of polyamine enzyme gene dosage and polyamine levels as well as emphasize that the correlation between polyamine levels and tumourigenesis is tissue-specific. Mice that overexpress the other major metabolic enzyme in the polyamine pathway, AdoMetDC, have also been examined. In one study, mice that ubiquitously overexpressed AdoMetDC exhibited no pronounced phenotypic changes (Ref. 70). In contrast, Shi et al. demonstrated that a targeted overexpression of AdoMetDC in the skin surprisingly resulted in fewer tumours when compared to control littermates (Ref. 71). A recent study suggests a tumour suppressive role for AdoMetDC by demonstrating that the heterozygous deletion of AdoMetDC and the translational protein eIF5A occur concurrently in human lymphoma and mouse lymphomagenesis (Ref. 72). Again, these data highlight the complexity of the polyamine pathway and show that changes in the polyamines themselves can have quite different effects on normal and tumour tissues.

Using the cytomegalovirus-immediate early gene enhancer/chicken B-actin promoter to overexpress human spermidine synthase in mice resulted in no marked phenotypic changes (Refs 73, 74). Moreover, mice that overexpressed spermine synthase displayed no changes in skin tumour susceptibility when compared to littermate controls and exhibited similar colon tumour multiplicity and size when compared to multiple intestinal neoplasia (Min) mice (Ref. 75). These data demonstrate that ubiquitously altered levels of the higher polyamines spermidine and spermine alone do not promote tumourigenesis.

Mice that overexpress AZ, the endogenous inhibitor of ODC, have also been developed. In a skin chemical carcinogenesis model, Feith et al. demonstrated that a tissue-specific overexpression of AZ in the skin resulted in the development of fewer papillomas when compared to normal littermate controls (Ref. 76). In addition, the overexpression of AZ in the forestomach of mice led to lower tumour incidence and multiplicity when compared to littermate controls after treatment with the carcinogen N-nitrosomethylbenzylamine (NMBA) (Ref. 77). These data support the causal role of ODC in tumourigenesis, and further highlight the potential of targeting the polyamine pathway as an anti-neoplastic strategy.

Catabolic enzyme animal models—Animal models for studying the catabolic enzymes in the polyamine pathway are less abundant, and no animal models for APAO or SMO have been reported to date. Mice that constitutively and ubiquitously overexpressed SSAT demonstrated a partial resistance to papilloma formation after the two-stage skin chemical carcinogenesis protocol (Ref. 78). However, mice that overexpressed SSAT specifically in the skin under the K6 promoter demonstrated a higher rate of tumour progression than their littermate controls (Ref. 79). In one study, Kee et al. crossed mice that overexpress SSAT with mice that are predisposed to prostate cancer (transgenic adenocarcinoma of the mouse prostate (TRAMP) mice). These TRAMP/SSAT mice displayed inhibited tumour outgrowth when compared to TRAMP mice, suggesting that the induction of SSAT can exert anti-tumour effects (Ref. 80). In a subsequent study, SSAT overexpressing mice were crossed with MIN mice. The progeny of this cross developed more intestinal adenomas than MIN mice. Interestingly, in a parallel study, SSAT knockout mice were crossed with MIN mice and resulted in mice that displayed fewer adenomas when compared to MIN mice (Ref. 81). The inconsistent data described for SSAT overexpressing mice might result from metabolic

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perturbations in the acetyl CoA pools of animals that overexpress SSAT in all of their tissues versus those models that have targeted expression. Further studies will be necessary to determine the role of SSAT in tumourigenesis in each of these transgenic models.

CLINICAL IMPLICATIONS AND APPLICATIONS

Cancer treatment: Targeting the polyamine pathway via enzyme inhibitors and polyamine analogues

Enzyme inhibitors—Targeting the polyamine biosynthetic pathway for anti-tumourigenic therapy started soon after the discovery that dysregulated polyamine levels were a hallmark for numerous tumour types (Figure 3). Thus, the original therapies that targeted the polyamine pathway were for the metabolic enzymes ODC and AdoMetDC. 2-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, was first shown in the 1970s to have anti-tumourigenic properties (Refs 82, 83). *In vitro*, DFMO depletes putrescine and spermidine, but has variable effects on spermine concentrations. In general, this depletion in the intracellular polyamine pools is associated with a cytostatic growth response (Ref. 84). However, the cytotoxic effects of DFMO on human small-cell lung cancer *in vivo* and *in vitro* prompted clinical trials for DFMO as a single agent (Refs 85, 86, 87, 88). Unfortunately, DFMO, alone or in combination with other agents, was largely ineffective as a chemotherapeutic agent. It was, however, demonstrated to be generally well-tolerated with mostly reversible toxicities including thrombocytopenia, gastrointestinal anomalies, and hearing loss (Ref. 9). The lack of effectiveness of DFMO as a chemotherapeutic agent is likely due to its poor transport into the cell, the fact that it is typically cytostatic and not cytotoxic, and compensatory mechanisms such as increased polyamine transport or upregulation of AdoMetDC that occur as a result of the depleted polyamine pools. Thus, tumour cells can overcome the effects of ODC inhibition.

In addition to targeting the biosynthetic enzyme ODC, inhibitors for the metabolic enzyme AdoMetDC have also been investigated. Methylglyoxal bis(guanylhydrazone) (MGBG) was used as a treatment for leukaemia before it was demonstrated to be a competitive inhibitor of AdoMetDC (Refs 89, 90). *In vitro*, MGBG decreases the levels of spermidine and spermine while increasing the levels of putrescine, and it inhibits cellular growth. Despite its potency as an AdoMetDC inhibitor, the role of MGBG as a chemotherapeutic agent was limited due to its mitochondrial toxicities (Ref. 91). Subsequently, a number of nucleoside-based inhibitors of the enzyme were synthesized, most of which were based on the structure of S-adenosylmethionine (Refs 92, 93). The most effective nucleoside-based AdoMetDC inhibitor, 5’([(Z)-4-amino-2-butenyl]methylamino)-5’-deoxy-adenosine (AbeAdo), is an irreversible inactivator of the enzyme (Ref. 94). AbeAdo has a charged nitrogen in place of the sulfonium centre and is significantly more potent than the corresponding sulphur-containing compounds. AbeAdo and its analogues have been shown to be effective against trypanosomiasis in animal models (Ref. 95). AbeAdo reduces the spermidine and spermine levels and increases putrescine. In L1210 cells, AbeAdo was shown to be cytostatic, but this was due to the loss of hypusinated eIF5A (Ref. 96).

More recently, 4-amidinoindan-1-one-2’-amidinhydrazone (SAM486A/CGP48664), a competitive inhibitor of AdoMetDC, was shown to produce low mitochondrial toxicity and

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has been tested in phase I and phase II clinical trials for multiple cancers, with partial responses exhibited in non-Hodgkin's lymphoma (Refs 97, 98, 99, 100). SAM486A/CGP48664 decreases the levels of both spermidine and spermine while increasing the levels of putrescine and thereby inhibits cell growth (Ref. 101).

Inhibitors have also been developed that target the aminopropyl transferases spermidine and spermine synthase. S-adenosyl-3-thio-1,8-diaminooctane (AdoDATO), a spermidine synthase inhibitor, caused little growth inhibition in cancer cell lines (Ref. 102). A similar analogue that inhibited spermine synthase was also created, but again showed little promise as a chemotherapeutic agent (Ref. 103).

Symmetrically substituted bis(alkyl) polyamine analogues—As indicated above, the initial focus of polyamine-targeted therapy was directed at inhibiting the biosynthetic enzymes ODC and AdoMetDC; however, this approach demonstrated minimal success in the clinic. More recently, an emphasis on exploiting the self-regulating nature of the polyamine pathway using polyamine analogues has occurred. This paradigm was initially articulated by Porter and Bergeron (Ref. 104). The rationale behind using polyamine analogues was to develop compounds that were able to utilize the polyamine transport system without evoking compensatory mechanisms or functionally substituting for the natural polyamines. The subsequent accumulation of polyamine analogues results in a downregulation of the polyamine biosynthetic pathway in conjunction with an increase in the catabolic pathway. Together, these actions result in a significant depletion of all 3 polyamines, including spermine, which is in contrast to what is seen with the inhibitors of polyamine biosynthesis. This depletion of the natural polyamine pool ultimately produces growth inhibition and, in specific cases, cell death.

The first polyamine analogues to be extensively studied were the bis(alkyl) polyamines. These compounds are similar to the polyamines spermidine and spermine; however, their primary amino termini are protected by symmetrical bis(alkyl) groups, thereby preventing their oxidation by multiple amine oxidases. This generation of analogues downregulates both ODC and AdoMetDC through a combination of multiple mechanisms, including product inhibition and changes in mRNA translation (Ref. 105). Furthermore, some of these analogues are able to induce polyamine catabolic enzyme activity, an induction that has been linked to cytotoxicity (Refs 39, 106, 107, 108).

One compound that highly induces SSAT, N^1, N^{11}-bis(ethyl)norspermine (BENSpm, also denoted as BE333 or DENSpm), uses the polyamine transport system to enter the cell and downregulates both ODC and AdoMetDC. In addition to SSAT, BENSpm also induces the catabolic enzyme SMO (Refs 108, 109, 110). Other examples of bis(alkyl) polyamine analogues include: N^1, N^{12}-bis(ethyl)spermine (BESpm), N^1, N^{14}-bis(ethyl)homospermine (BEHSpm), N^1, N^{15}-bis-[3-(ethylamino)-propyl]-1-17-heptane diamine (BE-3-7-3), and N^1, N^{19}-bis(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4) (Ref. 106).

Impressive *in vitro* work has demonstrated that BENSpm displays significant anti-tumour activity (Refs 106, 110, 111). Phases I and II clinical trials were conducted with this compound (Refs 111, 112, 113); however, the results were somewhat disappointing. The

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initial phase I trials were conducted with 3 different dosing schedules (Refs 114, 115), of which the most tolerated dosage was 1/day × 5 days. However, it is still not known if this dosing schedule is optimal. It is possible that alternative dosing schedules, including once weekly for a longer duration, would be more effective. The long biological half-life of these agents suggests that such scheduling might be both better tolerated and more efficacious. Although BENSpm was well tolerated with the once daily dosing, the compound failed to show significant anti-tumour effects against breast or lung cancer (Refs 112, 113, 115, 116). However, it should be noted that despite its failure as a single agent, studies that combine BENSpm with standard chemotherapeutic agents (Refs 117, 118, 119) appear promising and are likely to serve as catalysts for future combination studies using standard chemotherapeutics and polyamine analogues.

Unsymmetrically substituted analogues—A variation on the theme of alkylated polyamine analogues led to a second generation of polyamine analogues that contained unsymmetrical substitutions to the spermine and norspermine backbone. These compounds included: N^1-propargyl-N^{11}-ethylnorspermine (PENSpm), N^1-cyclopropyl-methyl-N^{11}-ethylnorspermine (CPENSpm), and N^1-cycloheptylmethyl-N^{11}-ethylnorspermine (CHENSpm). Both CPENSpm and PENSpm have demonstrated cytotoxicity and SSAT induction (Ref. 120). The data from studies with these compounds demonstrate that minor structural modifications can result in significantly altered biological responses to the individual polyamine analogues. Additionally, these studies have validated the strategy that reactive functional groups can be added to the polyamine backbone to facilitate the entry of such conjugates into the cell through the polyamine transport system, leading to the synthesis of multiple compounds exploiting this strategy (Refs 121, 122, 123, 124).

Conformationally restricted analogues—Another variation of the bis(alkyl) polyamine analogues is characterized by synthesized compounds that are rotationally restricted at the central carbons of the polyamine chain. From this subset of compounds, CGC-11047 (PG-11047) and CGC-11093 (PG-11093) have been the most encouraging therapeutic agents (Refs 125, 126). CGC-11047 is based on BESpm and is conformationally restricted by a double bond between the central 2 carbons of the 4-carbon methylene bridge. CGC-11093 is a BEHSpm analogue that contains a cyclopropyl ring at the central 2 carbons of the 4-carbon methylene bridge. These alterations to BESpm and BEHSpm increased the anti-proliferative activity of these compounds as well as reduced the overall non-specific toxicity. CGC-11047 inhibited growth in both small cell and non-small cell lung cancer cell lines and delayed the progression of established tumours in nu/nu mice (Ref. 126). CGC-11047 has also been reported to inhibit the growth of basal-subtype breast cancers and colon cancers *in vitro* (Refs 127, 128). CGC-11047 has been studied in phase I clinical trials as a single agent and is also in a phase Ib clinical trial in combination with bevacizumab (Avastin; Genetech), erlotinib (Tarceva; OSI Pharmaceuticals), docetaxel (Taxotere; Sanofi-Aventis), or gemcitabine (Gemzar; Eli Lilly) (Ref. 129). Likewise, CGC-11093 has been studied in phase I clinical trials (Ref. 9).

Oligoamines—Polyamines have long been known to bind to DNA and other macromolecules (Refs 3, 4). At physiological pH and concentrations of 50-100 uM,

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spermine causes DNA to condense (Ref. 130). Thus, it was hypothesized that increasing the number of protonatable nitrogens in the polyamine analogues would inherently increase the affinity of these molecules for DNA (Ref. 131). The oligoamines, synthesized by Frydman and colleagues, contain 8-14 amines and have representative saturated, unsaturated, and conformationally restricted forms. The oligoamine CGC-11144 (PG-11144) exhibited growth inhibition in a panel of prostate cancer cell lines, and this growth inhibition correlated with the ability of the analogue to aggregate DNA (Ref. 131). CGC-11144 has also demonstrated anti-tumour effects in both *in vivo* and *in vitro* models of breast cancer (Ref. 132). Additionally, as will be discussed below, CGC-11144 has been demonstrated to have profound epigenetic effects, leading to changes in gene expression of specific tumour cell types. However, to date there have been no clinical trials for CGC-11144.

Polyamine analogues targeting epigenetic modifications—The modification of chromatin structure is a mechanism for regulating gene expression. Post-translational modifications to histone tails facilitate the epigenetic remodelling of chromatin. For example, the mediation of histone acetylation by histone acetyltransferases (HATs) and histone deacetylases (HDACs) is crucial for chromatin structure and the regulation of gene expression. The acetylation of the cationic lysine tails of histones neutralizes the charge and promotes a relaxed chromatin structure, thereby increasing chromatin accessibility and potentiating gene transcription. In contrast, deacetylation of the lysine residues results in condensed chromatin, reduced accessibility, and, consequently, transcriptional repression. In some types of cancer, a hypoacetylation of histones is observed. This results in the underexpression of important transcripts, such as the tumour suppressing cell cycle regulator p21, and can promote tumourigenesis (Ref. 133). HDAC inhibitors such as entinostat (MS-275) and suberoylanilide hydroxamic acid (SAHA) increase histone acetylation and induce growth arrest in a variety of cancer cell lines. Unfortunately, these HDAC inhibitors lack specificity and can produce unacceptable levels of toxicity. Based on these observations, Varghese et al. synthesized polyaminohydroxamic acid (PAHA) derivatives that incorporated structural features of the polyamines spermidine and spermine and the hydroxamic acid moiety that is common amongst potent HDAC inhibitors. The rationale for this design was based on the observation that polyamine analogues maintain a high affinity for DNA and can enter the cell via the polyamine transport system. Varghese et al. observed that, at a 1 uM concentration, 3 of these compounds inhibited HDAC activity by at least 60% (Ref. 122). In a subsequent study, 15 additional PAHA analogues and polyaminobenzamides (PABAs) were studied. PABAs incorporated the benzamide moiety of MS-275 as well as some of the structural features of spermidine and spermine. Two PABA compounds and 7 PAHA compounds inhibited HDAC activity by over 50%. Compound 17, a PAHA compound, was shown to selectively inhibit HDAC 6. Interestingly, PABA compound 21 demonstrated the ability to utilize the polyamine transport system, although it was not particularly strong as an HDAC inhibitor (Ref. 121). It should be noted that in trying to produce effective epigenetic modulators, it is hypothetically preferable to produce drugs that restore the normal growth control mechanisms of tumour cells, rather than inducing outright cytotoxic activity. Therefore, it is significant that none of the compounds tested were cytotoxic at a dose of 100 uM. The cytotoxicity data, as well as the data showing that the PABA and PAHA compounds induced cellular effects that differed

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from previously identified HDAC inhibitors, provide an additional rationale for the synthesis and study of this compound family.

In cancer, in addition to histone acetylation alterations, promoter-region DNA hypermethylation, either alone or in combination with other histone modifications, including histone methylation, is associated with tumour suppressor gene silencing (Ref. 134). Until recently, the activity of histone methyltransferases and the replacement of methylated histones with non-methylated histones was the central dogma for the dynamic balance of methylated lysines on the histone tails. The discovery of lysine-specific demethylase 1 (LSD1) that demethylates mono- and dimethyl lysine 4 of histone H3 demonstrated that histone methylation is a dynamic and complex process similar to that previously observed for histone acetylation and deacetylation. LSD1 is a FAD-dependent amine oxidase that contains an active site with 60% homology to SMO, and the demethylation of lysine residues by LSD1 occurs through this oxidase activity (Ref. 135). These structural and functional similarities between LSD1 and SMO suggested another potential target for polyamine analogues. Based on previous data indicating that guanidine compounds are capable of inhibiting the polyamine oxidases, studies were conducted to test whether (bis)guanidine or biguanide polyamine analogues could inhibit LSD1 *in vitro* (Refs 136, 137). Of the 13 compounds initially tested, 9 were able to noncompetitively inhibit recombinant LSD1 activity by over 50% at 1 uM concentrations (Ref. 138). Treatment with the most potent LSD1 inhibitor from this study, the biguanide polyamine analogue **2d**, resulted in the significant re-expression of aberrantly silenced tumour suppressor genes. Four members of the secreted frizzled-related protein family (SFRP1, SFRP2, SFRP4, and SFRP5) and two GATA family transcription factors (GATA4 and GATA5) were re-expressed in **2d**-treated HCT116 cells. In RKO colon cancer cells, **2d** exposure caused the re-expression of SFRP4 and SFRP5 as well as induced global levels of the LSD1 substrate H3K4me2 (Ref. 106). Recent studies have led to the synthesis of a series of urea- and thiourea isosteres of **2d** that in some cases are significantly more potent (Refs 139, 140).

In addition to the (bis)guanidine and biguanide inhibitors of LSD1, specific oligoamine analogues have also been demonstrated to be effective inhibitors of LSD1. Most importantly, both classes of inhibitors have demonstrated significant *in vivo* inhibition of tumour growth in established tumours, and both have demonstrated a synergistic anti-tumour response when combined with the DNA methyltransferase inhibitor 5-azacitidine (Ref. 141). Interestingly, a very recent study has demonstrated a synergistic effect on gene expression when CGC-11144 and DFMO were used in combination in HCT116 cells (Ref. 123).

Most recently, a series of small amidoximes demonstrated modest inhibition of recombinant LSD1. However, these compounds exhibited profound changes to the methylation status of histone H3 at lysine 4, a well-known LSD1 target. Moreover, these compounds increased expression of the GATA4, SFRP2, and H-cadherin (HCAD) tumour suppressor genes. In this particular study, compound 22 promoted the re-expression of the aberrantly silenced genes in Calu-6 cells as well as increased the global H3K4me2 levels, suggesting that the amidoxime compound 22 is a good histone demethylase inhibitor (Ref. 142).

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To further substantiate the link between polyamines and DNA promoter methylation it has been demonstrated that the inhibition of ODC by AZ1 causes the accumulation of decarboxylated dcSAM which subsequently acts as a competitive inhibitor for methylation reactions resulting in a genome-wide hypomethylation of CpG islands (Ref. 143). Additionally, Yamamoto et al. showed that AZ1 causes histone H3 lysine 9 dimethylation in UMI human oral cancer cells (Ref. 144). These data further support a role for polyamines in epigenetic changes.

The above data highlight a tremendous effort by researchers in targeting polyamine metabolism, function, and transport as a strategy for the treatment of cancer. The most recent efforts using polyamine analogues and polyamine-like structures to target epigenetic regulators are of particular interest because the polyamine structure naturally targets these agents to chromatin. This property is unique amongst the many compounds and drugs that have been designed to inhibit the various chromatin modifiers. Therefore, it is likely that there will be a continued effort to exploit the polyamine structure in the design of future anti-neoplastic agents.

Targeting the polyamine pathway as a strategy for chemoprevention

In addition to the focus on chemotherapy, there has been a recent increase in the interest of targeting polyamine metabolism and function as a strategy for chemoprevention. Numerous studies have described the preventative effects of various polyamine inhibitors on combating both skin and colon cancers. For example, in a phase IIb clinical trial, the lowest oral dose of DFMO that was effective in reducing the polyamine content in human rectal mucosa was determined with minimal side effects, suggesting that DFMO is a safe chemopreventive agent (Ref. 145). In a phase IIb/III clinical trial, the combination of DFMO and sulindac, a non-selective inhibitor of both COX-1 and COX-2, was shown to be effective in reducing the number of metachronous colorectal adenomas in patients with a history of adenomas (Ref. 146). These data suggest that DFMO can act as an effective chemopreventive agent for colon cancer, particularly in combination with a non-steroidal anti-inflammatory agent (NSAID). It should be noted that a 4-arm clinical trial with sulindac and DFMO is currently underway. Additionally, other investigators are attempting to develop NSAIDs that are as efficacious as sulindac without some of the untoward effects; however, further studies will be necessary to determine if the newer compounds have clear benefits over sulindac (Refs 147, 148).

A phase III clinical trial of daily DFMO oral intake (0.5 $g/m^2/day$) was conducted over 5 years in patients with a history of non-melanoma skin cancer (NMSC) in an attempt to determine if DFMO reduced NMSC occurrence. The studies showed a trend towards the reduction of NMSC in individuals taking DFMO, and a significant reduction in basal cell carcinomas was also recorded in those taking DFMO (Ref. 149). Furthermore, in a study in which DFMO was administered topically to individuals with a history of actinic keratosis (AK), a squamous cell carcinoma precursor, it was shown that treatment with DFMO significantly decreased the number of AKs (Ref. 150). These data support the role of DFMO as a chemopreventive agent for various epithelial neoplasias.

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Recently, a single-arm study was conducted over 6 months for individuals with Barrett's oesophagus and mucosal dysplasia with a dosing schedule of 0.5 g/m^2/day of DFMO. In those who were given DFMO (n=10), 8 individuals showed stable disease, 1 individual exhibited disease regression, and 1 individual showed disease progression. Although encouraging, further clinical trials are needed to test the toxicity and efficacy of DFMO in treating this disease (Ref. 151).

Simoneau et al. conducted a phase IIb clinical trial to test DFMO as a chemopreventive agent for prostate cancer. The use of DFMO correlated with a smaller increase in prostate volume as well as a decrease in the level of putrescine. Significantly, no grade 3 or grade 4 toxicity was reported. Similar to the DFMO trial for Barrett's oesophagus, further studies are needed to determine the effects of using DFMO as a chemopreventive agent for prostate cancer (Ref. 152).

Induction of SMO in response to inflammatory stimuli and the subsequent production of reactive oxygen species (ROS), molecules that are capable of causing DNA damage, have been documented in multiple systems (Refs 46, 153). Together, these data suggest that SMO may be a rational chemopreventive target. *H. pylori* infection of gastric epithelial cells results in a rapid induction of SMO, increased ROS, and significant DNA damage (Ref. 63). Additionally, patients with active *H. pylori* infections demonstrate uniformly increased SMO expression compared to matched samples after eradication of infection through antibiotic treatment. Babbar et al. demonstrated that SMO induction by inflammatory cytokines could be a general inflammatory response in non-tumorigenic lung epithelial cells, suggesting a role for SMO in lung and other epithelial cancers (Ref. 46). In an *in vivo* colon cancer model, Goodwin et al. demonstrated that in response to enterotoxigenic *Bacteroides fragilis* (ETBF) infection, there was an increase in the levels of ROS that was concurrent with increased SMO expression. Treatment of animals with the SMO inhibitor MDL 72,527 significantly reduced the ETBF-induced burden consistent with SMO activity a major contributor to tumourigenesis in this model (Ref. 47).

The ongoing studies with DFMO and the new findings suggesting the involvement of SMO in inflammation-associated carcinogenesis underscore this new and exciting avenue for exploiting polyamine metabolism and catabolism for therapeutic benefit.

RESEARCH IN PROGRESS AND OUTSTANDING RESEARCH QUESTIONS

Over the past 40 years, much progress has been made in understanding the role of the polyamine pathway in normal cell functioning and tumourigenesis. The knowledge gained is now being used to develop new strategies for the treatment and prevention of cancer.

One important area in the polyamine field that continues to be poorly understood is the polyamine transport system. Very little is currently known regarding the molecular components of the mammalian transport system. To fully exploit the polyamine pathway and to optimize the use of polyamine analogues in a therapeutic setting will require greater knowledge in this area. As indicated above, the polyamine transport system has been used for cellular entry of molecules that are conjugated to a polyamine backbone, and several

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attempts using this approach have been investigated (Refs 154, 155, 156); however, a greater understanding of the transporter will be necessary to fully exploit this strategy.

The recent findings that the chromatin remodelling enzyme LSD1 is similar in structure to SMO and APAO and uses the same catalytic mechanism for the oxidative demethylation of the lysine tail of histone H3, combined with the findings that polyamine analogues can be effective inhibitors of this enzyme, opens up an entirely new avenue for polyamine-based anti-tumour drug development. The natural affinity of polyamines for chromatin make them a perfect backbone for targeting agents such as HDAC inhibitors or other chromatin remodelling compounds.

The recognition that polyamine catabolism may play a significant role in inflammation-associated carcinogenesis has implicated SMO as a rational target for chemoprevention. The best inhibitor of SMO reported to date, MDL 72,527, has demonstrated the potential of such inhibition in a valid model for colon carcinogenesis. However, because MDL 72,527 is also an effective inhibitor of APAO, a more selective inhibitor may be preferable. Regardless, it is intriguing to consider the potential for chemoprevention in multiple inflammation-associated epithelial cancers including, colon, prostate, gastric, and lung, each of which appear to have a link with increased SMO activity.

Finally, as the field moves forward and we gain a better understanding of the roles that polyamines play in growth and differentiation in the normal setting, as well as their dysregulation in neoplastic disease, it is likely that more rational targets and better agents to target them will be discovered. There is no doubt that the newly generated animal models, along with a continuing stream of polyamine-based compounds will aid in this endeavour.

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References

1. Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. Cancer research. 1988; 48(4):759–74. [PubMed: 3123052]
2. Gerner EW, Meyskens FL Jr. Polyamines and cancer: old molecules, new understanding. Nature reviews. Cancer. 2004; 4(10):781–92.
3. Wallace HM, Fraser AV, Hughes A. A perspective of polyamine metabolism. The Biochemical journal. 2003; 376(Pt 1):1–14. [PubMed: 13678416]
4. Matthews HR. Polyamines, chromatin structure and transcription. Bioessays. 1993; 15(8):561–6. [PubMed: 8135771]
5. Feuerstein BG, et al. Implications and concepts of polyamine-nucleic acid interactions. Journal of cellular biochemistry. 1991; 46(1):37–47. [PubMed: 1874798]
6. Ha HC, et al. The natural polyamine spermine functions directly as a free radical scavenger. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95(19): 11140–5. [PubMed: 9736703]
7. Kurata HT, Marton LJ, Nichols CG. The polyamine binding site in inward rectifier K+ channels. The Journal of general physiology. 2006; 127(5):467–80. [PubMed: 16606689]

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8. Schuber F. Influence of polyamines on membrane functions. The Biochemical journal. 1989; 260(1):1–10. [PubMed: 2673211]
9. Casero RA Jr. Marton LJ. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nature reviews. Drug discovery. 2007; 6(5):373–90.
10. Pegg AE. Mammalian polyamine metabolism and function. IUBMB Life. 2009; 61(9):880–94. [PubMed: 19603518]
11. Wallace HM. The physiological role of the polyamines. Eur J Clin Invest. 2000; 30(1):1–3. [PubMed: 10619994]
12. Russell D, Snyder SH. Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. Proceedings of the National Academy of Sciences of the United States of America. 1968; 60(4):1420–7. [PubMed: 4299947]
13. Manni A, et al. Involvement of the polyamine pathway in breast cancer progression. Cancer Lett. 1995; 92(1):49–57. [PubMed: 7757960]
14. Gupta S, et al. Chemoprevention of prostate carcinogenesis by alpha- difluoromethylornithine in TRAMP mice. Cancer research. 2000; 60(18):5125–33. [PubMed: 11016639]
15. Gilmour SK. Polyamines and nonmelanoma skin cancer. Toxicol Appl Pharmacol. 2007; 224(3): 249–56. [PubMed: 17234230]
16. Upp JR Jr. et al. Polyamine levels and gastrin receptors in colon cancers. Annals of surgery. 1988; 207(6):662–9. [PubMed: 3389934]
17. Tobias KE, Shor J, Kahana C. c-Myc and Max transregulate the mouse ornithine decarboxylase promoter through interaction with two downstream CACGTG motifs. Oncogene. 1995; 11(9): 1721–7. [PubMed: 7478599]
18. Shantz LM, Levin VA. Regulation of ornithine decarboxylase during oncogenic transformation: mechanisms and therapeutic potential. Amino acids. 2007; 33(2):213–23. [PubMed: 17443268]
19. Holtta E, Sistonen L, Alitalo K. The mechanisms of ornithine decarboxylase deregulation in c-Ha-ras oncogene-transformed NIH 3T3 cells. The Journal of biological chemistry. 1988; 263(9):4500–7. [PubMed: 3279036]
20. Ignatenko NA, et al. Suppression of polyamine catabolism by activated Ki-ras in human colon cancer cells. Molecular carcinogenesis. 2004; 39(2):91–102. [PubMed: 14750214]
21. Shantz LM. Transcriptional and translational control of ornithine decarboxylase during Ras transformation. The Biochemical journal. 2004; 377(Pt 1):257–64. [PubMed: 14519103]
22. Pegg AE. Regulation of ornithine decarboxylase. The Journal of biological chemistry. 2006; 281(21):14529–32. [PubMed: 16459331]
23. Nowotarski SL, Shantz LM. Cytoplasmic accumulation of the RNA-binding protein HuR stabilizes the ornithine decarboxylase transcript in a murine nonmelanoma skin cancer model. The Journal of biological chemistry. 2010; 285(41):31885–94. [PubMed: 20685649]
24. Pendeville H, et al. The ornithine decarboxylase gene is essential for cell survival during early murine development. Molecular and cellular biology. 2001; 21(19):6549–58. [PubMed: 11533243]
25. Katz A, Kahana C. Transcriptional activation of mammalian ornithine decarboxylase during stimulated growth. Molecular and cellular biology. 1987; 7(7):2641–3. [PubMed: 3614203]
26. Verma AK, Hsieh JT, Pong RC. Mechanisms involved in ornithine decarboxylase induction by 12-O-tetradecanoylphorbol-13-acetate, a potent mouse skin tumor promoter and an activator of protein kinase C. Advances in experimental medicine and biology. 1988; 250:273–90. [PubMed: 3076326]
27. Abrahamsen MS, et al. Multiple DNA elements responsible for transcriptional regulation of the ornithine decarboxylase gene by protein kinase A. The Journal of biological chemistry. 1992; 267(26):18866–73. [PubMed: 1356108]
28. Zhao B, Butler AP. Core promoter involvement in the induction of rat ornithine decarboxylase by phorbol esters. Molecular carcinogenesis. 2001; 32(2):92–9. [PubMed: 11746821]
29. Bello-Fernandez C, Packham G, Cleveland JL. The ornithine decarboxylase gene is a transcriptional target of c-Myc. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90(16):7804–8. [PubMed: 8356088]

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30. Packham G, Cleveland JL. The role of ornithine decarboxylase in c-Myc-induced apoptosis. Current topics in microbiology and immunology. 1995; 194:283–90. [PubMed: 7895500]
31. Murakami Y, et al. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature. 1992; 360(6404):597–9. [PubMed: 1334232]
32. Nilsson J, et al. Polyamines regulate both transcription and translation of the gene encoding ornithine decarboxylase antizyme in mouse. European journal of biochemistry / FEBS. 1997; 250(2):223–31. [PubMed: 9428668]
33. Matsufuji S, et al. Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. Cell. 1995; 80(1):51–60. [PubMed: 7813017]
34. Stanley BA, Pegg AE, Holm I. Site of pyruvate formation and processing of mammalian S-adenosylmethionine decarboxylase proenzyme. The Journal of biological chemistry. 1989; 264(35):21073–9. [PubMed: 2687270]
35. Ikeguchi Y, Bewley MC, Pegg AE. Aminopropyltransferases: function, structure and genetics. Journal of biochemistry. 2006; 139(1):1–9. [PubMed: 16428313]
36. Korhonen VP, et al. Molecular cloning of a cDNA encoding human spermine synthase. DNA and cell biology. 1995; 14(10):841–7. [PubMed: 7546290]
37. Wahlfors J, et al. Human spermidine synthase: cloning and primary structure. DNA and cell biology. 1990; 9(2):103–10. [PubMed: 2344393]
38. Casero RA Jr. Pegg AE. Spermidine/spermine N1- acetyltransferase--the turning point in polyamine metabolism. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1993; 7(8):653–61. [PubMed: 8500690]
39. Casero RA, Pegg AE. Polyamine catabolism and disease. The Biochemical journal. 2009; 421(3): 323–38. [PubMed: 19589128]
40. Xie X, Gillies RJ, Gerner EW. Characterization of a diamine exporter in Chinese hamster ovary cells and identification of specific polyamine substrates. The Journal of biological chemistry. 1997; 272(33):20484–9. [PubMed: 9252359]
41. Vujcic S, et al. Genomic identification and biochemical characterization of the mammalian polyamine oxidase involved in polyamine back-conversion. The Biochemical journal. 2003; 370(Pt 1):19–28. [PubMed: 12477380]
42. Wu T, Yankovskaya V, McIntire WS. Cloning, sequencing, and heterologous expression of the murine peroxisomal flavoprotein, N1-acetylated polyamine oxidase. The Journal of biological chemistry. 2003; 278(23):20514–25. [PubMed: 12660232]
43. Wang Y, et al. Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analogue exposure. Cancer research. 2001; 61(14):5370–3. [PubMed: 11454677]
44. Vujcic S, et al. Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. The Biochemical journal. 2002; 367(Pt 3):665–75. [PubMed: 12141946]
45. Wang Y, et al. Properties of purified recombinant human polyamine oxidase, PAOh1/SMO. Biochemical and biophysical research communications. 2003; 304(4):605–11. [PubMed: 12727196]
46. Babbar N, Casero RA Jr. Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. Cancer research. 2006; 66(23):11125–30. [PubMed: 17145855]
47. Goodwin AC, et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(37):15354–9. [PubMed: 21876161]
48. Bardocz S, et al. The importance of dietary polyamines in cell regeneration and growth. The British journal of nutrition. 1995; 73(6):819–28. [PubMed: 7632663]
49. Kahana C. Regulation of cellular polyamine levels and cellular proliferation by antizyme and antizyme inhibitor. Essays in biochemistry. 2009; 46:47–61. [PubMed: 20095969]
50. Poulin R, Casero RA, Soulet D. Recent advances in the molecular biology of metazoan polyamine transport. Amino acids. 2012; 42(2-3):711–23. [PubMed: 21814785]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

51. Lessard M, et al. Hormonal and feedback regulation of putrescine and spermidine transport in human breast cancer cells. The Journal of biological chemistry. 1995; 270(4):1685–94. [PubMed: 7829504]
52. Mitchell JL, et al. Antizyme induction mediates feedback limitation of the incorporation of specific polyamine analogues in tissue culture. The Biochemical journal. 2004; 384(Pt 2):271–9. [PubMed: 15315476]
53. Soulet D, et al. A fluorescent probe of polyamine transport accumulates into intracellular acidic vesicles via a two-step mechanism. J Biol Chem. 2004; 279(47):49355–66. [PubMed: 15208319]
54. Belting M, et al. Glypican-1 is a vehicle for polyamine uptake in mammalian cells: a pivital role for nitrosothiol-derived nitric oxide. The Journal of biological chemistry. 2003; 278(47):47181–9. [PubMed: 12972423]
55. Roy UK, et al. Activated K-RAS increases polyamine uptake in human colon cancer cells through modulation of caveolar endocytosis. Molecular carcinogenesis. 2008; 47(7):538–53. [PubMed: 18176934]
56. Uemura T, et al. Polyamine transport is mediated by both endocytic and solute carrier transport mechanisms in the gastrointestinal tract. American journal of physiology. Gastrointestinal and liver physiology. 2010; 299(2):G517–22. [PubMed: 20522643]
57. Casero RA Jr. et al. The role of polyamine catabolism in anti-tumour drug response. Biochemical Society transactions. 2003; 31(2):361–5. [PubMed: 12653639]
58. Giardiello FM, et al. Ornithine decarboxylase and polyamines in familial adenomatous polyposis. Cancer research. 1997; 57(2):199–201. [PubMed: 9000553]
59. Martinez ME, et al. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(13):7859–64. [PubMed: 12810952]
60. Elmets CA, Athar M. Targeting Ornithine Decarboxylase for the Prevention of Nonmelanoma Skin Cancer in Humans. Cancer Prev Res (Phila Pa). 2010; 3(1):8–11.
61. Manni A, et al. Prognostic influence on survival of increased ornithine decarboxylase activity in human breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 1996; 2(11):1901–6. [PubMed: 9816147]
62. Chaturvedi R, et al. Spermine oxidase mediates the gastric cancer risk associated with Helicobacter pylori CagA. Gastroenterology. 2011; 141(5):1696–708. e1–2. [PubMed: 21839041]
63. Xu H, et al. Spermine oxidation induced by Helicobacter pylori results in apoptosis and DNA damage: implications for gastric carcinogenesis. Cancer research. 2004; 64(23):8521–5. [PubMed: 15574757]
64. Goodwin AC, et al. Increased spermine oxidase expression in human prostate cancer and prostatic intraepithelial neoplasia tissues. The Prostate. 2008; 68(7):766–72. [PubMed: 18302221]
65. Hong SK, et al. Increased expression and cellular localization of spermine oxidase in ulcerative colitis and relationship to disease activity. Inflammatory bowel diseases. 2010; 16(9):1557–66. [PubMed: 20127992]
66. Alhonen L, et al. Life-long over-expression of ornithine decarboxylase (ODC) gene in transgenic mice does not lead to generally enhanced tumorigenesis or neuronal degeneration. International journal of cancer. Journal international du cancer. 1995; 63(3):402–4. [PubMed: 7591239]
67. Guo Y, et al. Conversion of C57Bl/6 mice from a tumor promotion- resistant to a -sensitive phenotype by enhanced ornithine decarboxylase expression. Molecular carcinogenesis. 1999; 26(1):32–6. [PubMed: 10487519]
68. Guo Y, Cleveland JL, O'Brien TG. Haploinsufficiency for odc modifies mouse skin tumor susceptibility. Cancer research. 2005; 65(4):1146–9. [PubMed: 15734996]
69. Nilsson JA, et al. Targeting ornithine decarboxylase in Myc-induced lymphomagenesis prevents tumor formation. Cancer cell. 2005; 7(5):433–44. [PubMed: 15894264]
70. Heljasvaara R, et al. Transgenic mice overexpressing ornithine and S-adenosylmethionine decarboxylases maintain a physiological polyamine homoeostasis in their tissues. The Biochemical journal. 1997; 323(Pt 2):457–62. [PubMed: 9163338]
71. Shi C, et al. S-adenosylmethionine decarboxylase overexpression inhibits mouse skin tumor promotion. Carcinogenesis. 2012; 33(7):1310–8. [PubMed: 22610166]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

72. Scuoppo C, et al. A tumour suppressor network relying on the polyamine-hypusine axis. Nature. 2012; 487(7406):244–8. [PubMed: 22722845]
73. Shi C, et al. Characterization of transgenic mice with overexpression of spermidine synthase. Amino acids. 2012; 42(2-3):495–505. [PubMed: 21809077]
74. Kauppinen L, et al. Transgenic mice over-expressing the human spermidine synthase gene. The Biochemical journal. 1993; 293(Pt 2):513–6. [PubMed: 8343131]
75. Welsh PA, et al. Spermine synthase overexpression in vivo does not increase susceptibility to DMBA/TPA skin carcinogenesis or Min-Apc intestinal tumorigenesis. Cancer biology & therapy. 2012; 13(6):358–68. [PubMed: 22258329]
76. Feith DJ, Shantz LM, Pegg AE. Targeted antizyme expression in the skin of transgenic mice reduces tumor promoter induction of ornithine decarboxylase and decreases sensitivity to chemical carcinogenesis. Cancer research. 2001; 61(16):6073–81. [PubMed: 11507056]
77. Fong LY, Feith DJ, Pegg AE. Antizyme overexpression in transgenic mice reduces cell proliferation, increases apoptosis, and reduces N-nitrosomethylbenzylamine-induced forestomach carcinogenesis. Cancer research. 2003; 63(14):3945–54. [PubMed: 12873989]
78. Pietila M, et al. Relation of skin polyamines to the hairless phenotype in transgenic mice overexpressing spermidine/spermine N-acetyltransferase. The Journal of investigative dermatology. 2001; 116(5):801–5. [PubMed: 11348473]
79. Coleman CS, et al. Targeted expression of spermidine/spermine N1-acetyltransferase increases susceptibility to chemically induced skin carcinogenesis. Carcinogenesis. 2002; 23(2):359–64. [PubMed: 11872645]
80. Kee K, et al. Activated polyamine catabolism depletes acetyl-CoA pools and suppresses prostate tumor growth in TRAMP mice. The Journal of biological chemistry. 2004; 279(38):40076–83. [PubMed: 15252047]
81. Tucker JM, et al. Potent modulation of intestinal tumorigenesis in Apcmin/+ mice by the polyamine catabolic enzyme spermidine/spermine N1-acetyltransferase. Cancer research. 2005; 65(12):5390–8. [PubMed: 15958588]
82. Weeks CE, et al. alpha-Difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits tumor promoter-induced polyamine accumulation and carcinogenesis in mouse skin. Proceedings of the National Academy of Sciences of the United States of America. 1982; 79(19):6028–32. [PubMed: 6821130]
83. Prakash NJ, et al. Effect of alpha-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on L1210 leukemia in mice. Cancer research. 1978; 38(9): 3059–62. [PubMed: 679213]
84. Mamont PS, et al. Anti-proliferative properties of DL-alpha-difluoromethyl ornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochemical and biophysical research communications. 1978; 81(1):58–66. [PubMed: 656104]
85. Luk GD, et al. Polyamines are necessary for the survival of human small-cell lung carcinoma in culture. Proceedings of the National Academy of Sciences of the United States of America. 1981; 78(4):2355–8. [PubMed: 6264474]
86. Luk GD, et al. Successful treatment with DL-alpha-difluoromethylornithine in established human small cell variant lung carcinoma implants in athymic mice. Cancer research. 1983; 43(9):4239–43. [PubMed: 6409400]
87. Abeloff MD, et al. Phase II trials of alpha-difluoromethylornithine, an inhibitor of polyamine synthesis, in advanced small cell lung cancer and colon cancer. Cancer treatment reports. 1986; 70(7):843–5. [PubMed: 3013400]
88. Abeloff MD, et al. Phase I trial and pharmacokinetic studies of alpha- difluoromethylornithine--an inhibitor of polyamine biosynthesis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1984; 2(2):124–30. [PubMed: 6422008]
89. Mihich E. Current Studies with Methylglyoxal-Bis(Guanylhydrazone). Cancer research. 1963; 23:1375–89. [PubMed: 14070391]
90. Williams-Ashman HG, Schenone A. Methyl glyoxal bis(guanylhydrazone) as a potent inhibitor of mammalian and yeast S-adenosylmethionine decarboxylases. Biochemical and biophysical research communications. 1972; 46(1):288–95. [PubMed: 4550082]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

91. Nass MM. Analysis of methylglyoxal bis(guanylhydrazone)-induced alterations of hamster tumor mitochondria by correlated studies of selective rhodamine binding, ultrastructural damage, DNA replication, and reversibility. Cancer research. 1984; 44(6):2677–88. [PubMed: 6722801]

92. Secrist JA 3rd. New substrate analogues as inhibitors of S-adenosylmethionine decarboxylase. Nucleosides and Nucleotides. 1987; 6:73–83.

93. Wu Y, Woster PM. S-(5'-deoxy-5'-adenosyl)-1-ammonio-4-(methylsulfonio)-2-cyclopentene: A potent, enzyme-activated irreversible inhibitor of S- adenosylmethionine decarboxylase. Journal of medicinal chemistry. 1992; 35(17):3196–201. [PubMed: 1507205]

94. Danzin C, Marchal P, Casara P. Irreversible inhibition of rat S-adenosylmethionine decarboxylase by 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxyadenosine. Biochemical pharmacology. 1990; 40(7):1499–503. [PubMed: 2222506]

95. Barker RH Jr. et al. Novel S-adenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. Antimicrobial agents and chemotherapy. 2009; 53(5):2052–8. [PubMed: 19289530]

96. Byers TL, et al. Effects of chronic 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxy-adenosine (AbeAdo) treatment on polyamine and eIF-5 A metabolism in AbeAdo-sensitive and -resistant L1210 murine leukaemia cells. The Biochemical journal. 1993; 290(Pt 1):115–21. [PubMed: 8439281]

97. Eskens FA, et al. Phase I and pharmacological study of weekly administration of the polyamine synthesis inhibitor SAM 486A (CGP 48 664) in patients with solid tumors. European Organization for Research and Treatment of Cancer Early Clinical Studies Group. Clinical cancer research : an official journal of the American Association for Cancer Research. 2000; 6(5):1736–43. [PubMed: 10815892]

98. Zhou H, et al. Population pharmacokinetics/toxicodynamics (PK/TD) relationship of SAM486A in phase I studies in patients with advanced cancers. Journal of clinical pharmacology. 2000; 40(3): 275–83. [PubMed: 10709156]

99. Paridaens R, et al. A phase I study of a new polyamine biosynthesis inhibitor, SAM486A, in cancer patients with solid tumours. British journal of cancer. 2000; 83(5):594–601. [PubMed: 10944598]

100. Pless M, et al. Clinical efficacy, tolerability, and safety of SAM486A, a novel polyamine biosynthesis inhibitor, in patients with relapsed or refractory non-Hodgkin's lymphoma: results from a phase II multicenter study. Clinical cancer research : an official journal of the American Association for Cancer Research. 2004; 10(4):1299–305. [PubMed: 14977828]

101. Regenass U, et al. CGP 48664, a new S-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. Cancer research. 1994; 54(12):3210–7. [PubMed: 8205541]

102. Pegg AE, Tang KC, Coward JK. Effects of S-adenosyl-1,8-diamino-3-thiooctane on polyamine metabolism. Biochemistry. 1982; 21(20):5082–9. [PubMed: 6291600]

103. Pegg AE, et al. Effect of S-adenosyl-1,12-diamino-3-thio-9-azadodecane, a multisubstrate adduct inhibitor of spermine synthase, on polyamine metabolism in mammalian cells. Biochemistry. 1989; 28(21):8446–53. [PubMed: 2605194]

104. Porter CW, Bergeron RJ. Regulation of polyamine biosynthetic activity by spermidine and spermine analogs--a novel antiproliferative strategy. Advances in experimental medicine and biology. 1988; 250:677–90. [PubMed: 3076344]

105. Casero RA Jr. Woster PM. Terminally alkylated polyamine analogues as chemotherapeutic agents. Journal of medicinal chemistry. 2001; 44(1):1–26. [PubMed: 11141084]

106. Casero RA Jr. Woster PM. Recent advances in the development of polyamine analogues as antitumor agents. Journal of medicinal chemistry. 2009; 52(15):4551–73. [PubMed: 19534534]

107. Ha HC, et al. The role of polyamine catabolism in polyamine analogue-induced programmed cell death. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(21):11557–62. [PubMed: 9326648]

108. Pledgie A, et al. Spermine oxidase SMO(PAOh1), Not N1- acetylpolyamine oxidase PAO, is the primary source of cytotoxic H2O2 in polyamine analogue-treated human breast cancer cell lines. The Journal of biological chemistry. 2005; 280(48):39843–51. [PubMed: 16207710]

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109. Casero RA Jr. Gabrielson EW, Pegg AE. Immunohistochemical staining of human spermidine/spermine N1-acetyltransferase superinduced in response to treatment with antitumor polyamine analogues. Cancer research. 1994; 54(15):3955–8. [PubMed: 8033120]
110. Gabrielson EW, Pegg AE, Casero RA Jr. The induction of spermidine/spermine N1-acetyltransferase (SSAT) is a common event in the response of human primary non-small cell lung carcinomas to exposure to the new antitumor polyamine analogue N1,N11-bis(ethyl)norspermine. Clinical cancer research : an official journal of the American Association for Cancer Research. 1999; 5(7):1638–41. [PubMed: 10430062]
111. Bernacki RJ, et al. Preclinical antitumor efficacy of the polyamine analogue N1, N11-diethylnorspermine administered by multiple injection or continuous infusion. Clinical cancer research : an official journal of the American Association for Cancer Research. 1995; 1(8):847–57. [PubMed: 9816054]
112. Hahm HA, et al. Phase I study of N(1),N(11)-diethylnorspermine in patients with non-small cell lung cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2002; 8(3):684–90. [PubMed: 11895896]
113. Streiff RR, Bender JF. Phase 1 study of N1-N11- diethylnorspermine (DENSPM) administered TID for 6 days in patients with advanced malignancies. Investigational new drugs. 2001; 19(1):29–39. [PubMed: 11291831]
114. Creaven PJ, et al. Unusual central nervous system toxicity in a phase I study of N1N11 diethylnorspermine in patients with advanced malignancy. Investigational new drugs. 1997; 15(3):227–34. [PubMed: 9387045]
115. Davidson NE, et al. Clinical aspects of cell death in breast cancer: the polyamine pathway as a new target for treatment. Endocrine-related cancer. 1999; 6(1):69–73. [PubMed: 10732790]
116. Wolff AC, et al. A Phase II study of the polyamine analog N1,N11- diethylnorspermine (DENSpm) daily for five days every 21 days in patients with previously treated metastatic breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003; 9(16 Pt 1):5922–8. [PubMed: 14676116]
117. Hector S, et al. Polyamine catabolism in platinum drug action: Interactions between oxaliplatin and the polyamine analogue N1,N11-diethylnorspermine at the level of spermidine/spermine N1-acetyltransferase. Molecular cancer therapeutics. 2004; 3(7):813–22. [PubMed: 15252142]
118. Choi W, et al. Combination of 5-fluorouracil and N1,N11- diethylnorspermine markedly activates spermidine/spermine N1-acetyltransferase expression, depletes polyamines, and synergistically induces apoptosis in colon carcinoma cells. The Journal of biological chemistry. 2005; 280(5):3295–304. [PubMed: 15546879]
119. Pledgie-Tracy A, et al. The role of the polyamine catabolic enzymes SSAT and SMO in the synergistic effects of standard chemotherapeutic agents with a polyamine analogue in human breast cancer cell lines. Cancer chemotherapy and pharmacology. 2010; 65(6):1067–81. [PubMed: 19727732]
120. Casero RA Jr. et al. Growth and biochemical effects of unsymmetrically substituted polyamine analogues in human lung tumor cells 1. Cancer chemotherapy and pharmacology. 1995; 36(1):69–74. [PubMed: 7720179]
121. Varghese S, et al. Polyaminohydroxamic acids and polyaminobenzamides as isoform selective histone deacetylase inhibitors. Journal of medicinal chemistry. 2008; 51(8):2447–56. [PubMed: 18348516]
122. Varghese S, et al. Alkyl-substituted polyaminohydroxamic acids: a novel class of targeted histone deacetylase inhibitors. Journal of medicinal chemistry. 2005; 48(20):6350–65. [PubMed: 16190761]
123. Wu Y, et al. Oligoamine analogues in combination with 2-difluoromethylornithine synergistically induce re-expression of aberrantly silenced tumour-suppressor genes. The Biochemical journal. 2012; 442(3):693–701. [PubMed: 22132744]
124. Huang Y, et al. Inhibition of lysine-specific demethylase 1 by polyamine analogues results in reexpression of aberrantly silenced genes. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(19):8023–8. [PubMed: 17463086]

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125. Carew JS, et al. The novel polyamine analogue CGC-11093 enhances the antimyeloma activity of bortezomib. Cancer research. 2008; 68(12):4783–90. [PubMed: 18559525]

126. Hacker A, et al. In vitro and in vivo effects of the conformationally restricted polyamine analogue CGC-11047 on small cell and non-small cell lung cancer cells. Cancer chemotherapy and pharmacology. 2008; 63(1):45–53. [PubMed: 18301893]

127. Kuo WL, et al. A systems analysis of the chemosensitivity of breast cancer cells to the polyamine analogue PG-11047. BMC medicine. 2009; 7:77. [PubMed: 20003408]

128. Ignatenko NA, et al. Gene expression analysis of HCT116 colon tumor-derived cells treated with the polyamine analog PG-11047. Cancer genomics & proteomics. 2009; 6(3):161–75. [PubMed: 19487545]

129. Dredge K, et al. The polyamine analog PG11047 potentiates the antitumor activity of cisplatin and bevacizumab in preclinical models of lung and prostate cancer. Cancer chemotherapy and pharmacology. 2009; 65(1):191–5. [PubMed: 19685053]

130. Osland A, Kleppe K. Polyamine induced aggregation of DNA. Nucleic acids research. 1977; 4(3): 685–95. [PubMed: 17099]

131. Valasinas A, et al. Long-chain polyamines (oligoamines) exhibit strong cytotoxicities against human prostate cancer cells. Bioorganic & medicinal chemistry. 2003; 11(18):4121–31. [PubMed: 12927874]

132. Huang Y, et al. A novel polyamine analog inhibits growth and induces apoptosis in human breast cancer cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003; 9(7):2769–77. [PubMed: 12855657]

133. Marks P, et al. Histone deacetylases and cancer: causes and therapies. Nature reviews. Cancer. 2001; 1(3):194–202.

134. Arrowsmith CH, et al. Epigenetic protein families: a new frontier for drug discovery. Nature reviews. Drug discovery. 2012; 11(5):384–400. [PubMed: 22498752]

135. Shi Y, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004; 119(7):941–53. [PubMed: 15620353]

136. Federico R, et al. Inhibition of pig liver and Zea mays L. polyamine oxidase: a comparative study. Journal of enzyme inhibition. 2001; 16(2):147–55. [PubMed: 11342283]

137. Bianchi M, et al. Inhibition of polyamine and spermine oxidases by polyamine analogues. The FEBS journal. 2006; 273(6):1115–23. [PubMed: 16519678]

138. Huang Y, et al. Inhibition of lysine-specific demethylase 1 by polyamine analogues results in reexpression of aberrantly silenced genes. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(19):8023–8. [PubMed: 17463086]

139. Sharma SK, et al. (Bis)urea and (bis)thiourea inhibitors of lysine-specific demethylase 1 as epigenetic modulators. Journal of medicinal chemistry. 2010; 53(14):5197–212. [PubMed: 20568780]

140. Sharma SK, et al. Polyamine-based small molecule epigenetic modulators. Med. Chem. Commun. 2012; 3:14–21.

141. Huang Y, et al. Novel oligoamine analogues inhibit lysine-specific demethylase 1 and induce reexpression of epigenetically silenced genes. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009; 15(23):7217–28. [PubMed: 19934284]

142. Hazeldine S, et al. Low Molecular Weight Amidoximes that Act as Potent Inhibitors of Lysine-Specific Demethylase 1. Journal of medicinal chemistry. 2012; 55(17):7378–91. [PubMed: 22876979]

143. Tsuji T, et al. Induction of epithelial differentiation and DNA demethylation in hamster malignant oral keratinocyte by ornithine decarboxylase antizyme. Oncogene. 2001; 20(1):24–33. [PubMed: 11244502]

144. Yamamoto D, et al. Ornithine decarboxylase antizyme induces hypomethylation of genome DNA and histone H3 lysine 9 dimethylation (H3K9me2) in human oral cancer cell line. PloS one. 2010; 5(9):e12554. [PubMed: 20838441]

145. Meyskens FL Jr. et al. Effect of alpha-difluoromethylornithine on rectal mucosal levels of polyamines in a randomized, double-blinded trial for colon cancer prevention. J Natl Cancer Inst. 1998; 90(16):1212–8. [PubMed: 9719082]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

146. Meyskens FL Jr. et al. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev Res (Phila Pa). 2008; 1(1):32–8.

147. Huang L, et al. Phospho-sulindac (OXT-922) inhibits the growth of human colon cancer cell lines: a redox/polyamine-dependent effect. Carcinogenesis. 2010; 31(11):1982–90. [PubMed: 20627873]

148. Mackenzie GG, et al. Phospho-sulindac (OXT-328), a novel sulindac derivative, is safe and effective in colon cancer prevention in mice. Gastroenterology. 2010; 139(4):1320–32. [PubMed: 20600034]

149. Bailey HH. A Randomized, Double-Blind, Placebo controlled Phase 3 Skin Cancer Prevention Study of α-Difluoromethylornithine in Subjects with Previous History of Skin Cancer. Cancer Prev Res (Phila Pa). 2010; 3(1):35–47.

150. Alberts DS, et al. Chemoprevention of human actinic keratoses by topical 2-(difluoromethyl)-dl-ornithine. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2000; 9(12):1281–6.

151. Sinicrope FA, et al. Evaluation of difluoromethylornithine for the chemoprevention of Barrett's esophagus and mucosal dysplasia. Cancer prevention research. 2011; 4(6):829–39. [PubMed: 21636549]

152. Simoneau AR, et al. The effect of difluoromethylornithine on decreasing prostate size and polyamines in men: results of a year-long phase IIb randomized placebo-controlled chemoprevention trial. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008; 17(2):292–9.

153. Babbar N, Murray-Stewart T, Casero RA Jr. Inflammation and polyamine catabolism: the good, the bad and the ugly. Biochemical Society transactions. 2007; 35(Pt 2):300–4. [PubMed: 17371265]

154. Holley JL, et al. Targeting of tumor cells and DNA by a chlorambucil spermidine conjugate. Cancer research. 1992; 52(15):4190–5. [PubMed: 1638533]

155. Phanstiel OI, et al. The effect of polyamine homologation on the transport and cytotoxicity properties of polyamine-(DNA-intercalator) conjugates. The Journal of organic chemistry. 2000; 65(22):7710. [PubMed: 11076643]

156. Wang C, et al. Molecular requirements for targeting the polyamine transport system. Synthesis and biological evaluation of polyamine-anthracene conjugates. Journal of medicinal chemistry. 2003; 46(13):2672–82. [PubMed: 12801231]

157. Here are journal review articles that were not featured above which provide useful information on the topic of polyamines.

158. Pegg AE. Spermidine/spermine-N(1)-acetyltransferase: a key metabolic regulator. American Journal of Physiology-Endocrinology and Metabolism. 2008; 294(6):E995–1010. [PubMed: 18349109]

159. Wallace HM, Niiranen K. Polyamine analogues-an update. Amino Acids. 2007; 33:261–265. [PubMed: 17443267]

160. Wallace HM. The polyamines: past, present and future. Essays in Biochemistry. 2009; 46:1–9. [PubMed: 20095966]

161. Wang Y, Casero RA. Mammalian polyamine catabolism: a therapeutic target, a pathological problem, or both? Journal of Biochemistry (Tokyo). 2006; 139:17–25.

162. Seiler N. Thirty years of polyamine-related approaches to cancer therapy. retrospect and prospect. part 2. structural analogues and derivatives. Current Drug Targets. 2003; 4:565–585.

163. Cohen, SS. A guide to the Polyamines. Oxford University Press; New York, USA: 1998.

164. Pegg, AE.; Casero, RA. Polyamines: Methods and Protocols. Humana Press/Springer Science +Business Media; New York, USA: 2011.

165. Wallace, HM. The Polyamines: Small Molecules in the Omics Era. Portland Press; London, UK: 2009.

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NIH-PA Author Manuscript

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166. Wang, JY.; Casero, RA. Polyamine Cell Signaling: Physiology, Pharmacology, and Cancer Research. Humana Press; New Jersey, USA: 2006.

167. Woster, PM.; Casero, RA. Polyamines Drug Discovery. Royal Chemical Society Publishing; London, UK: 2011.

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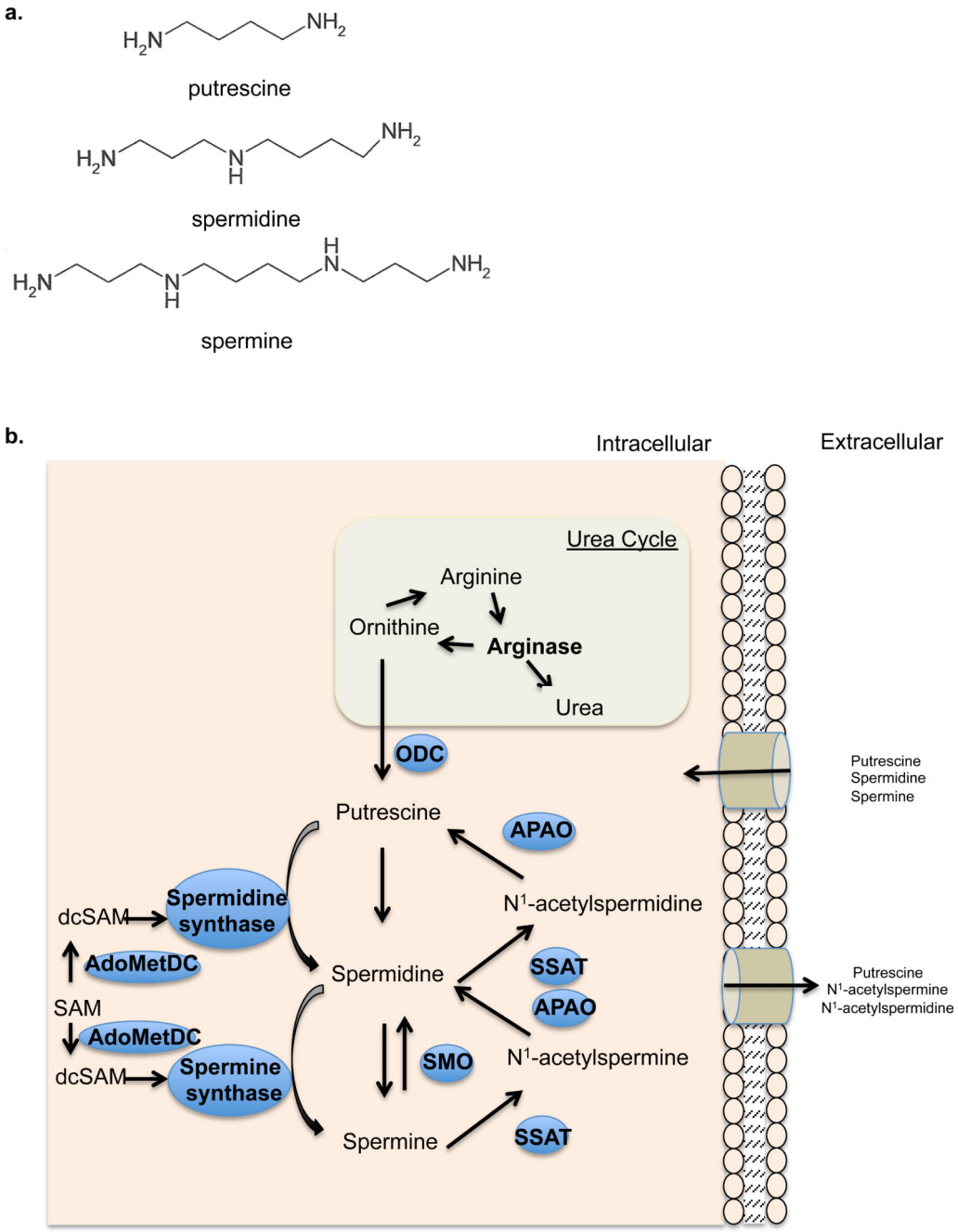

Figure 1. The polyamine pathway

(a) Schematic of putrescine and the higher polyamines spermidine and spermine. (b) The amino acid ornithine is a product of the urea cycle. Ornithine is converted to the diamine putrescine by the enzyme ornithine decarboxylase (ODC). Putrescine is then converted to the higher polyamines spermidine and spermine via spermidine synthase and spermine synthase, respectively. The decarboxylation of S-adenosylmethionine (SAM) by S-adenosylmethionine decarboxylase (AdoMetDC) produces decarboxylated SAM (dcSAM), which acts as the propyl amine donor for the formation of spermidine and spermine via the

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spermidine and spermine synthases. Spermidine/spermine N^1-acetyltransferase (SSAT) is a propylamine acetyltransferase that converts spermine and spermidine to N^1-acetylspermine and N^1-acetylspermidine, respectively. The acetylated polyamines can be either exported out of the cell via an undetermined transport system, or act as substrates for the polyamine oxidase (APAO). APAO catalyses the conversion of N^1-acetylspermine to spermidine and N^1-acetylspermidine to putrescine. Spermine oxidase (SMO) oxidizes non-acetylated spermine to form spermidine. Putrescine, spermidine, and spermine can also be imported into the cell via a poorly understood transport mechanism. All polyamine pathway enzymes are in blue.

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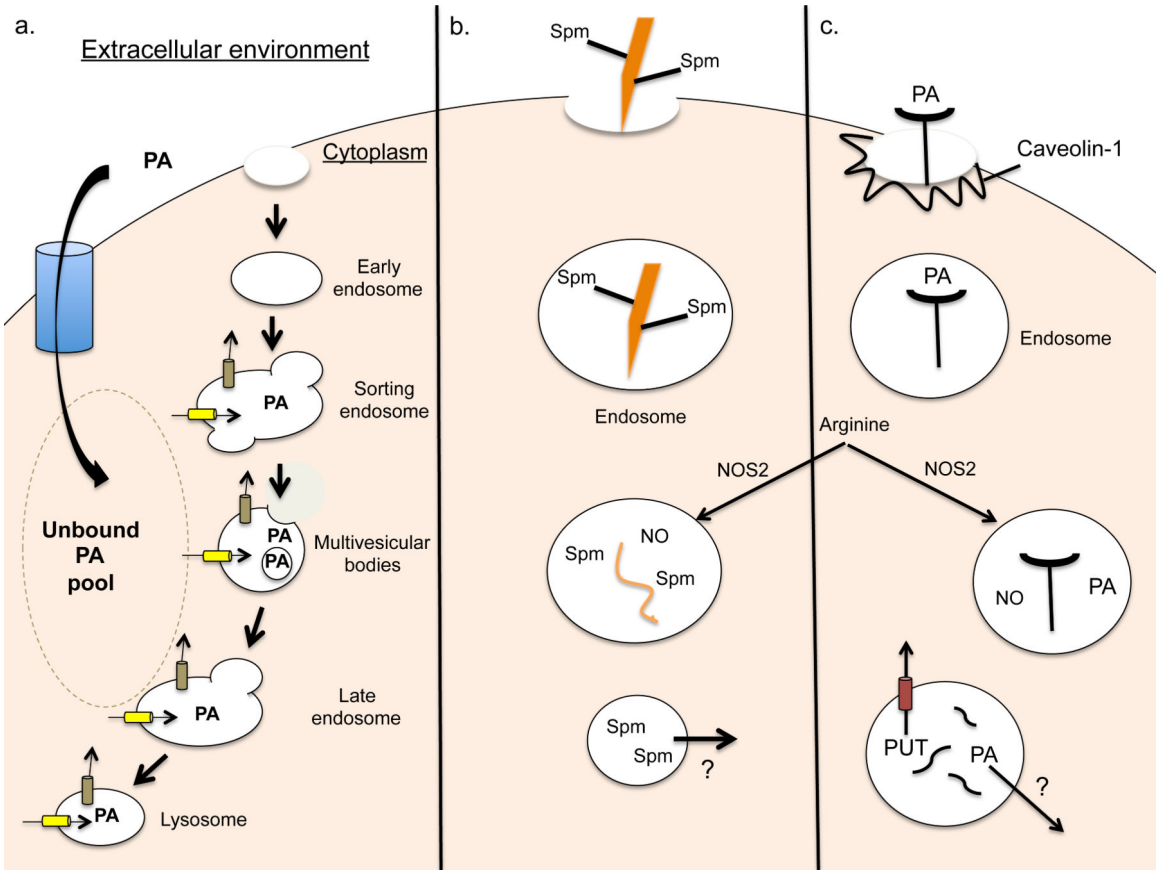

Figure 2. Theoretical models of the polyamine transport system

(a) This model proposes that polyamines (PA) are transported into cells via a yet to be identified transporter that is powered by membrane potential. Accumulated polyamines localize to polyamine-sequestering vesicles. This process is dependent on a vacuolar ATPase pH gradient and proton exchange. Yellow transporters represent vesicular polyamine exporters and brown transporters represent vesicular polyamine antiporters. A plasma membrane polyamine permease is shown in blue. (b) This model describes a mechanism by which heparan sulphate and glypican 1 (GPC1) work together to transport spermine (Spm). Spermine binds to heparan sulphate groups in GPC1 on the cell surface and is subsequently internalized (the GPC1 cell surface receptor is depicted in orange). Inside the cell, the spermine is freed by a NO-oxidation-mediated process through nitric oxide synthase-2 (NOS2). (c) This mechanism hypothesizes a caveolin-1-dependent internalization of polyamines that are bound to an unknown polyamine membrane receptor. In this model, only putrescine (PUT) is released from the vesicles through a SLC3A2 exporter. A NOS2-dependent reaction destabilizes the spermine-receptor complex in this model. This figure was adapted from Poulin et al. (Ref. 50).

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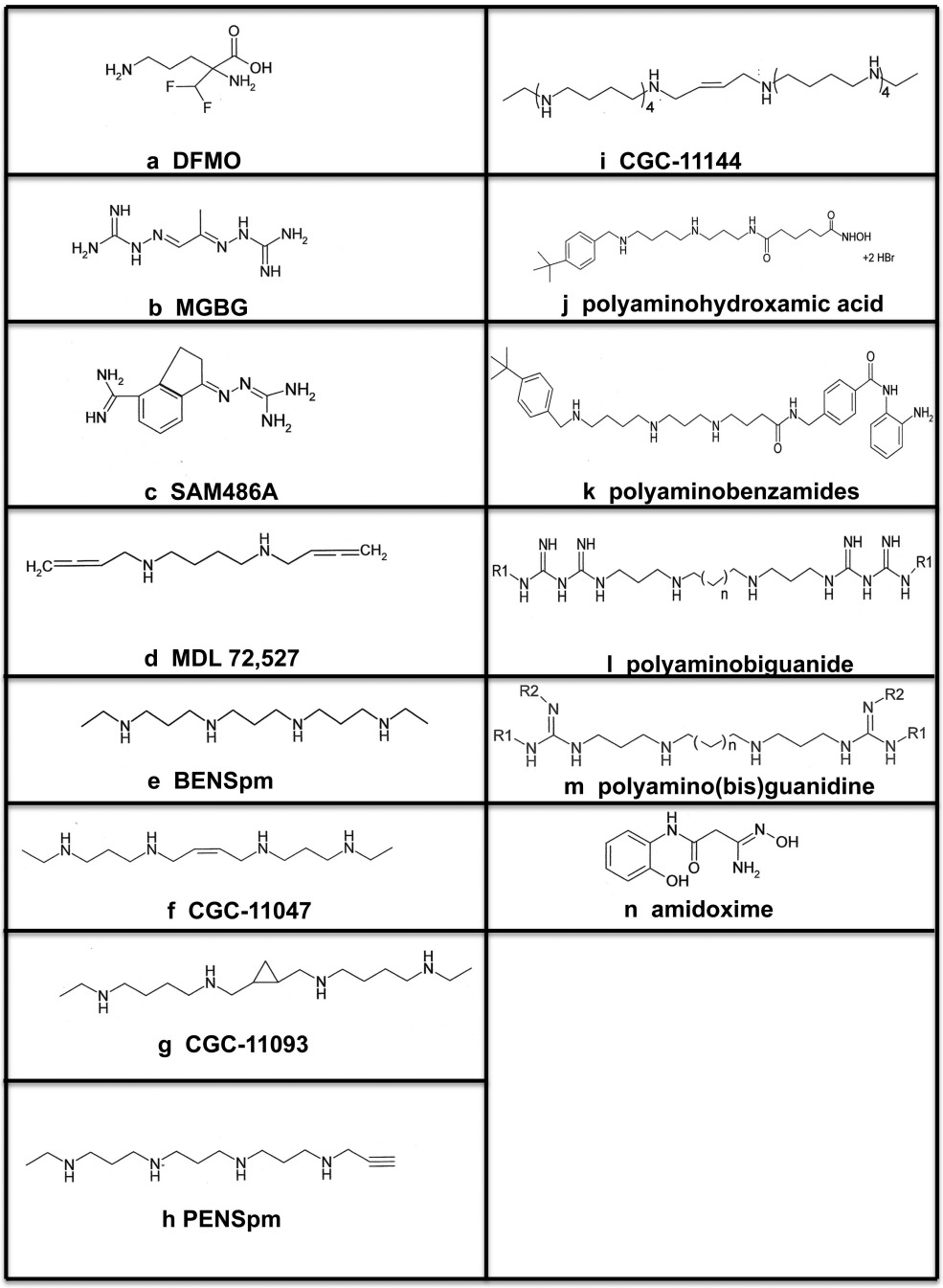

Figure 3. Representative agents targeting the polyamines, polyamine metabolism, and polyamine catabolism

(a) 2-difluoromethylornithine (DFMO) is an enzyme inhibitor for ODC. It causes a decrease in intracellular putrescine and spermidine pools. (b) Methylglyoxal bis(guanylhydrazone) (MGBG) is an enzyme inhibitor of AdoMetDC. It causes a decrease in spermidine and spermine levels while increasing the level of putrescine. It competes with the natural polyamines for uptake and is a mitochondrial toxin. (c) 4-amidoinoindan-1-one-2'-amidinhydrazone (SAM486A) is an enzyme inhibitor of AdoMetDC that has lower mitochondrial toxicity than MGBG. It decreases the levels of spermidine and spermine

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while increasing the level of putrescine. (d) N,N^1-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72,527) is an active site inhibitor of APAO that also inhibits SMO. (e) N^1,N^{11}-bis(ethyl)norspermine (BENSpm) is a symmetrically substituted spermine analogue. It induces SSAT and SMO and causes the depletion of putrescine, spermidine, and spermine. (f) CGC-11047 is a conformationally restricted polyamine analogue. It induces both SSAT and SMO and also decreases the levels of putrescine, spermidine, and spermine. (g) CGC-11093 is a conformationally restricted polyamine analogue but, unlike CGC-11047, it does not induce SSAT or SMO. (h) PENSpm is a non-symmetrically substituted polyamine analogue. It causes an induction of SSAT and cell type-specific cytotoxicity. (i) CGC-11144 is an oligoamine that has a high affinity for DNA. It competes with the natural polyamines for uptake and inhibits SMO activity and LSD1 activity. (j) Polyaminohydroxamic acids (PAHAs) incorporate features of the polyamines spermidine and spermine and the hydroxamic acid moiety of the HDAC inhibitors SAHA and Trichostatin A. (k) Polyaminobenzamides (PABAs) incorporate the benzamide moiety of MS-275 as well as features of spermidine and spermine. (l) Polyaminobiguanides are trypanocidal inhibitors and have recently been determined to inhibit LSD1 activity as well as cause the re-expression of some aberrantly silenced genes. (m) Polyamino(bis)guanidines are trypanocidal inhibitors and have recently been determined to inhibit LSD1 activity in cancer cells as well as cause the re-expression of aberrantly silenced genes. (n) Amidoximes were synthesized based on the success of certain polyaminobiguanide and polyamino(bis)guanidine compounds. Some from this series of compounds exhibited LSD1 inhibition as well as caused the re-expression of some aberrantly silenced genes.