Molecular mechanisms of polyamine analogs in cancer cells

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The natural polyamines are aliphatic cations with multiple functions and are essential for cell growth. Soon after the critical requirement of polyamines for cell proliferation was recognized, the metabolism of polyamines was pursued as a target for antineoplastic therapy. Initially, much attention was focused on the development of inhibitors of polyamine biosynthesis as a means to inhibit tumor growth. The best-characterized inhibitor is α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase. While compensatory mechanisms in polyamine metabolism reduce the effectiveness of DFMO as a single chemotherapeutic agent, it is currently undergoing extensive testing and clinical trials for chemoprevention and other diseases. There has been increasing interest over the last two decades in the cytotoxic response to agents that target the regulation of polyamine metabolism rather than directly inhibiting the metabolic enzymes in tumor cells. This interest resulted in the development of a number of polyamine analogs that exhibit effective cytotoxicity against tumor growth in preclinical models. The analogs enter cells through a selective polyamine transport system and can be either polyamine antimetabolites that deplete the intracellular polyamines or polyamine mimetics that displace the natural polyamines from binding sites, but do not substitute in terms of growth-promoting function. Synthesis of the first generation of symmetrically substituted bis(alkyl)polyamine analogs in the mid-1980s was based on the theory that polyamines may utilize feedback mechanisms to auto-regulate their synthesis. In the 1990s, unsymmetrically substituted bis(alkyl) polyamine analogs were developed. These compounds display structure-dependent and cell type-specific cellular effects and regulation on polyamine metabolism. More recently, a novel class of analogs has been synthesized, which include conformationally restricted, cyclic and long-chain oligoamine analogs. The development and use of these analogs have provided valuable information for understanding the molecular mechanisms of targeting the polyamine pathway as a means of cancer therapy. *Anti-Cancer Drugs* 16:229–241 © 2005 Lippincott Williams & Wilkins.

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Introduction

Polyamines (putrescine, spermidine and spermine) are naturally occurring, polycationic alkylamines that are absolutely required for cell growth. Most living organisms are able to synthesize intracellular polyamines from the precursor amino acids. The metabolic enzymes controlling polyamine concentrations are highly regulated and respond to changing environmental conditions [1–3]. The significance of polyamines for cell growth and function is reflected by the complex coordination of polyamine transport, uptake, synthesis and catalysis in mammalian cells. It has been realized for more than three decades that there is a strong connection between high levels of intracellular polyamines and increased cell proliferation. Since depletion of natural polyamine pools in cancer cells may result in the disruption of several important cellular functions and cytotoxicity, the polyamine metabolic pathway represents an important target for cancer therapy.

Initially, inhibitors of essentially all of the polyamine biosynthetic enzymes were developed and their antineoplastic activities were evaluated. Although these inhibitors may interrupt the polyamine metabolic pathway and lead to the cessation of tumor cell growth in *in vitro* and animal studies, the encouraging preclinical results did not translate well into the clinic [4]. Currently, the most actively pursued approach is the development and use of polyamine analogs as tumor growth inhibitors based upon the rationale that natural polyamines have several feedback mechanisms to regulate their own synthesis by reducing the rate of polyamine transport and increasing the rate of polyamine degradation and export [2,5]. Some polyamine analogs can mimic these regulatory properties of the natural polyamines and induce the catabolic process, inhibit polyamine biosynthesis, deplete the polyamine pools, and ultimately lead to the cessation of tumor cell growth [2,3,6]. In this review, we discuss the complexity of

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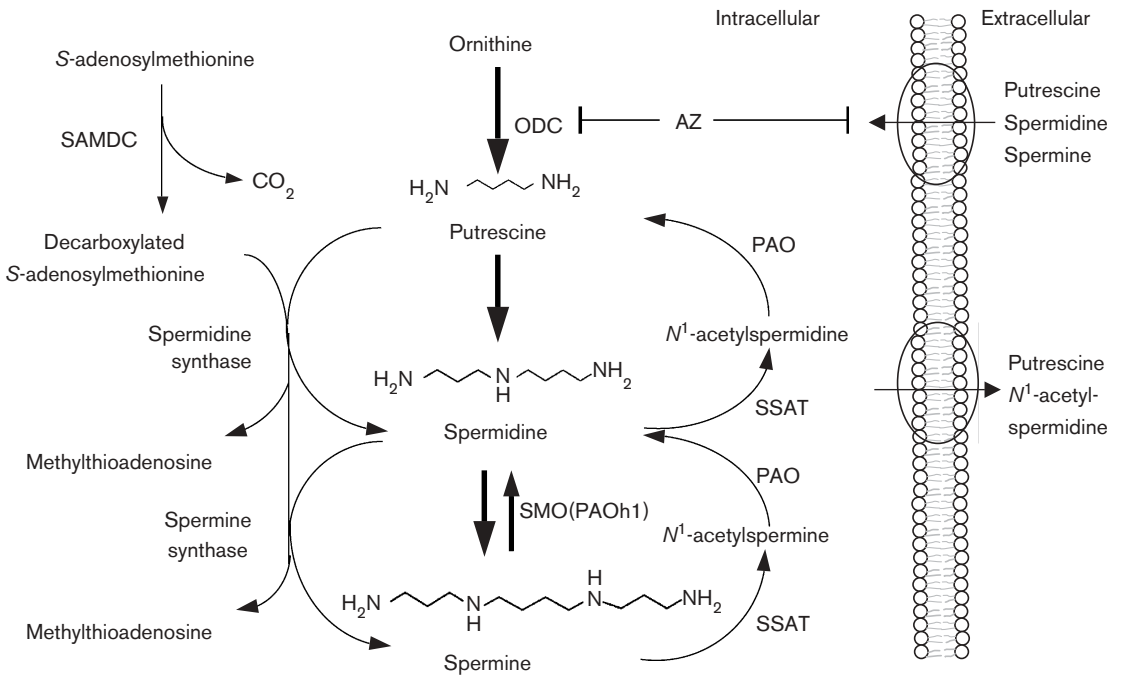
polyamine metabolism, the roles of polyamines in cell growth and tumorigenesis, and the mechanisms of action of different classes of polyamine analogs in cancer cells.

Polyamine function, metabolism and transport

The natural polyamines, putrescine, spermidine and spermine, are found in all eukaryotic cells and are essential for cell growth. Positively charged polyamines can interact electrostatically with negatively charged moieties such as DNA, RNA, proteins and phospholipids to stabilize and alter the structure and function of these molecules [2]. Polyamine-mediated DNA conformational changes play an important role in DNA structural stabilization and may be essential for the normal transcriptional activity of genes [7]. Functional interactions between polyamines and cellular anions may extend to DNA–protein interaction, post-translational modification, protein–protein interaction and enzyme activity [8]. Polyamines have been demonstrated to regulate membrane K^+ channel activity that is important in maintaining normal membrane electrical activity [9]. Polyamines may also function as second messengers in protein phosphorylation and signal transduction by promoting the activity of several important kinases in cell proliferation like MAP kinase and casein kinase II [10,11]. Other important cellular roles of polyamines include anti-oxidant, anti-apoptotic and metabolic regulatory functions [12].

The natural polyamines are synthesized in a highly regulated biochemical pathway [1–3]. As shown in Figure 1, polyamine biosynthesis is rate-limited by the polyamine biosynthetic enzymes, ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (SAMDC) (see the end of the article for a full list of abbreviations). The amino acid, ornithine, is converted to putrescine in a decarboxylation reaction catalyzed by ODC. SAMDC forms decarboxylated *S*-adenosylmethionine (dcAdoMet) that serves as an aminopropyl donor in the synthesis of spermidine and spermine by separable aminopropyl transferases. The regulatory protein ODC-antizyme (AZ) can bind to ODC and facilitate the degradation of AZ–ODC complex by the 26S proteasome in a non-ubiquitin-dependent manner [13]. AZ mRNA contains two overlapping open reading frames (1 and 2) and is regulated in a post-transcriptional manner. The presence of increased intracellular polyamine or analog levels can lead to enhanced efficiency of AZ mRNA frameshifting from reading frame 1 which contains an initiation codon to reading frame 2 where most of the functionally active AZ protein is produced [14]. Four forms of AZ (AZ1–4) have been identified, but only AZ1 has strong association with degradation of ODC [14]. The aminopropyltransferase reactions that form natural spermidine and spermine are essentially irreversible, but the catabolism of spermine to putrescine can be accomplished by the activity of two catabolic pathways. The first pathway relies on the acetylation of spermine and

Fig. 1



Polyamine metabolic pathways. See 'Abbreviations' for definitions.

spermidine by the rate-limiting enzyme spermidine/spermine N^1-acetyltransferase (SSAT) to form N^1-acetylspermine and N^1-acetylspermidine, respectively. Acetyl derivatives are then cleaved into 3-acetamidopropanal, H_2O_2, spermidine and putrescine through the action of FAD-dependent polyamine oxidase (PAO). Wang *et al.* and Vujcic *et al.* recently identified a second polyamine catabolic pathway by cloning a variably spliced human spermine oxidase SMO(PAOh1). SMO(PAOh1) uses unacetylated spermine as substrate and is inducible by specific polyamine analogs [15,16]. These findings indicate a complex polyamine catabolic pathway that may contribute to polyamine homeostasis and response to polyamine analogs.

Eukaryotic polyamine uptake is known to be an energy-dependent and carrier-mediated process, and is critical in maintaining cellular polyamine homeostasis. Although much is recognized about the polyamine transport system in prokaryotes, considerably less is understood about the cell membrane polyamine transport system in eukaryotic cells. Soulet *et al.* recently proposed a putative two-step process of eukaryotic polyamine transport where polyamines first enter the cells by a plasma membrane carrier and are then sequestered into pre-existing polyamine-sequestering vesicles via a mechanism that requires an outwardly directed H^+ gradient [17]. It is important to note that the feedback mechanism may regulate the polyamine transport system to ensure intracellular polyamine homeostasis. One example is that AZ induction has recently been found to negatively regulate the eukaryotic polyamine transport system [18].

Polyamines and cancer

Polyamine concentrations and biosynthetic enzyme activities are generally higher in tumor cells as compared to their normal counterparts [19,20]. The key polyamine synthesis enzyme, ODC, is considered to be a proto-oncogene product, and a marker of carcinogenesis and tumor progression. Enhanced levels of ODC activity compared with normal tissues have been detected in several solid tumors [21,22]. Overexpression of ODC in NIH 3T3 cells led to the transformation of these cells [23]. Transgenic mice overexpressing the ODC gene developed skin cancer with typical administration of carcinogen alone, while normal mice required the administration of carcinogen and a tumor promoter [24]. In tissue specimens of prostate cancer patients, ODC expression and activity were substantially higher in cancerous tissues as compared with the normal surrounding tissues [25]. Colorectal mucosa specimens from familial adenomatous polyposis (FAP) patients show higher ODC activity and polyamine levels than those from genotypic normal relatives [26]. In estrogen receptor (ER)-positive breast cancer cells, estradiol up-regulates ODC and increases polyamine levels, which promotes the breast cell proliferation [27]. Antiestrogen tamoxifen-induced growth inhibition of breast cancer cells is associated with the down-regulation of ODC activity and polyamine biosynthesis [28]. Moreover, treatment with ODC or SAMDC inhibitors decreased intracellular polyamine pools and inhibited the growth of a variety of tumor cells [29,30]. In mice bearing human breast cancer MDA-MB-435 xenografts, DFMO almost completely prevented the development of pulmonary metastasis, which supports a role of ODC and polyamines in promoting the distant metastasis of breast cancer [31].

The above studies demonstrate that polyamines are important for tumor development. It is noteworthy that a high level of polyamines has also been found in diseases other than malignant conditions, such as cystic fibrosis, muscular dystrophy, psoriasis and diabetes [32–35]. Elevated polyamines have also been detected after stress including metabolic, mechanical, chemical and ischemia injury [36,37]. These clinical findings suggest that the application of polyamine measurements in body fluids as a diagnostic tool for cancer patients will be limited. Nevertheless, the clear association between elevated polyamine content and tumorigenesis implies that depletion of polyamines could be a practical strategy for antineoplastic therapy, and measuring polyamine levels could be a useful tool in predicting therapeutic efficacy.

Development of polyamine analogs as effective antineoplastic agents

Polyamine biosynthesis inhibitors

Because of the requirement for polyamines in mammalian cell growth and the demonstration of dysregulated polyamine metabolism in tumor cells, polyamine metabolism became a logical target for cancer therapy. Initially, inhibitors of essentially all of the biosynthetic enzymes were developed and their antineoplastic activities were evaluated [2]. However, most work focused on the rate-limiting polyamine biosynthesis enzymes, ODC and SAMDC, as targets. The best characterized among the inhibitors is α-difluoromethylornithine (DFMO), an enzyme-activated, irreversible inhibitor of ODC [38]. Despite success in *in vitro* and preclinical animal models, clinical trials with DFMO were disappointing and the compound failed to demonstrate lasting antineoplastic effect [4]. The major problems of DFMO as a monotherapeutic strategy include poor transport, complex regulatory mechanisms leading to the compensatory changes in metabolism, rapid turnover of ODC and toxicity (thrombocytopenia, anemia, gastrointestinal reaction and ototoxicity) at high therapeutic doses [29]. Currently, DFMO is the front-line agent in the treatment of African trypanosomiasis and is undergoing clinical trials as a potential chemopreventive drug [29,39]. Other representative biosynthesis inhibitors include the ODC inhibitors α-monofluoromethylornithine (MFMO) and

Table 1 Inhibitors of polyamine biosynthesis enzymes

Ornithine decarboxylase	α-difluoromethylornithine (Eflornithine, Ornidyl) (DFMO)
	α-monofluoromethylornithine (MFMO)
	(2*R*,5*R*)-δ- methylacetylenicputrescine (MAP or MDL 72,175)
S-Adenosylmethionine decarboxylase	methylglyoxal bis(guanylhydrazone) (MGBG)
	4-amidinoindan-a-one 2′-amidinohydrazone (CGP-48664 or SAM486A)
	S-(5′-deoxy-5′-adenosyl)-methylthioethylhydroxylamine (AMA)
	5′-{[(*Z*)-4-amino-2-butenyl] methylamino}-5′-deoxy-adenosine (AbeAdo)
	[2,2-bipyridine]-6,6′-dicarboximidamide (CGP-39937)
Spermidine synthase	*S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD)
Spermine synthase	*S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO)

(2*R*,5*R*)-δ-methylacetylenicputrescine (MAP or MDL 72,175), the SAMDC inhibitors methylglyoxal bis (guanylhydrazone) (MGBG), 4-amidinoindan-a-one 2′-amidinohydrazone (CGP-48664 or SAM486A), *S*-(5′-deoxy–5′-adenosyl)-methylthioethylhydroxylamine (AMA), 5′-{[(*Z*)-4-amino–2-butenyl]methylamino}-5′-deoxyadenosine (AbeAdo) and [2,2-bipyridine]-6,6′-dicarboximidamide (CGP-39937), the spermidine synthase inhibitor *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD) and the spermine synthase inhibitor *S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) (Table 1). Unfortunately, these compounds were also of limited clinical usefulness [2,40].

Symmetrically alkylated polyamine analogs

Because of the limited success of polyamine biosynthetic inhibitors as effective chemotherapeutic agents, interest has been turned to the development of polyamine analogs based upon the theory that natural polyamines have several feedback mechanisms to regulate their own synthesis. Symmetrically substituted bis(alkyl)polyamine analogs represented the first generation of these analogs. Examples includes *N*,*N*′-bis(ethyl)polyamines, which include N^1,N^{11}-bis(ethyl)norspermine (BENSpm or BE-3-3-3), N^1,N^{12}-bis(ethyl)spermine (BESpm, or BE-3-4-3), N^1,N^{14}-bis(ethyl) homospermine (BEHSpm or BE-4-4-4), N^1,N^{15}-bis-[3-(ethylamino)-propyl]-1-17-heptane diamine (BE-3-7-3), N^1,N^{15}-bis(ethylamino)-4,8,12-triazapentadecane (BE-3-3-3-3) and N^1,N^{19}-bis(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4) (Fig. 2) [41]. These analogs utilize the energy-dependent polyamine transport system and are readily accumulated by tumor cells. Their accumulation generally results in a net reduction of the natural polyamine pool, decrease in ODC and SAMDC activities, as well as cytotoxicity in certain types of tumor cells [6,42]. In specific instances in human non-small cell lung cancer (NSCLC), melanoma and breast cancer cells, these analogs result in tremendous induction of SSAT by as much as several thousand fold ('superinduction') [43–45]. The induction of SSAT enzymatic activity is a result of analog-induced transcription, increased message stability, enhanced translational efficacy and protein stability [46–49]. Studies using melanoma and pancreatic adenocarcinoma cells suggested that cytotoxicity produced by symmetrical bis(ethyl)polyamines analogs is correlated with the induction of SSAT activity in these cells [44,50]. Perhaps the most successful alkypolyamine to date is BENSpm, an analog that shows exceptional promise as an antitumor agent in both *in vitro* and *in vivo* studies. However, a recent phase II clinical trial of BENSpm in breast cancer patients showed that this analog can be safely administered to women with advanced refractory breast cancer, but was not effective as a single agent [51]. Combination of BENSpm with standard cytotoxic agents is currently being examined [52].

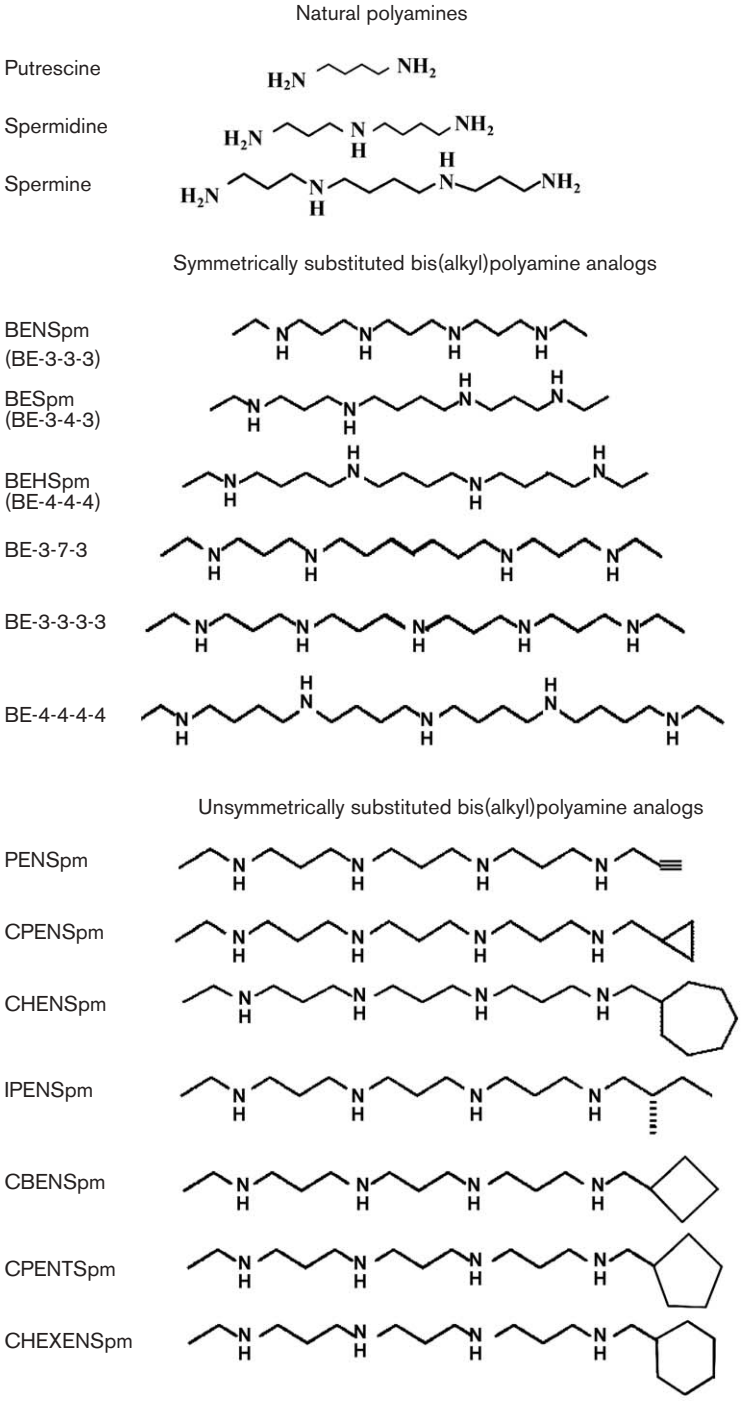
Unsymmetrically substituted polyamine analogs

In the 1990s, a second generation of polyamine analogs termed unsymmetrically substituted bis(alkyl)polyamines was developed by Woster *et al.* at Wayne State University [53]. The first synthesized unsymmetrically substituted analogs were N^1-propargyl-N^{11}-ethylnorspermine (PENSpm) and N^1-(cyclopropylmethyl)-N^{11}-ethyl-4,8-diazaundecane (CPENSpm) (Fig. 2). Both compounds exhibited cytotoxicity and induction of SSAT similar to or greater than symmetrically substituted analogs like BESpm [53]. As with symmetrically substituted analog-treated tumor cells, the cytotoxicity of PENSpm and CPENSpm was correlated with the induction of SSAT mRNA levels and activity [53]. Two other representatives of this class of compounds, N^1-(cycloheptylmethyl)-N^{11}-ethyl-4,8-diazaundecane (CHENSpm) and (*S*)-N^1-(2-methyl-1-butyl)-N^{11}-ethyl-4,8-diazaundecane (IPENSpm), exhibited a more potent growth-inhibitory effect against *in vitro* tumor cell growth, but with only modest SSAT induction and polyamine depletion [54]. In addition, CHENSpm and IPENSpm, but not CPENSpm, could alter microtubule polymerization and induce G_2/M cell cycle arrest in tumor cells [55]. These data imply that small modifications in backbone structure of unsymmetrically substituted polyamine analogs have profound consequences on cellular effects and cytotoxicity produced by these compounds.

Conformationally restricted, cyclic and long-chain oligoamine analogs

Recently, a third generation of conformationally restricted analogs has been developed [56–58]. These agents incorporate alterations into the free rotation of the single bonds in otherwise flexible molecules such as spermine or the analogs, thus restricting the molecular conformation that they may assume. Such modifications can alter the ability of analogs to bind DNA, tRNA or other polyamine-binding sites by introducing bends, kinks or loops at their binding domains [56,59]. One class of conformationally restricted analogs comprises the tetramines (homospermine analogs) (Fig. 3). In these compounds, the external

Fig. 2



Chemical structures of natural polyamines, and symmetrically and unsymmetrically substituted bis(alkyl)polyamine analogs.

aminopropyl residues are replaced by aminobutyl residues, making a homospermine backbone. By introduction of alicyclic residues or one or two *cis* double bonds in the homospermine backbone, free rotation at the central part of the molecule is restricted [58,60]. Another class of conformational restricted analogs is termed pentamine analogs. Their free rotating conformation is also restricted by the introduction of *cis* or *trans* double bonds into the

hydrocarbon skeleton or hydrophilic groups into the pentamine structure [58,60] (Fig. 3). Oligoamine analogs have longer chains than natural polyamines and each NH_2^+ residue is separated by four CH_2 residues [57,58]. Oligoamines consist of synthetic octa-, deca-, dodeca- and tetradecamines. The rationale behind the synthesis of oligoamines is that spermine at a concentration range of 50–100 μM and at near physiological ionic strength, leads to the collapse of DNA [61]. Oligoamines were found to initiate DNA aggregation at much lower concentrations (around 2–4 μM) than spermine and are more cytotoxic against several different types of cultured cancer cells as compared to other analogs tested so far [56,59,62]. In human prostate carcinoma cells, oligoamines are markedly more cytotoxic against tumor cells in culture than many previously described polyamine analogs [57]. A recent study from our group demonstrated that oligoamines effectively inhibit growth of human breast cancer cell lines in cell culture and nude mouse xenografts [62].

Alteration of polyamine metabolic enzyme activities by polyamine analogs

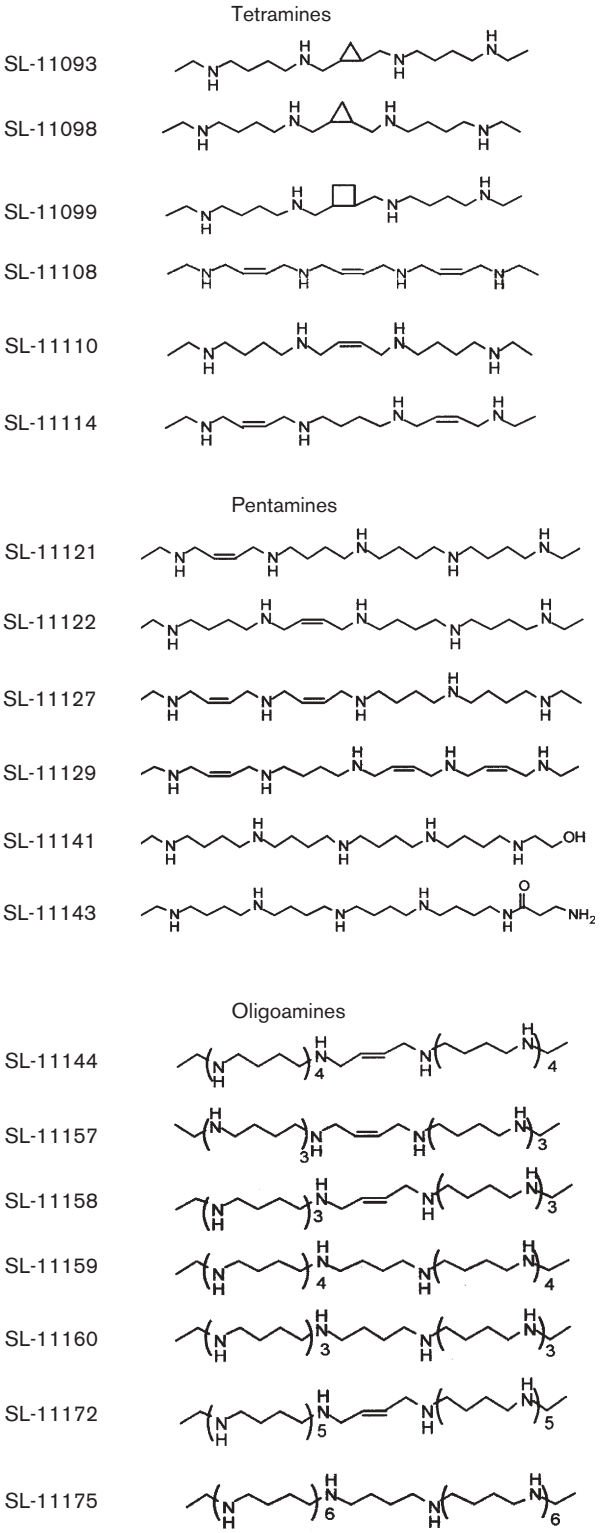
The requirement for polyamine action in cell growth and cancer progression has underscored the rationale of targeting polyamine metabolism as a therapeutic strategy. Although the *in vitro* success of DFMO did not translate well into the clinic, it indeed provides a proof of principle for targeting the polyamine metabolic pathway as a strategy for antineoplastic intervention. New strategies using the polyamine analogs may circumvent some problems encountered with DFMO for the following reasons: (i) unlike DFMO, polyamine analogs utilize the polyamine transporter system and compete with the uptake of the natural polyamines; (ii) polyamine analogs indirectly down-regulate ODC activity through induction of ODC antizyme, which may prevent the rapid turnover of ODC seen in DFMO-treated cells and therefore produce a steady net reduction in natural polyamines; and (iii) some polyamine analogs-induce the activity of the rate-limiting catabolic enzymes, SSAT and SMO(PAOh1), that produce cytotoxic H_2O_2, and therefore are more effective in tumor growth inhibition.

A recent study of 24 polyamine analogs, including tetramines, pentamines and various oligoamines, demonstrated that almost all of these analogs stimulated ODC-AZ synthesis and there was a good correlation between AZ induction and cytotoxicity [60]. Although the AZ induction contributes to down-regulation of ODC activity and depletion of polyamines by many of these analogs, it may also inhibit the activity of polyamine transport system and therefore limit the uptake and effectiveness of polyamine analogs [18]. Better understanding of the mechanisms underlying the inhibition of AZ on polyamine transport system may aid in the development of more effective agents that circumvent this problem.

Recently, there has been increasing interest in the role that polyamine catabolism plays in determining tumor cell response to analogs. This interest is a direct result of the findings that several different polyamine analogs such as BENSpm and CPENSpm significantly up-regulate the activities of polyamine catabolic enzymes. As described above, induction of SSAT in response to analogs and, in some cases, 'superinduction' of several thousand fold are the most interesting features of the response of this enzyme to the polyamine analogs [3,41]. The molecular mechanisms of the transcriptional regulation of SSAT are currently not fully understood. By using deletion/mutation and reporter construct strategies, a 9-bp consensus sequence (5′-TATGACTAA-3′) that functions as a polyamine response element (PRE) and its associated transcription factors have been recently identified in the SSAT promoter [63]. This element was subsequently used to probe an expression library produced from BENSpm-induced lung cancer cells, which led to the identification of NF-E2-related transcription factor (Nrf-2), a protein that was constitutively bound to the PRE [63]. Importantly, Nrf-2 expression was only observed in the analog-responsive cell lines with increased SSAT expression, suggesting that Nrf-2 is a specific regulator of analog mediated SSAT induction. A yeast two-hybrid screen using the leucine-zipper domain of Nrf-2 as bait identified a transcription co-factor designated polyamine-modulated factor-1 (PMF-1) [64]. Like SSAT, PMF-1 expression significantly increased with analog treatment in analog-sensitive tumor cell lines. Because of the lack of a DNA-binding domain, PMF-1 appears to modulate SSAT transcription indirectly through a unique leucine-zipper–coiled-coil interaction with Nrf-2 [65].

During the search for the human PAO gene, Wang *et al.* identified and characterized a protein, named human polyamine oxidase (PAOh1) [15], with a molecular weight similar to that of the PAO previously found by Holtta [66]. Vujcic *et al.* used a similar technique to find the identical clone termed spermine oxidase (SMO) [16]. The function of SMO(PAOh1) as a spermine oxidase has been confirmed [15,67,68]. To date, at least four splice variants of human and mouse SMO(PAOh1) genes have been identified [69,70]. These variants have unique biochemical properties that are capable of using multiple substrates including spermine, spermidine and acetylspermine [69]. However, the *in situ* relevance is not known. Rapid increase of SMO(PAOh1) mRNA levels in response to polyamine analog results in the enhanced oxidase activity, which occurs in a tumor and agent specific manner [71]. These findings suggest the potential of the newly identified SMO(PAOh1) family as a target for antineoplastic intervention. The contribution of analog-induced SMO(PAOh1) activity to analog cytotoxicity is currently being assessed and the preliminary results suggest that it does play a role in tumor cell response to specific agents. Understanding of the

Fig. 3



Chemical structures of tetramines, pentamines and oligoamines.

regulation of both SSAT and SMO(PAOh1) will provide valuable information in more selectively targeting cancer cells with newly designed analogs.

Disruption of polyamine–DNA interaction by polyamine analogs

Although the rapid cell death produced by several polyamine analogs has been well characterized, the exact mechanisms involved in this cytotoxicity have not been fully elucidated. A number of studies have suggested that DNA is a major target for the function of polyamines [72]. Polyamine–DNA interactions play a pivotal role in DNA conformational transitions, condenstation/decondensation and stabilization [73,74]. Functional interaction between DNA and polyamines extends to DNA–protein binding, particularly those involving gene regulatory proteins [75]. As a result of the structural similarity, polyamine analogs might play a similar function leading to interference with the normal interactions of polyamine and DNA. Tumor cells treated with cytotoxic analogs are more sensitive than untreated or non-toxic analog-treated cells to nuclease digestion, suggesting a correlation between the ability of an analog to alter the chromatin structure and the analog cytotoxicity [76]. On the basis of computer modeling and physical/chemical studies of polyamine–DNA interactions, several polyamine analogs were synthesized by modifying the charge distribution along the surface of the aliphatic polyamine backbone. These compounds exhibited enhanced cell killing activity in human brain tumor cell lines [77]. A recent study has demonstrated a general correlation between the effects of newly developed long-chain oligoamine analogs on *in vitro* DNA aggregation and their cytotoxic effects in prostate cancer DuPro cells. By comparing the concentrations of oligoamines required to induce DNA aggregation with the concentrations necessary to kill tumor cells, it is apparent that oligoamines with a greater ability to aggregate DNA are more cytotoxic [57]. These studies provide evidence to suggest that modification of polyamine analog structures may affect their DNA binding abilities and, in turn, their cytotoxicity.

Effect of polyamine analogs on cell cycle regulation

It has been known for many years that natural polyamines are needed for normal cell cycle progression. Cellular polyamine levels and ODC activity peak at specific points during the cell cycle. ODC is activated in a biphasic manner with a first burst at late G_1 phase and a second one during the S/G_2 transition [78]. Early studies indicated that polyamines were essential for cells to enter S phase and polyamine depletion led to the inhibition of G_1/S phase transition [79]. Recent studies using an ODC inhibitor demonstrated that inhibition of ODC activity can lead to G_1 arrest in a variety of cell lines including tumor cells [80,81]. In human melanoma cells expressing wild-type p53, treatment with BENSpm activated the p53/p21/pRb cell cycle regulatory pathway leading to cell cycle block at G_1 phase, but in human melanoma cells lacking p53, no G_1 arrest was observed after BENSpm treatment [82]. Other studies indicated that treatment with BENSpm caused a delay of S phase progression and prolongation of the other cell cycle phases occurred at later time points [83]. Our recent studies indicated that the oligoamine analogs down-regulated cyclin D1 protein, inhibited CDK activity, activated the p53/p21/pRb pathway and induced cell cycle arrest at G_1 phase in breast cancer MCF-7 cells (Huang *et al.*, unpublished data). These results clearly suggest that the polyamines and regulatory enzymes play important roles in the progression of each phase of cell cycle and disruption of polyamine metabolism by polyamine biosynthesis inhibitors or analogs may lead to the cell cycle arrest in cancer cells.

Webb *et al.* found that the unsymmetrically substituted polyamine analogs, CHENSpm and IPENSpm, produced a significant G_2/M arrest in human NSCLC H157 cells [55]. As compared with spermine, these analogs exhibited different effects on tubulin polymerization, both in the presence and absence of microtubule-associated proteins (MAPs). Using immunohistochemical staining for tubulin, CHENSpm was found to alter the microtubule density in the putative centrosome adjacent to the nucleus, but did not affect the cytoplasmic microtubules. The mechanism underlying the effect of these analogs on tubulin polymerization is not clear. It is possible that the loss of mitochondrial membrane potential is correlated with the observed alteration of tubulin polymerization in analog-treated tumor cells [84]. These findings indicate a completely novel antitumor mechanism for a subclass of structurally similar unsymmetrically substituted analogs. Their ability to change cell cycle transition and tubulin polymerization is evidently structure dependent.

Polyamine analog-induced programmed cell death

Interaction with chromatin, displacement of natural polyamines from their cellular binding sites, induction of polyamine catabolism and depletion of mitochondrial DNA have been proposed as possible mechanisms underlying the antitumor action of polyamine analogs [41,85]. Recent studies indicated that programmed cell death might be a common characteristic for polyamine analog-induced cytotoxicity in cancer cells. Polyamine analog-induced apoptotic cell death was first observed by our group following CPENSpm treatment of the breast cancer cell lines, MCF-7 and MDA-MB-468, as well as the H157 NSCLC human lung tumor cell line [45,86]. To date, most of the bis(ethyl)polyamine analogs have been found to induce apoptosis in a variety of tumor cell lines [87–90]. However, the mechanisms involved in

polyamine analog-induced apoptosis have not been adequately defined. Catalysis of polyamines through analog-induced SSAT and PAO activities produces H_2O_2 as a byproduct, suggesting that analog-induced apoptosis may be, in part, due to oxidative stress resulting from H_2O_2 production. This hypothesis is supported by the finding that co-addition of catalase reduced high-molecular-weight DNA fragmentation, and inhibition of PAO activity by the PAO inhibitor, MDL 72,527, significantly reduced DNA damage and delayed apoptosis in CHENSpm-treated H157 cells [86]. In a recent study, Chen *et al.* demonstrated that SSAT siRNA, which specifically suppressed polyamine analog-induced SSAT activity, prevented the depletion of polyamine pools by analog and inhibited analog-induced apoptosis in human melanoma SKMEL-28 cells [91]. This mechanism of analog-induced cytotoxicity has important implications for the development of new analogs because SSAT induction occurs in some important solid tumors, but not generally in normal tissues.

Although the production of H_2O_2 by SSAT and PAO activity evidently plays an important role in apoptosis induced by some analogs, it is obviously not the only mechanism by which polyamine analogs kill tumor cells. For example, CHENSpm does not superinduce SSAT, but still induces significant apoptotic cell death in tumor cells [84]. Also, a novel oligoamine analog, SL-11144, induced oligonucleosome DNA fragmentation and activated apoptotic pathways in several human breast cancer cell lines without superinduction of SSAT activity. Multiple apoptotic mechanisms were associated with SL-11144 cytotoxic effects in specific breast cancer cell lines [62]. These results show that polyamine analog-induced apoptosis may occur through multiple structure-related and cell type-specific mechanisms. Typical features of apoptosis including cytochrome *c* release, activation of caspase-3 and cleavage of poly(ADP-ribose) polymerase (PARP) have been observed in oligoamine SL-11144-treated breast cancer MDA-MB-435 cells [62], BENSpm-treated melanoma SK-MEL-28 cells [87], and CPENSpm- and CHENSpm-treated lung cancer NCI H157 cells [86]. In H157 cells stably transfected with a Bcl-2 cDNA expression vector, CHENSpm-induced cytochrome *c* release, caspase-3 activation and PARP cleavage were significantly inhibited [84], suggesting that Bcl-2 activity may modulate the effect of polyamine analog on mitochondrial function and the activities of downstream apoptotic effectors. However, Bcl-2 did not abrogate CHENSpm-induced DNA fragmentation, indicating that a caspase-independent pathway also existed and could be activated by analog treatment. Similar results were observed in caspase-3-deficient MCF-7 breast cancer cells treated with oligoamine SL-11144, indicating that capase-3 activation is not necessary for DNA fragmentation in this cell line [62]. These results demonstrate that both classical and non-classical apoptotic response pathways are operative in polyamine analog-induced programmed cell death. Treatment with the c-Jun NH_2-terminal kinase (JNK) inhibitor, SP600125, or overexpression of dominant-negative mutant c-Jun (TAM67) in human breast cancer MDA-MB-435 cells significantly increased their susceptibility to oligoamine-induced apoptosis, suggesting that an oligoamine-inducible JNK/AP-1 signaling pathway plays an anti-apoptotic role in this line in response to polyamine analog cytotoxicity [92]. In another study, the combination of the MAPK/ERK specific inhibitor, PD98059, with BENSpm nearly doubled the proportion of apoptotic cells in three melanoma lines as compared to BENSpm treatment alone [93]. These results indicate that the combination of polyamine analogs and other agents that target analog resistance mechanisms may significantly enhance the efficacy of tumor cell responsiveness to polyamine analogs.

Effects of polyamine analogs on cancer gene expression and ligand–receptor interaction

Polyamine analogs are known to alter the expression of a number of genes that are associated with the regulation of tumor cell proliferation, differentiation and apoptosis. BESpm treatment of the colon cancer cell line, $CaCO_2$, decreased c-*myc* oncoprotein in association with ODC inhibition, SSAT induction and polyamine depletion [94]. Gundogus-Ozcanli *et al.* reported that polyamines stimulated casein kinase II (CKII)-mediated phosphorylation of *myc* by 2- to 20-fold, and the polyamine analogs, BESpm and BE4x4, inhibited cancer cell proliferation partially through interference with CKII activity, suggesting that inhibition of cell proliferation by these polyamine analogs may involve the down-regulation of CKII activity [95]. Polyamines can also enhance binding of the transcription factor NF-κB to its response element (NRE) in the promoter of a variety of target genes, a possible mechanism for the ability of polyamines to promote proliferation of certain cancer cell lines [96]. Treatment with the polyamine analogs, BENSpm and BESpm, significantly inhibited the transactivation of NF-κB with a loss of the anti-apoptotic protein Bcl-2 in human breast cancer cells, suggesting that polyamine analog-induced down-regulation of NF-κB signaling pathway may contribute to growth inhibition of breast cancer cells [97].

The effect of polyamine analogs on ligand–receptor interactions and function has also been examined. ERs are critical transcription factors in regulating the expression of a number of genes involved in cell proliferation and differentiation [98]. Polyamine biosynthesis inhibitors disrupted several ER–co-activator interactions and exogenous spermidine reversed this inhibition, indicating a specific role of polyamines in the interaction of ER with its co-activators to regulate ER mediated gene expression

[99]. Further studies showed that increased polyamine concentrations may alter DNA structure harboring estrogen response elements (ERE) to promote conformational changes and enhance the binding of ER to ERE [100]. Treatment of MCF-7 cells with the analogs, BENSpm and BESpm, resulted in transcriptional inhibition of an ERE-driven reporter plasmid through the disruption of the association between ERα and co-activator CBP/p300 [97]. The impact of polyamine analogs on other ligand–receptor systems has also been evaluated. Spermine, a natural tetramine, has been shown to exhibit concentration-dependent biphasic effect of binding of MK-801 to *N*-methyl-D-aspartate (NMDA) receptor [101]. Bergeron *et al.* used a group of spermine homologs and analogs to demonstrate that the effect of these analogs on MK-801 binding in the NMDA channel was related to their length and charge state [102]. This finding provides a structure–activity relationship to guide the design of more effective MK-801 agonists and antagonists for cancer therapy.

Summary and future directions

A number of polyamine analogs exhibit encouraging effects against tumor growth in both cell culture and animal studies. A wide range of molecular targets for polyamine analogs has been unveiled and the regulation of analogs on the activity of these targets is summarized in Table 2. AZ induction has been found in response to the treatment with many analogs, suggesting that this is perhaps a common mechanism shared by different classes of analogs. The association of 'superinduction' of SSAT activity with a cytotoxic response to certain polyamine analogs has been noted. The complexity of the polyamine catabolic pathway is suggested by the identification of the SMO(PAOh1) genes. Cell cycle arrest and programmed cell death appear to be the common features of analog cytotoxicity in cancer cells. Alteration of DNA structure, production of toxic chemical and oxidative stress by catabolic enzyme induction, altered cancer-related gene expression, and activation of classic or non-classic apoptotic pathways have been proposed as the likely mechanisms by which polyamine analogs induce cell cycle arrest and apoptosis in tumor cells. These effects are clearly both cell type specific and structure dependent.

Despite the disappointing results obtained from early clinical trials of DFMO and BENSpm, polyamine metabolism remains a rational target for cancer therapy. Increasing knowledge about the molecular mechanisms of polyamine analogs in cancer cells over the last two decades should guide optimum development and use of polyamine analogs in cancer treatment. Development of analogs that target a unique function of polyamine metabolism in tumor cells may possibly provide selectivity in cancer therapy. However, several barriers remain. For example, the molecular characterization of the eukaryotic polyamine transport system is still elusive.

Table 2 Molecular targets of polyamine analog action

Target	Active analog	Cellular effect	References
Polyamine metabolism			
AZ	symmetrically substituted bis(alkyl)polyamines; tetramines; pentamines; oligoamines	AZ induction; ODC inhibition; transport inhibition; polyamine depletion	[14,60]
ODC/SAMDC	symmetrically substituted bis(alkyl)polyamines; oligoamines	ODC inhibition, polyamine depletion; growth inhibition; cell cycle arrest	[3,6,42,62]
SSAT/PAO/SMO(PAOh1)	symmetrically and unsymmetrically bis(alkyl)polyamines	elevated enzyme activity; H_2O_2 production; growth inhibition; apoptosis	[3,15,16,41]
DNA	symmetrically substituted bis(alkyl)polyamines; oligoamines; 5me-4-4-4; Q-Amm-4-4-4; BE-Q-Amm-4-4-4	altered chromatin structure; DNA collapse; altered transcription; apoptosis	[57,76,77]
Cell cycle	symmetrically and unsymmetrically bis(alkyl)polyamines	cell cycle arrest; altered tubulin function; growth inhibition	[55,82–84]
Apoptosis			
SSAT/PAO	symmetrically and unsymmetrically bis(alkyl)polyamines	production of H_2O_2, 3-acetamidopropanal and other toxic chemicals	[86,91]
caspases/PARP	oligoamines; symmetrically and unsymmetrically bis(alkyl) polyamines	induction of apoptosis	[62,87,86]
Bcl-2	CHENSpm; SL-11144	down-regulation of Bcl-2; inhibition of cytochrome *c* release; caspase-3 activation	[63,84]
AP-1	oligoamines	cell survival; apoptosis resistance	[62,92]
ERK	BENSpm	apoptosis resistance	[93]
Cancer genes			
c-*myc*	BENSpm	ODC inhibition; SSAT induction; polyamine depletion	[94]
casein kinase II	BESpm; BE4x4	growth inhibition	[95]
NF-κB	BENSpm	disruption of NRE activity; loss of Bcl-2 function	[96,97]
Ligand–receptor			
ERα	BENSpm; BESpm	disruption of the association of ERα with co-activator; down-regulation of ERE activity	[97,100]
NMDA	BENSpm; BESpm; BEHSpm; tetraazaoctadecanes (5,4,5)	growth inhibition	[102]

Since elevated polyamine transport levels usually occur in rapidly dividing tumor cells, a better understanding of transport pathways may help in design of potent drugs to inhibit the transport system and disrupt the polyamine metabolism in tumor cells with minimal effects on normal tissues. It is possible that new techniques such as cDNA microarray and proteomic analysis will facilitate the identification and assessment of other specific genes involved in polyamine analog-mediated tumor growth inhibition. In addition, the combination of a polyamine analog with classic anticancer agents may enhance the effectiveness of these agents without increased toxicity [103]. Information from these studies will be important to lay the framework for further clinical trials.

Abbreviations

AbeAdo, 5′-{[(*Z*)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine; AdoDATAD, *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane; AdoDATO, *S*-adenosyl-1,8-diamino-3-thiooctane; AMA, *S*-(5′-deoxy-5′-adenosyl)-methylthioethylhydroxylamine; AZ, antizyme; BEHSpm, N^1,N^{14}-bis(ethyl)homospermine; BENSpm, N^1,N^{11}-bis(ethyl)-norspermine; BESpm, N^1,N^{12}-bis(ethyl)spermine; CGP-48664, 4-amidinoindan-a-one 2′-amidinohydrazone; CHEMSpm, N^1-(cycloheptylmethyl)-N^{11}-ethyl-4,8-diazaundecane; CPENSpm, N^1-(cyclopropylmethyl)-N^{11}-ethyl-4,8-diazaundecane; DFMO, α-difluoromethylornithine; ER, estrogen receptor; ERE, estrogen response elements; IPENSpm, (*S*)-N^1-(2-methyl-1-butyl)-N^{11}-ethyl-4,8-diazaundecane; MAP, (2*R*,5*R*)-δ-methylacetylenicputrescine; MAPs, microtubule-associated proteins; MFMO, α-monofluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone); NMDA, *N*-methyl-D-aspartate; Nrf-2, NF-E2-related transcription factor; ODC, ornithine decarboxylase; PAO, polyamine oxidase; PENSpm, N^1-propargyl-N^{11}-ethylnorspermine; PRE, polyamine response elements; PMF-1, polyamine-modulated factor-1; SAMDC, *S*-adenosylmethionine decarboxylase; SMO(PAOh1), spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase.

References

1 Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res* 1988; **48**:759–774.
2 Marton LJ, Pegg AE. Polyamines as targets for therapeutic intervention. *Annu Rev Pharmacol Toxicol* 1995; **35**:55–91.
3 Casero Jr RA, Pegg AE. Spermidine/spermine N^1–acetyltransferase—the turning point in polyamine metabolism. *FASEB J* 1993; **7**:653–661.
4 Abeloff MD, Rosen ST, Luk GD, Baylin SB, Zeltzman M, Sjoerdsma A. Phase II trials of alpha-difluoromethylornithine, an inhibitor of polyamine synthesis, in advanced small cell lung cancer and colon cancer. *Cancer Treat Rep* 1986; **70**:843–845.
5 Porter CW, Sufrin JR. Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. *Anticancer Res* 1986; **6**:525–542.
6 Bergeron RJ, Neims AH, McManis JS, Hawthorne TR, Vinson JR, Bortell R, *et al.* Synthetic polyamine analogues as antineoplastics. *J Med Chem* 1988; **31**:1183–1190.
7 Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cell Mol Life Sci* 2001; **58**:244–258.
8 Panagiotidis CA, Artandi S, Calame K, Silverstein SJ. Polyamines alter sequence-specific DNA–protein interactions. *Nucleic Acids Res* 1995; **23**:1800–1809.
9 Lopatin AN, Makhina EN, Nichols CG. Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature* 1994; **372**:366–369.
10 Manni A, Wechter R, Verderame MF, Mauger D. Cooperativity between the polyamine pathway and HER-2*neu* in transformation of human mammary epithelial cells in culture: role of the MAPK pathway. *Int J Cancer* 1998; **76**:563–570.
11 Leroy D, Heriche JK, Filhol O, Chambaz EM, Cochet C. Binding of polyamines to an autonomous domain of the regulatory subunit of protein kinase CK2 induces a conformational change in the holoenzyme. A proposed role for the kinase stimulation. *J Biol Chem* 1997; **272**: 20820–20827.
12 Wallace HM. The physiological role of the polyamines. *Eur J Clin Invest* 2000; **30**:1–3.
13 Coffino P. Regulation of cellular polyamines by antizyme. *Nat Rev Mol Cell Biol* 2001; **2**:188–194.
14 Coffino P. Antizyme, a mediator of ubiquitin-independent proteasomal degradation. *Biochimie* 2001; **83**:319–323.
15 Wang Y, Devereux W, Woster PM, Stewart TM, Hacker A, Casero Jr RA. Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analogue exposure. *Cancer Res* 2001; **61**: 5370–5373.
16 Vujcic S, Diegelman P, Bacchi CJ, Kramer DL, Porter CW. Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. *Biochem. J* 2002; **367**:665–675.
17 Soulet D, Gagnon B, Rivest S, Audette M, Poulin R. A fluorescent probe of polyamine transport accumulates into intracellular acidic vesicles via a two-step mechanism. *J Biol Chem* 2004; **279**:49355–49366.
18 Mitchell JL, Simkus CL, Thane TK, Tokarz P, Bonar MM, Frydman B, *et al.* Antizyme induction mediates feedback limitation of the incorporation of specific polyamine analogues in tissue culture. *Biochem J* 2004; **384**: 271–279.
19 Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. *Biochim Biophys Acta* 1978; **473**:241–293.
20 Russell DH. Clinical relevance of polyamines. *Crit Rev Clin Lab Sci* 1983; **18**:261–311.
21 LaMuraglia GM, Lacaine F, Malt RA. High ornithine decarboxylase activity and polyamine levels in human colorectal neoplasia. *Ann Surg* 1986; **204**:89–93.
22 Porter CW, Herrera-Ornelas L, Pera P, Petrelli NF, Mittelman A. Polyamine biosynthetic activity in normal and neoplastic human colorectal tissues. *Cancer* 1987; **60**:1275–1281.
23 Moshier JA, Dosescu J, Skunca M, Luk GD. Transformation of NIH/3T3 cells by ornithine decarboxylase overexpression. *Cancer Res* 1993; **53**:2618–2622.
24 O'Brien TG, Megosh LC, Gilliard G, Soler AP. Ornithine decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res* 1997; **57**:2630–2637.
25 Mohan RR, Challa A, Gupta S, Bostwick DG, Ahmad N, Agarwal R, *et al.* Overexpression of ornithine decarboxylase in prostate cancer and prostatic fluid in humans. *Clin Cancer Res* 1999; **5**:143–147.
26 Giardiello FM, Hamilton SR, Hylind LM, Yang VW, Tamez P, Casero Jr RA. Ornithine decarboxylase and polyamines in familial adenomatous polyposis. *Cancer Res* 1997; **57**:199–201.
27 Thomas T, Thomas TJ. Estradiol control of ornithine decarboxylase mRNA, enzyme activity, and polyamine levels in MCF-7 breast cancer cells: therapeutic implications. *Breast Cancer Res Treat* 1993; **29**:189–201.
28 Cohen FJ, Manni A, Glikman P, Bartholomew M, Demers L. Involvement of the polyamine pathway in antiestrogen-induced growth inhibition of human breast cancer. *Cancer Res* 1988; **48**:6819–6825.
29 Meyskens Jr FL, Gerner EW. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. *Clin Cancer Res* 1999; **5**:945–951.
30 Thomas T, Faaland CA, Adhikarakunnathu S, Thomas TJ. Structure–activity relations of *S*-adenosylmethionine decarboxylase inhibitors on the growth of MCF-7 breast cancer cells. *Breast Cancer Res Treat* 1996; **39**: 293–306.
31 Manni A, Washington S, Griffith JW, Verderame MF, Mauger D, Demers LM, *et al.* Influence of polyamines on *in vitro* and *in vivo* features of aggressive and metastatic behavior by human breast cancer cells. *Clin Exp Metastasis* 2002; **19**:95–105.
32 Russell DH, Rosenblum MG, Beckerman RC, Durie BG, Taussig LM, Barnett DR. Altered polyamine metabolism in cystic fibrosis. *Pediatr Res* 1979; **13**:1137–1140.

33 Russell DH, Stern LZ. Altered polyamine excretion in Duchenne muscular dystrophy. *Neurology* 1981; **31**:80–83.

34 Russell DH, Combest WL, Duell EA, Stawiski MA, Anderson TF, Voorhees JJ. Glucocorticoid inhibits elevated polyamine biosynthesis in psoriasis. *J Invest Dermatol* 1978; **71**:177–181.

35 Seghieri G, Anichini R, Ciuti M, Gironi A, Bennardini F, Franconi F. Raised erythrocyte polyamine levels in non-insulin-dependent diabetes mellitus with great vessel disease and albuminuria. *Diabetes Res Clin Pract* 1997; **37**:15–20.

36 Dienel GA, Cruz NF. Induction of brain ornithine decarboxylase during recovery from metabolic, mechanical, thermal, or chemical injury. *J Neurochem* 1984; **42**:1053–1061.

37 de Vera N, Artigas F, Serratosa J, Martinez E. Changes in polyamine levels in rat brain after systemic kainic acid administration: relationship to convulsant activity and brain damage. *J Neurochem* 1991; **57**:1–8.

38 Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C.$^{+}$.1.1.17) by substrate and product analogs. *J Am Chem Soc* 1978; **100**: 2551–2553.

39 Burri C, Brun R. Eflornithine for the treatment of human African trypanosomiasis. *Parasitol Res* 2003; **90**(**suppl 1**):S49–S52.

40 Regenass U, Mett H, Stanek J, Mueller M, Kramer D, Porter CW. CGP 48664, a new *S*-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. *Cancer Res* 1994; **54**:3210–3217.

41 Casero Jr RA, Woster PM. Terminally alkylated polyamine analogues as chemotherapeutic agents. *J Med Chem* 2001; **44**:1–26.

42 Porter CW, Berger FG, Pegg AE, Ganis B, Bergeron RJ. Regulation of ornithine decarboxylase activity by spermidine and the spermidine analogue N^1N^8-bis(ethyl)spermidine. *Biochem J* 1987; **242**:433–440.

43 Casero Jr RA, Celano P, Ervin SJ, Porter CW, Bergeron RJ, Libby PR. Differential induction of spermidine/spermine N^1-acetyltransferase in human lung cancer cells by the bis(ethyl)polyamine analogues. *Cancer Res* 1989; **49**:3829–3833.

44 Porter CW, Ganis B, Libby PR, Bergeron RJ. Correlations between polyamine analogue-induced increases in spermidine/spermine N^1-acetyltransferase activity, polyamine pool depletion, and growth inhibition in human melanoma cell lines. *Cancer Res* 1991; **51**:3715–3720.

45 McCloskey DE, Casero Jr RA, Woster PM, Davidson NE. Induction of programmed cell death in human breast cancer cells by an unsymmetrically alkylated polyamine analogue. *Cancer Res* 1995; **55**:3233–3236.

46 Casero Jr RA, Mank AR, Xiao L, Smith J, Bergeron RJ, Celano P. Steady-state messenger RNA and activity correlates with sensitivity to N^1,N^{12}-bis(ethyl)spermine in human cell lines representing the major forms of lung cancer. *Cancer Res* 1992; **52**:5359–5363.

47 Xiao L, Casero Jr RA. Differential transcription of the human spermidine/spermine N^1-acetyltransferase (SSAT) gene in human lung carcinoma cells. *Biochem J* 1996; **313**:691–696.

48 Fogel-Petrovic M, Vujcic S, Brown PJ, Haddox MK, Porter CW. Effects of polyamines, polyamine analogs, and inhibitors of protein synthesis on spermidine-spermine N^1-acetyltransferase gene expression. *Biochemistry* 1996; **35**:14436–14444.

49 Coleman CS, Pegg AE. Polyamine analogues inhibit the ubiquitination of spermidine/spermine N^1-acetyltransferase and prevent its targeting to the proteasome for degradation. *Biochem J* 2001; **358**:137–145.

50 Chang BK, Bergeron RJ, Porter CW, Vinson JR, Liang Y, Libby PR. Regulatory and antiproliferative effects of *N*-alkylated polyamine analogues in human and hamster pancreatic adenocarcinoma cell lines. *Cancer Chemother Pharmacol* 1992; **30**:183–188.

51 Wolff AC, Armstrong DK, Fetting JH, Carducci MK, Riley CD, Bender JF, *et al.* A Phase II study of the polyamine analog N^1,N^{11}-diethylnorspermine (DENSpm) daily for five days every 21 days in patients with previously treated metastatic breast cancer. *Clin Cancer Res* 2003; **9**:5922–5928.

52 Hector S, Porter CW, Kramer DL, Clark K, Prey J, Kisiel N, *et al.* Polyamine catabolism in platinum drug action: interactions between oxaliplatin and the polyamine analogue N^1,N^{11}-diethylnorspermine at the level of spermidine/spermine N^1-acetyltransferase. *Mol Cancer Ther* 2004; **3**:813–822.

53 Saab NH, West EE, Bieszk NC, Preuss CV, Mank AR, Casero Jr RA, *et al.* Synthesis and evaluation of unsymmetrically substituted polyamine analogues as modulators of human spermidine/spermine-N^1-acetyltransferase (SSAT) and as potential antitumor agents. *J Med Chem* 1993; **36**:2998–3004.

54 Wu R, Saab NH, Huang H, Wiest L, Pegg AE, Casero Jr RA, *et al.* Synthesis and evaluation of a polyamine phosphinate and phosphonamidate as transition-state analogue inhibitors of spermidine/spermine-N^1-acetyltransferase. *Bioorg Med Chem* 1996; **4**:825–836.

55 Webb HK, Wu Z, Sirisoma N, Ha HC, Casero Jr RA, Woster PM. 1-(*N*-alkylamino)-11-(*N*-ethylamino)-4,8-diazaundecanes: simple synthetic polyamine analogues that differentially alter tubulin polymerization. *J Med Chem* 1999; **42**:1415–1421.

56 Reddy VK, Valasinas A, Sarkar A, Basu HS, Marton LJ, Frydman B. Conformationally restricted analogues of ^1N,^{12}N-bisethylspermine: synthesis and growth inhibitory effects on human tumor cell lines. *J Med Chem* 1998; **41**:4723–4732.

57 Valasinas A, Reddy VK, Blokhin AV, Basu HS, Bhattacharya S, Sarkar A, *et al.* Long-chain polyamines (oligoamines) exhibit strong cytotoxicities against human prostate cancer cells. *Bioorg Med Chem* 2003; **11**: 4121–4131.

58 Bacchi CJ, Weiss LM, Lane S, Frydman B, Valasinas A, Reddy V, *et al.* Novel synthetic polyamines are effective in the treatment of experimental microsporidiosis, an opportunistic AIDS-associated infection. *Antimicrob Ag Chemother* 2002; **46**:55–61.

59 Valasinas A, Sarkar A, Reddy VK, Marton LJ, Basu HS, Frydman B. Conformationally restricted analogues of ^1N,^{14}N-bisethylhomospermine (BE-4-4-4): synthesis and growth inhibitory effects on human prostate cancer cells. *J Med Chem* 2001; **44**:390–403.

60 Mitchell JL, Leyser A, Holtorff MS, Bates JS, Frydman B, Valasinas AL, *et al.* Antizyme induction by polyamine analogues as a factor of cell growth inhibition. *Biochem J* 2002; **366**:663–671.

61 Osland A, Kleppe K. Polyamine induced aggregation of DNA. *Nucleic Acids Res* 1977; **4**:685–695.

62 Huang Y, Hager ER, Phillips DL, Dunn VR, Hacker A, Frydman B, *et al.* A novel polyamine analog inhibits growth and induces apoptosis in human breast cancer cells. *Clin Cancer Res* 2003; **9**:2769–2777.

63 Wang Y, Xiao L, Thiagalingam A, Nelkin BD, Casero Jr RA. The identification of a *cis*-element and a *trans*-acting factor involved in the response to polyamines and polyamine analogues in the regulation of the human spermidine/spermine N^1-acetyltransferase gene transcription. *J Biol Chem* 1998; **273**:34623–34630.

64 Wang Y, Devereux W, Stewart TM, Casero Jr RA. Cloning and characterization of human polyamine-modulated factor-1, a transcriptional cofactor that regulates the transcription of the spermidine/spermine N^1-acetyltransferase gene. *J Biol Chem* 1999; **274**:22095–22101.

65 Wang Y, Devereux W, Woster PM, Casero Jr RA. Cloning and characterization of the mouse polyamine-modulated factor-1 (mPMF-1) gene: an alternatively spliced homologue of the human transcription factor. *Biochem J* 2001; **359**:387–392.

66 Holtta E. Oxidation of spermidine and spermine in rat liver: purification and properties of polyamine oxidase. *Biochemistry* 1977; **16**:91–100.

67 Wang Y, Murray-Stewart T, Devereux W, Hacker A, Frydman B, Woster PM, *et al.* Properties of purified recombinant human polyamine oxidase, PAOh1/SMO. *Biochem Biophys Res Commun* 2003; **304**:605–611.

68 Vujcic S, Liang P, Diegelman P, Kramer DL, Porter CW. Genomic identification and biochemical characterization of the mammalian polyamine oxidase involved in polyamine back-conversion. *Biochem J* 2003; **370**: 19–28.

69 Murray-Stewart T, Wang Y, Devereux W, Casero Jr RA. Cloning and characterization of multiple human polyamine oxidase splice variants that code for isoenzymes with different biochemical characteristics. *Biochem J* 2002; **368**:673–677.

70 Cervelli M, Bellini A, Bianchi M, Marcocci L, Nocera S, Polticelli F, *et al.* Mouse spermine oxidase gene splice variants. Nuclear subcellular localization of a novel active isoform. *Eur J Biochem* 2004; **271**: 760–770.

71 Devereux W, Wang Y, Stewart TM, Hacker A, Smith R, Frydman B, *et al.* Induction of the PAOh1/SMO polyamine oxidase by polyamine analogues in human lung carcinoma cells. *Cancer Chemother Pharmacol* 2003; **52**:383–390.

72 Basu HS, Smirnov IV, Peng HF, Tiffany K, Jackson V. Effects of spermine and its cytotoxic analogs on nucleosome formation on topologically stressed DNA *in vitro*. *Eur J Biochem* 1997; **243**:247–258.

73 Thomas TJ, Messner RP. Structural specificity of polyamines in left-handed *Z*-DNA formation. Immunological and spectroscopic studies. *J Mol Biol* 1988; **201**:463–467.

74 Feuerstein BG, Williams LD, Basu HS, Marton LJ. Implications and concepts of polyamine-nucleic acid interactions. *J Cell Biochem* 1991; **46**:37–47.

75 Panagiotidis CA, Artandi S, Calame K, Silverstein SJ. Polyamines alter sequence-specific DNA–protein interactions. *Nucleic Acids Res* 1995; **23**:1800–1809.

76 Basu HS, Sturkenboom MC, Delcros JG, Csokan PP, Szollosi J, Feuerstein BG, *et al.* Effect of polyamine depletion on chromatin structure in U-87 MG human brain tumour cells. *Biochem J* 1992; **282**:723–727.

77 Basu HS, Marton LJ, Pellarin M, Deen DF, McManis JS, Liu CZ, *et al.* Design and testing of novel cytotoxic polyamine analogues. *Cancer Res* 1994; **54**:6210–6214.

78 Fredlund JO, Johansson MC, Dahlberg E, Oredsson SM. Ornithine decarboxylase and *S*-adenosylmethionine decarboxylase expression during the cell cycle of Chinese hamster ovary cells. *Exp Cell Res* 1995; **216**: 86–92.

79 Heby O, Sarna GP, Marton LJ, Omine M, Perry S, Russell DH. Polyamine content of AKR leukemic cells in relation to the cell cycle. *Cancer Res* 1973; **33**:2959–2964.

80 Marty C, Mori G, Sabini L, Rivarola V. Effects of alpha-difluoromethylornithine on the cyclin A expression in Hep-2 cells. *Biocell* 2000; **24**:49–52.

81 Kramer DL, Chang BD, Chen Y, Diegelman P, Alm K, Black AR, *et al.* Polyamine depletion in human melanoma cells leads to G_1 arrest associated with induction of p21WAF1/CIP1/SDI1, changes in the expression of p21-regulated genes, and a senescence-like phenotype. *Cancer Res* 2001; **61**:7754–7762.

82 Kramer DL, Vujcic S, Diegelman P, Alderfer J, Miller JT, Black JD, *et al.* Polyamine analogue induction of the p53–$p21^{WAF1/CIP1}$–Rb pathway and G_1 arrest in human melanoma cells. *Cancer Res* 1999; **59**:1278–1286.

83 Alm K, Berntsson PS, Kramer DL, Porter CW, Oredsson SM. Treatment of cells with the polyamine analog N,N^{11}-diethylnorspermine retards S phase progression within one cell cycle. *Eur J Biochem* 2000; **267**:4157–4164.

84 Ha HC, Woster PM, Casero Jr RA. Unsymmetrically substituted polyamine analogue induces caspase-independent programmed cell death in Bcl-2-overexpressing cells. *Cancer Res* 1998; **58**:2711–2714.

85 Thomas T, Balabhadrapathruni S, Gallo MA, Thomas TJ. Development of polyamine analogs as cancer therapeutic agents. *Oncol Res* 2002; **13**:123–135.

86 Ha HC, Woster PM, Yager JD, Casero Jr RA. The role of polyamine catabolism in polyamine analogue-induced programmed cell death. *Proc Natl Acad Sci USA* 1997; **94**:11557–11562.

87 Chen Y, Kramer DL, Diegelman P, Vujcic S, Porter CW. Apoptotic signaling in polyamine analogue-treated SK-MEL-28 human melanoma cells. *Cancer Res* 2001; **61**:6437–6444.

88 Faaland CA, Thomas TJ, Balabhadrapathruni S, Langer T, Mian S, Shirahata A, *et al.* Molecular correlates of the action of bis(ethyl)polyamines in breast cancer cell growth inhibition and apoptosis. *Biochem Cell Biol* 2000; **78**:415–426.

89 Hu RH, Pegg AE. Rapid induction of apoptosis by deregulated uptake of polyamine analogues. *Biochem J* 1997; **328**:307–316.

90 Fraser AV, Woster PM, Wallace HM. Induction of apoptosis in human leukaemic cells by IPENSpm, a novel polyamine analogue and anti-metabolite. *Biochem J* 2002; **367**:307–312.

91 Chen Y, Kramer DL, Jell J, Vujcic S, Porter CW. Small interfering RNA suppression of polyamine analog-induced spermidine/spermine N^1-acetyltransferase. *Mol Pharmacol* 2003; **64**:1153–1159.

92 Huang Y, Keen JC, Hager E, Smith R, Hacker A, Frydman B, *et al.* Regulation of polyamine analogue cytotoxicity by c-Jun in human MDA-MB-435 cancer cells. *Mol Cancer Res* 2004; **2**:81–88.

93 Chen Y, Alm K, Vujcic S, Kramer DL, Kee K, Diegelman P, *et al.* The role of mitogen-activated protein kinase activation in determining cellular outcomes in polyamine analogue-treated human melanoma cells. *Cancer Res* 2003; **63**:3619–3625.

94 Berchtold CM, Tamez P, Kensler TW, Casero Jr RA. Inhibition of cell growth in $CaCO_2$ cells by the polyamine analogue N^1,N^{12}-bis(ethyl)spermine is preceded by a reduction in MYC oncoprotein levels. *J Cell Physiol* 1998; **174**:380–386.

95 Gundogus-Ozcanli N, Sayilir C, Criss WE. Effects of polyamines, polyamine synthesis inhibitors, and polyamine analogs on casein kinase II using Myc oncoprotein as substrate. *Biochem Pharmacol* 1999; **58**: 251–254.

96 Shah N, Thomas T, Shirahata A, Sigal LH, Thomas TJ. Activation of nuclear factor κB by polyamines in breast cancer cells. *Biochemistry* 1999; **38**:14763–14774.

97 Shah N, Thomas TJ, Lewis JS, Klinge CM, Shirahata A, Gelinas C, *et al.* Regulation of estrogenic and nuclear factor kappa B functions by polyamines and their role in polyamine analog-induced apoptosis of breast cancer cells. *Oncogene* 2001; **20**:1715–1729.

98 Nicholson R, McClelland R, Robertson J, Gee J. Involvement of steroid hormone and growth factor cross-talk in endocrine response in breast cancer. *Endocr Relat Cancer* 1999; **6**:373–387.

99 Thomas T, Shah N, Klinge CM, Faaland CA, Adihkarakunnathu S, Gallo MA, *et al.* Polyamine biosynthesis inhibitors alter protein–protein interactions involving estrogen receptor in MCF-7 breast cancer cells. *J Mol Endocrinol* 1999; **22**:131–139.

100 Thomas T, Kulkarni GD, Gallo MA, Greenfield N, Lewis JS, Shirahata A, *et al.* Effects of natural and synthetic polyamines on the conformation of an oligodeoxyribonucleotide with the estrogen response element. *Nucleic Acids Res* 1997; **25**:2396–2402.

101 Williams K, Romano C, Molinoff PB. Effects of polyamines on the binding of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. *Mol Pharmacol* 1989; **36**:575–581.

102 Bergeron RJ, Weimar WR, Wu Q, Feng Y, McManis JS. Polyamine analogue regulation of NMDA MK-801 binding: a structure–activity study. *J Med Chem* 1996; **39**:5257–66.

103 Hahm HA, Dunn VR, Butash KA, Deveraux WL, Woster PM, Casero Jr RA, *et al.* Combination of standard cytotoxic agents with polyamine analogues in the treatment of breast cancer cell lines. *Clin Cancer Res* 2001; **7**: 391–399.