Spermine Metabolism and Anticancer Therapy

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Abstract: The natural polyamines (PA), putrescine (PUT), spermidine (SPD) and spermine (SPM) are ubiquitous constituents of eukaryotic cells. The increase of PA in malignant and proliferating cells attracted the interest of scientists during last decades, addressing PA depletion as a new strategy to inhibit cell growth. Selective enzyme inhibitors were developed for decreasing PA metabolism and to act as chemotherapeutic anticancer agents. Indeed, the complexity of the PA homoeostasis overcomes the PA perturbation by a single enzyme to take effect therapeutically. Recently, an increasing interest has been posed on spermine-oxidase (SMO), the only catabolic enzyme able to specifically oxidise SPM. Interestingly, the absence of SPM is compatible with life, but its accumulation and degradation is lethal. Augmented SMO activity provokes an oxidative stress rendering cells prone to die, and appears to be important in the cell differentiation pathway. Extra-cellular SPM is cytotoxic, but its analogues are capable of inhibiting cell growth at low concentrations, most likely by intracellular SPM depletion. These pivotal roles seem to evoke the biological processes of stress response, wherein balance is mandatory to live or to die. Thus, altering SPM metabolism could allow a multi-tasking therapeutic strategy, addressed not only to inhibit PA metabolism. Several tetramines are presently in early phases (I and II) of clinical trials, and it will be a matter of a few more years to understand whether SPM-related therapeutic approaches would be of benefit for composite treatment protocols of cancer.

Keywords: Spermine, spermine oxidase, polyamine, cancer, inhibitors, analogues, ROS.

INTRODUCTION

Polyamine Metabolism Overview

The natural polyamines (PA), putrescine (PUT), spermidine (SPD) and spermine (SPM) are ubiquitous low-molecular weight aliphatic cations constituents of eukaryotic cells [1, 2]. Intracellular PA concentration is around millimolar, and their molecular functions are accomplished by reversible ionic interactions with negatively charged macromolecules. For this reason, the intracellular free PA concentration is very low. PA bind to nucleic acids, thus to interfere with chromatin conformation and gene expression, and to proteins and membrane phospholipids, targeting ion-channel modulation and stability. Schematic representation of PA metabolism showing enzymes network, substrate interconversion and inhibitor targets are depicted in Fig. (**1**). In mammals, PA directly affect cell growth, differentiation, and apoptosis [3, 4]. To adequately preserve PA concentrations, cells strictly regulate the *de novo* synthesis from amino acid precursors and PA uptake from diet, with the balancing interconversion stepwise degradation and efflux. PA biosynthesis is step-limited by the action of two enzymes [2]. Initially, the pyridoxal phosphate-dependent ornithine decarboxylase enzyme (ODC) produces PUT by decarboxylation of ornithine. The second biosynthetic step involves the concerted action of the pyruvoyl-containing S-adenosylmethionine decarboxylase enzyme (AdoMetDC), producing the aminopropyl moiety. The two specific aminopropyl transferase, Spermidine synthase (SPDS) and Spermine synthase (SMS) synthesize SPD and SPM adding the aminopropyl group, respectively, to PUT and to SPD, Fig. (**1**). The PA catabolism is dependent on the activity of the inducible enzyme spermidine/spermine N^1-acetyltransferase (SSAT), able to transfer acetyl group from acetyl-coenzyme A (AcCoA) to the N^1 position of both SPD and SPM. The N^1-acetylspermidine (AcSPD) and the N^1-acetylspermine (AcSPM) are then oxidized by the peroxisomal FAD-dependent enzyme N^1-acetylpolyamine oxidase (APAO) to produce respectively SPD and SPM, 3-aceto-aminopropanal and hydrogen peroxide (H_2O_2), Fig. (**1**). In parallel of the acetylating SSAT activity, SPM is directly oxidized by the spermine oxidase enzyme (SMO, EC number 1.5.3.3), a flavoprotein formerly cloned in human and characterized as a human polyamine oxidase (PAO-h1) [5], and then designated as SMO both in human [6] and in mouse [7]. SMO specifically recognize SPM as substrate to produce SPD, 3-aminopropanal and H_2O_2, Fig. (**1**). In order to maintain adequately the intracellular PA content, PA transport is an active energy-dependent process, crucial for both the diet uptake and the efflux *via* the intestinal flora [8, 9].

Since the PA content is strictly related to cell growth, and a consistent number of evidences relates PA metabolism dysfunction with cancer [10, 11], the increase of PA in malignant and proliferating cells attracted the interest of scientists during last decades, addressing PA depletion as a new strategy to inhibit carcinogenesis. Potentially, each biosynthetic and catabolic step is a target for therapies, as well as PA transport across cell membrane, to minimize the homoeostatic effects of the PA metabolism [12].

Alteration of Polyamine Metabolism and Therapeutic Approaches

The principal irreversible inhibitor of PA synthesis is the ODC-inhibitor 2-fluoromethylornithine (DFMO), Fig. (**1**). DFMO was the first drug rationally designed to halt cell pro-

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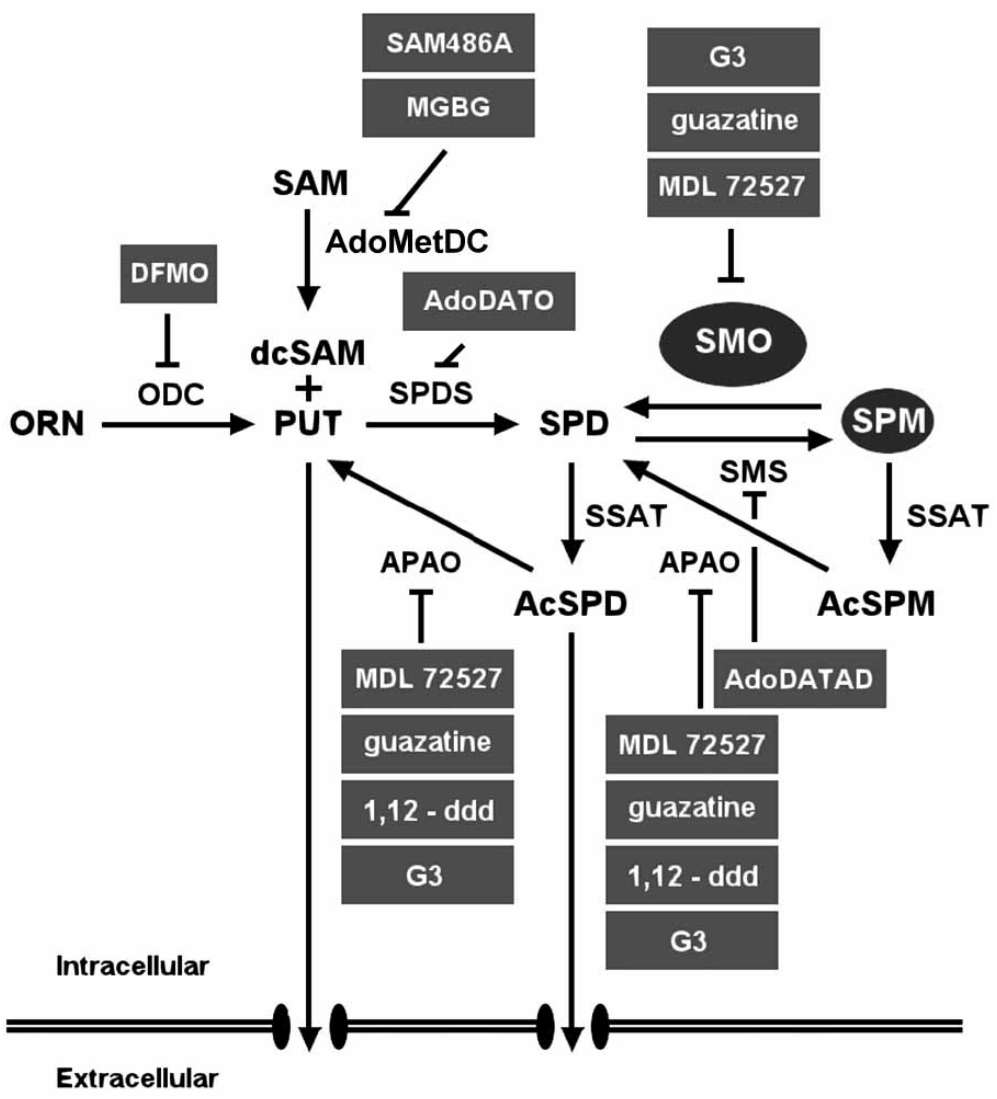

Fig. (1). Targets in the polyamine metabolic pathway. Schematic representation of PA metabolism showing enzymes network, substrate inter-conversion and inhibitor targets. The PA interconversion pathway as shown in this figure is the most recently studied and mainly related to mammalian species. In black, SPM and specific catabolic enzyme SMO. In grey, the inhibitors described in more details in the text (Alteration of PA metabolism and therapeutic approaches section). PUT and AcSPD are the preferred exported PA from intracellular moiety. Abbreviation used in the figure are listed in the text of the manuscript.

liferation interfering with polyamine metabolism. DFMO is a substrate for ODC and binds permanently within the active site at lys69 and lys360, apart from to the ODC cleavage domain. [13, 14]. DFMO administration on several normal and malignant cell lines diminishes growth rate and depletes PUT and SPD intracellular contents, but both the augmented compensatory PA catabolic steps and the cellular PA uptake, produces a cytostatic rather than cytotoxic effects *in vivo*. DFMO is a well tolerated anti-cancer drug, and has been phase I and phase II clinically evaluated against gliomas, melanomas, and other solid tumours, alone and in combinatorial treatment protocols [15-21]. Unfortunately, DFMO as single drug exerted limited therapeutic effect, likely for the high concentration needed to inhibit ODC and the poor cellular uptake [22]. On the contrary, encouraging results came up from the association with the PA transport inhibitor MQT 1426 in treating a mouse model of squamous-cell carcinoma [23]. Recently, DFMO alone or combined with cyclo-oxygenase inhibitors went through several clinical trial as chemo-preventive agent [24]. In both cases, the DFMO administration produced clear positive clinical results.

The effect of DFMO alone administered in a year-long phase IIb randomized placebo chemo-prevention study was a decrease of prostate volume associated with a lower prostate PUT level [25], shedding new light on the prevention of prostate hyperplasia or cancer. Far more enthusiastic clinical results were obtained on the prevention of colorectal adenomas when DFMO has been used in combination with the non steroidal anti-inflammatory molecule sulindac [26]. In this three years randomized placebo-controlled double-bind trial, a drastic decrease of colorectal adenomas recurrence was registered in patients receiving daily DFMO plus sulindac,

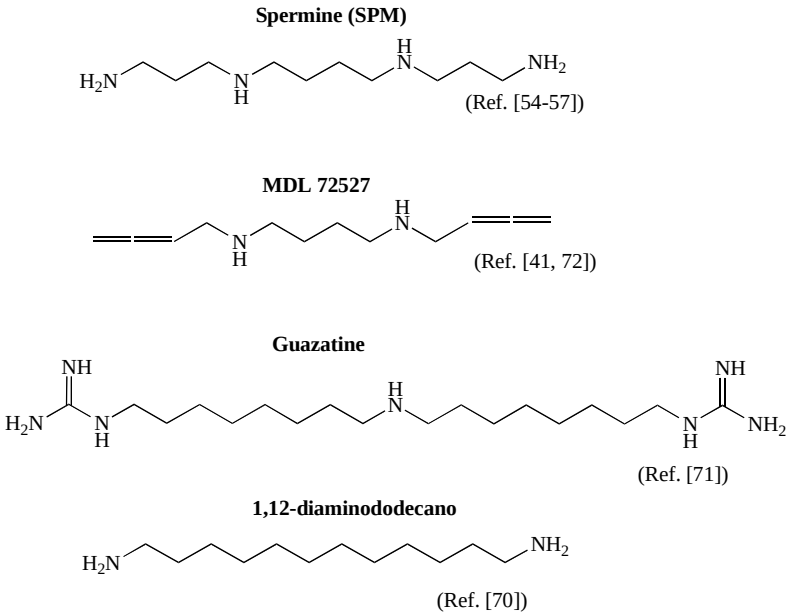

Fig. (2). Chemical structures of SMO substrates and inhibitors. Chemical structures of SPM, the specific substrate of SMO enzyme. Structures of two active inhibitors of SMO enzymatic activity: MDL 72527 [72]; guazatine [73, 74], and the inactive SPM-derivative analogue 1,12 ddd [73]. Guazatine was formerly isolated from *Verbesina caracasana* [73], and then synthetically synthesized [74]. (GChemPaint Chemical Structure Editor, GNU software). Abbreviation used in the figure are listed in the text of the manuscript.

representing a milestone in the synergistic action of two ineffective drugs when administered alone [27].

Studies on the inhibition of AdoMetDC began serendipitously during a clinical treatment of leukaemia with methylglyoxal bis(guanylhydrazone) (MGBG), without any previous knowledge on its effect as inhibitor of the polyamine biosynthesis [28]. MGBG strongly inhibits AdoMetDC, structurally resembling SPD, one of the AdoMetDC substrates, Fig (**1**). MGBG treatment depletes SPD and SPM with a high increase of PUT concentration [29]. The SPD decrement appears to be mandatory for the MGBG inhibitory effect, since it is reversed upon SPD exogenous treatment [30]. MGBG is extremely toxic, and causes severe ultrastructural mitochondrial damage [31], but promising observations on synergistic positive outcomes in leukemia led to the design of several new compounds structurally related to MGBG, such as ethylglyoxal-bis(guanylhydrazone) (EGBG), and 4-(aminoiminomethyl)-2,3-dihydro-1H-inden-1-one-diaminomethylenehydrazone (SAM486A) [32, 33]. The use of SAM486A decreased SPD and SPM content with less mitochondrial toxicity, entering in a clinical trial to assess the threshold of the administration tolerance [34].

SPD and SPM biosynthesis take place by the concerted action of a cascade of enzymatic reactions involving both the SPDS and the SMS, as illustrated in Fig. (**1**). Pursuing the goal to adverse cell growth by lowering the PA level, both the above mentioned enzymes have been targeted by analogues of the by-product 5'-deoxy-5'-(methylthio) adenosine (MTA) [35]. Both SPDS and SMS were successfully inhibit by the respectively transition-state analogues S-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) [36], and S-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD) [37], Fig. (**1**). Unfortunately, when experimentally tested, both of them did not show no appreciable effect on diminishing cell proliferation, mainly for the homoeostatic intrinsic regulation of the cellular pool of PA [22, 38].

Lowering PA intracellular level by recycling metabolism, inducers of catabolic enzymes SSAT, APAO and SMO should be investigated. Unfortunately, no compound directly and selectively affecting SSAT activity has been described [14]. On the other hand, since APAO activity has been modulated [38, 39] and its activity is low in several tumours [40], its induction by exogenous treatments deserves interest as a novel approach to fight cancer. On the contrary, the inhibition of both FAD-dependent enzymes APAO and SMO has been used to better understand and define the PA catabolic steps. The most relevant inhibitor of APAO and SMO is MDL 72527 (N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine), that selectively inhibits FAD-containing enzymes [41], Fig. (**1**) and Fig. (**2**). MDL 72527 is cytotoxic with a permanent lysosomotropic effect, leading ultimately to apoptosis [42] although the dosage requested to inhibit APAO and SMO activities in neuroblastoma cell line did not influence cell survival [43].

Several cell-specific membrane transport systems exist for the uptake of PA. PUT, SPD and SPM can cross the cell membrane by a single unique channel and by distinct ones [44]. Isolation of deficient mutants that were selected for resistance to the toxicity of MGBG demonstrated that PA permeates the cell membrane by an active, energy-dependent system [45]. The importance of the transport system in PA-related therapeutic methods is underlined by the evidence that the positive outcome of DFMO treatment is increased in leukaemia cell, with impaired PA uptake system [46]. Since PA transport system have been discovered influencing also the polyamine-like molecules and PA analogues uptake, [47, 48], several drugs able to inhibit transport were developed [49, 50]. Regrettably, the PA uptake inhibition was found to

be only a transitory response after a long term exposure. Once again, these findings suggest a crucial role played by the adaptive cellular countermeasure to maintain PA homoeostasis [48].

Perturbation of Polyamine Homoeostasis

Dealing with what above mentioned and described, the target inhibition of a single biosynthetic or catabolic step of PA metabolism did not guarantee a significant therapeutic success, although a set of encouraging results permitted a growing sense to be close to the target. Recently, most of the effort has been addressed to perturbation of PA *via* treatment with PA analogues. This strategy seems hypothetically more appropriate to overcome the PA homoeostasis mechanisms. The design of the best analogue should take into account the easiness of cellular access, the down-regulation of biosynthetic steps and the up-regulation of the catabolism to decrease PA intracellular level, the competition with natural PA for uptake, and the selective activity on cancer [22]. Prototypic examples of PA analogues were N^1,N^8 bis(ethyl)SPD (BESPD), and N^1, N^8 bis(propyl)SPD, symmetrically substituted analogues based on the structure of SPD [51]. The inhibitory effect was obtained by a decrease of ODC activity and PUT and SPD levels, promising a novel anticancer therapy by the perturbation of PA homoeostasis *via* PA analogues [52].

Soon after the beginning of experimentation of PA analogues, it was evident the higher inhibitory effect on cell proliferation of SPM analogues as compared to SPD analogues, thus addressing the design of a novel class of anticancer drugs based on alteration of SPM backbone [22].

Recently, an increasing interest has been posed on the SMO enzyme activity, since its ability to produce oxidative stress able to drive cells to death. Interestingly, the absence of SPM is compatible with life, but its accumulation and degradation is lethal. The pivotal roles played by SMO and SPM concentration seem to evoke the biological processes of stress response, wherein balance is mandatory to live or to die. Thus, the alteration mediated by SPM analogues and SMO inhibitors could represent a multi-tasking anticancer strategy, addressed not only to inhibit PA metabolism.

SPERMINE AND POLYAMINES METABOLISM

SPM is a tetramine, the largest molecule involved in the PA metabolism and it is present in all eukaryotic cells. SPM is found in a wide variety of organisms and tissues albeit not generally present in prokaryotes, it is an essential growth factor in some bacteria. Its level is tightly regulated and kept within a narrow range by a combined action of a complex network of enzymes. SPM synthesis from its precursor SPD and the reverse conversion to SPD by the enzymatic activity of SMO [5-7], represents a bright example of circular interconversion and enzymatic crosstalk, which seems to juxtapose to the PA metabolism, to finely regulate intracellular SPD content [2, 53].

Crystals of SPM phosphate were discovered and described in human semen, by Anton van Leeuwenhoek in 1678 [54]. The name ***spermin*** was first used by the German chemists Ladenburg and Abel in 1888 [55], but the correct structure of SPM was not definitely established until 1925, almost simultaneously in two independent laboratories [56, 57].

Since SPM is a polycation at physiological pH, its association with negatively charged nucleic acids was postulated around sixty years later [58], then resulting in the strongest PA able to stabilize the DNA helical structure . Nowadays, with the growing knowledge of the capacity of SPM to interact with negatively charged moieties, it is well known that SPM can play several cellular mandatory roles in the cellular physiological status, independently from its function in the PA metabolism. SPM can act as a regulator of DNA synthesis, cellular proliferation, modulation of ion channel function, as a second messenger in cellular signalling, as a modulator of the synthesis of nitric oxide, as an inhibitor in immune responses [2, 22, 59]. Increased SPM levels have been measured in tissues from hyper-proliferative diseases and in tissues following injury, inflammation and infection [2, 22]. On the contrary, a very low amount of SPM in tissues and body fluids is compatible with life, albeit associated with severe cellular dysfunctions, thus indicating the compulsory physiological roles for SPM [53]. An elegant demonstration of these findings were obtained, as a coincidence, by the production of transgenic hemizygous gyro male mice lacking the *Phex* gene, a phosphate-regulating gene with homologies with endopeptidases on the X chromosome, (*Gy*/Y mice) [60]. The mouse model engineered to delete the *Phex* gene harboured also the deletion of the neighbouring *SMS* gene, resulting in a mouse with SPM deficiency. This SPM deficiency shows a dramatic phenotype, as smaller size, sterility, inner-ear abnormality, deafness, decrease viability from birth, sudden death in adults, head shaking, and lack of postural reflexes [60]. The name *gyro* mice derived from their circling behavior, depending both on ear defect as well as neurological disorders related to the low content of SPM as a modulator of ion channels in the brain [61].

Spermin Catabolism and SMO Activity

As above mentioned, SPM is directly oxidised by SMO enzyme [5-7]. Noteworthy, SPM can be also oxidized by the serum amino oxidases (SAOs), which contain copper and 2,4,5-trihydroxyphenylalanine quinone (TPQ), as co-factors. The SAOs preferentially oxidise SPM and SPD at the primary amino group in addition to and others aliphatic and aromatic monoamines, always producing H_2O_2 and aldehyde as toxic by-products. Contrarily to SMO, SAOs localize in serum, in biological fluids, and in human are expressed at considerable level only in pregnancy and during cancer diseases [62]. SAOs belong to the terminal oxidative catabolism while SMO, as a FAD amino oxidize, is involved in the PA inter-conversion pathway. Probably, any possible enzymatic SAOs variation could be counteract by SMO activity.

The single copy mammalian *SMO* gene encodes for many splice variants, both in human and mouse [63, 64]. In particular, in mouse, among nine isoforms isolated so far, the SMO isoform named *alfa* (SMOα) and the isoform named *mu* (SMOμ) are the only splice variants with catalytic activity [63]. Interestingly, these two proteins have different subcellular localization, SMOα localizes in the cytoplasm, and

SMOμ proved to be also nuclear localized [63]. Since the primary enzymatic activity of SMO is the oxidation of SPM with the production of hydrogen peroxide, an increased interest has been focused in the cascade of events provoked by the unbalancing of ROS due to SMO induction. SPM catabolic degradation has been found to be closely related to DNA oxidation and apoptosis, mainly *via* H_2O_2 production and deleterious effects in several cellular models [65-67]. The ectopically expression of SMO in a mouse neuroblastoma cell line demonstrated once more the pivotal role of its activity. SMO over expression caused augmented DNA oxidative damage, but with no increase in cell mortality and below the threshold of the activation of cell cycle arrest [43]. Unexpectedly, a cell repair system was activated as evidenced by the enhanced phosphorylation of the histone H2AX and the activation of the apurinic/apyrimidinic endonuclease (APE1 or REF1), both markers of DNA repair mechanisms [68]. Thus, SMO over activity induced a chronic sub-lethal DNA damage, which, in turn was not able to stimulate an adaptive response, as expected. Contrarily, irradiation drastically enhanced cell death in cells expressing both isoforms, indicating an acquired hypersensitivity to radiation [43].

SMO over activity, both ectopically driven and endogenous induced, diminishes the amount of free SPM and, subsequently, the shielding function on DNA, leading to radiosensitivity, augmented mutagenesis and cell death. It is still unclear to what extent these effects are due to ROS production by oxidative metabolism, or to the depletion of SPM as a reactive oxygen species scavenger and DNA shielding molecule. Prospectively, coupling PA analogues treatments [67] with radiotherapy in the presence of SMO over activity could provoke a synergistic apoptotic stimulus for therapeutic purposes. In particular, in respiratory tract cancer, colon carcinoma, colorectal cancer and multiple myelomas a high content of SPM has been measured, while a low APAO activity has been observed thus justifying the assumption that the induction of SMO and APAO in response to a chemotherapeutic agent may be a combined approach for the treatment of cancer [14]. A tissues variability of the level of SMO activities have been described [63], and preliminary experiments on conditional transgenic mice over expressing SMOα in the brain cortex suggest a specific tissues sensitivity related to SMO activity, both in terms of biochemical and behaviour issues (unpublished data)[1].

Experiments of translation of the recombinant human and mouse SMO proteins were performed in different *in vitro* heterologous systems [5-7]. The expression of the recombinant enzyme allowed to elucidate in more details the biochemical properties of SMO and its structure/function characteristics, facilitating the study of the inhibitors as therapeutic drugs. Consistently with the inhibition of SMO over activity in neuroblastoma cells [43, 68], *in vitro* recombinant SMO activity was reverted by treatment with the inhibitor MDL 72527 [69, 70], whose inhibition has been initially debated [5-7].

[1]Conditionally transgenic mouse ovrexpressing SMO in the brain cortex shows extensive neuronal depletion and accentuate defensive behavior. Mice model were obtained driving SMO construct under the control of the conditionally cortex specific Dach promoter (Amendola, R.; Cervelli, M.; Cecconi, F.; Mariottini, P., work in progress).

THE SPERMINE OXIDASE ACTIVITY: AN *IN SILICO* CONFORMATIONAL STUDY

In spite of the growing interest raised up by SPM analogues designed to inhibit SPM metabolism and utilized as multi-targeting drugs, still there are very few information on the real targets and key-actions of SPM analogues. Knowledge on their precise role would be fundamental to set up an anticancer drug development design in order to avoid dramatic toxic side effects. The *in vitro* expression of a set of mammalian recombinant catabolic enzymes, as SMO and APAO, was extremely useful to understand SPM analogues physiological features. In this regard, the K_i values of four class of polyamine catabolic enzyme inhibitors have been compared on animal purified SMO and APAO enzymes [69, 70]. In detail, the inhibitors used are: (a) guazatine, a compound formed by two octane linear chains connected by a secondary nitrogen atom with two terminal guanidine moieties, belonging to the di-guanidino class of plant PA inhibitors [71] (b) MDL 72527, a SPM analogue lacking terminal amino groups [72] (c) linear primary diamines lacking secondary amino groups 1,8-diamminooctane and 1,12 diaminododecane (1,12-ddd) [70]; (d) N^3 -prenylagmatine (G3), an agmatine derivative characterized by the presence of one guanidine moiety and one primary amino group [73, 74]. Table (**1**) summarizes the K_i values for those inhibitors [70] and Fig. (**2**) shows the chemical structures of SPM, MDL 72527, 1,12-ddd, and guazatine. Guazatine represents the most powerful characterized SMO inhibitor ($K_i = 4.0 \times 10^{-7}$ M), showing a very low K_i value also for APAO enzyme ($K_i = 4.5 \times 10^{-7}$ M) [70]. The high affinity exhibited by guazatine for SMO and APAO could be related to the high number of polar and hydrophobic interactions that the long linear chain of guazatine can establish with active site residues of SMO and APAO enzymes. Although its affinity is lower than that of guazatine, MDL 72527, represents the most studied mammalian APAO inhibitor. MDL 72527 inhibits both SMO and APAO activities, showing a comparable K_i value, of 6.3×10^{-5} M and 2.1×10^{-5} M for SMO and APAO, respectively. The SPD analogue 1,8-diamminooctane does not inhibit either SMO or APAO activities. Indeed the affinity of SMO for SPD is about 3000 fold lower than that for the preferred substrate SPM. This analogue does not inhibit the catalytic activity of APAO as well, most probably reflecting the lower affinity displayed by APAO for N^1-acetyl-spermidine ($K_m = 3.7 \times 10^{-5}$ M), as compared to N^1-acetyl-spermine ($K_m = 1.8 \times 10^{-6}$ M) [75]. Interestingly, 1,12-ddd does not inhibit SMO activity ($K_i > 10^{-3}$ M) [70], notwithstanding SMO specificity for SPM as a substrate ($K_m = 9 \times 10^{-5}$ M) [7], although it inhibits competitively APAO ($K_i = 8.0 \times 10^{-6}$ M) [70].

Along with 1,12-ddd, G3 is an other interesting inhibitors that shows a different affinity between SMO and APAO ($K_i = 4.6 \times 10^{-5}$ M and $K_i = 8.0 \times 10^{-7}$ M respectively), being highly specific for APAO. These results demonstrated that different analogues inhibit specifically or with different efficiency polyamine catabolic enzymes, thus allowing to foresee the design of a more specific enzyme-directed inhibition. In order to further analyse the inhibition properties of these analogues, an *in silico* comparative analyzis of the complexes formed by SMO with the substrate SPM and with the

Table 1. Values of Binding to ZmPAO, APAO and SMO for MDL 72527, Guazatine, G3, 1,8-diaminooctane, and 1,12-diaminododecane (Ki)

	K_i (M)		
	ZmPAO	**APAO**	**SMO**
Inhibitors			
MDL 72527 [72]	5.5×10^{-7}	2.1×10^{-5}	6.3×10^{-5}
Guazatine [71]	7.5×10^{-9}	4.5×10^{-7}	4.0×10^{-7}
N^3 -prenylagmatine (G3) [73, 74]	1.5×10^{-8}	8.0×10^{-7}	4.6×10^{-5}
1,8-diaminootano [70]	3.0×10^{-7}	$> 10^{-2}$	$> 10^{-2}$
1,12-diaminododecane [70]	1.7×10^{-7}	8.0×10^{-6}	$> 10^{-3}$

inhibitors MDL 72527 and 1,12-ddd has been performed. The structures of the complexes are the results of 1 nanosecond molecular dynamics simulations in explicit solvent using CHARMM [76] (see legend to Fig. (**3**) for technical details). The molecular model of SMO has been built by homology with the *Zea mays* polyamine oxidase (ZmPAO), whose crystal structure is available [77].

Starting SPM conformation within SMO active site as been modeled by analogy with yeast FMS1-SPM complex structure [78]. As above mentioned, SMO is not inhibited by the substrate analogue 1,12 diaminododecane, contrarily to ZmPAO (K_i = 1.7×10^{-7} M for ZmPAO). At variance with, both enzymes are inhibited by the MDL 72527 inhibitor, K_i =6.3×10^{-5} for SMO and K_i =5.5×10^{-7} M for ZmPAO [70]. Molecular dynamics simulations of the putative complexes formed by SMO with the substrate SPM, and the inhibitors MDL 72527 and 1,12-ddd provide the structural basis for the above outlined differences. In fact, as can be seen from Fig. (**3A-B**), both SPM and MDL 72527 establish several electrostatic and hydrophobic interactions with SMO active site residues. These interactions are almost completely absent in the putative SMO-1,12-ddd complex. In detail, SPM N^1 atom forms hydrogen bonds with the carbonyl oxygen and the side-chain hydroxyl group of Ser527, SPM N^5 atom forms hydrogen bonds with the amide carbonyl of Gln180 and the Nδ1 of His82, and SPM N^{14} atom forms an hydrogen bond with the backbone carbonyl group of Asp104 and a charge-charge interaction with Glu402, Fig. (**3A**). Hydrophobic interactions are also observed between Trp80 and the aliphatic carbon atoms C^7, C^8, and C^9 of SPM. Several of these interactions are conserved in the SMO-MDL 72527 complex, since the central portion of these inhibitors occupies the same SPM position within the SMO active site, Fig. (**3A**). In addition, the terminal diene moiety of MDL 72527 binds in a hydrophobic pocket formed by residues Val99, Leu171 and Ala172. None of the interactions observed in the SMO-SPM

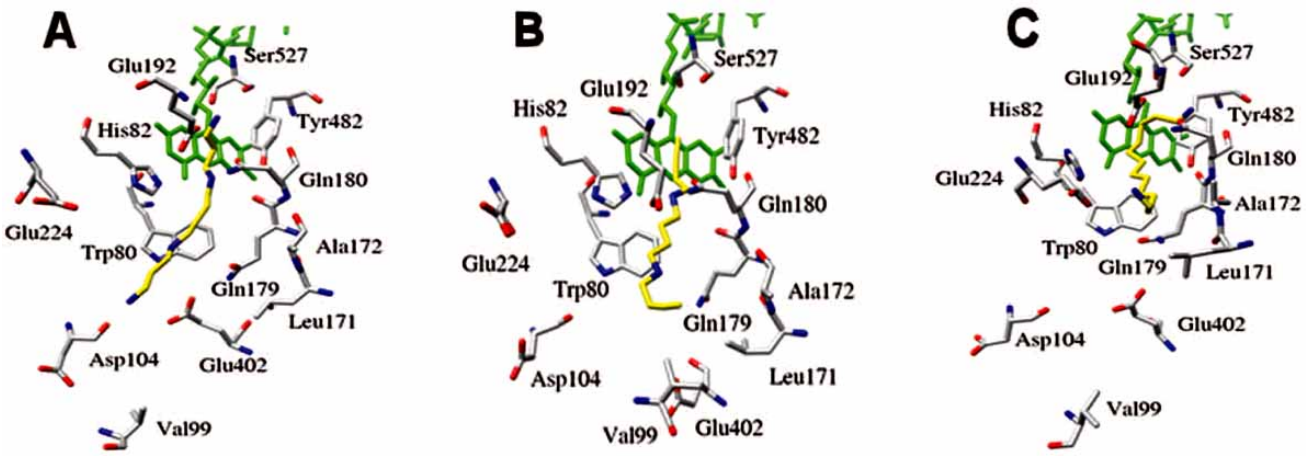

Fig. (3). Schematic representation of the putative complexes formed by mouse spermine oxidase (SMO). Schematic representation of SMO in complex with the substrate SPM (A), and the inhibitors MDL 72527 (B) and 1,12 ddd (C). Model building of the SMO three-dimensional structure has been previously reported [7]. The structures shown are the result of 1 ns molecular dynamics simulations in explicit solvent using CHARMM macromolecular mechanics package [76]. In detail, complexes were placed in a simulation box containing approximately 15000 water molecules, removing molecules overlapping with protein atoms (cut off 2.8 Å). The resulting simulation systems were energy minimized to an energy gradient of 0.05 kcal/mol and then subjected to a 1 ns molecular dynamics simulation. The starting SPM conformation within the SMO active site has been modelled by best fit of the SMO model with the X-ray structure of yeast polyamine oxidase FMS1 in complex with SPM [78]. Starting SMO-MDL and SMO-1,12 ddd complexes have been modelled by superimposing the inhibitor molecules to the substrate SPM. The FAD co-factor is depicted in green, and the substrate/inhibitors backbone in yellow.

complex are present in the putative SMO-1,12-ddd complex. The 1,12-ddd inhibitor, in the early phase of the molecular dynamics simulation moves away from the position occupied by SPM and after 1 nanosecond simulation the only relevant interaction observed is a hydrogen bond between the N^1 atom of the inhibitor and the amide carbonyl of Gln180, Fig. (**3C**). On the basis of this preliminary comparative inhibition studies and with increasing knowledge of the structural relationships between substrate analogue and enzyme active site, it could be possible to design novel and more selective inhibitors that could be applied in *in vitro* and *in vivo* experiments devoted to elucidate the physiological role of these enzymes in PA homoeostasis.

SPERMINE ANALOGUES AND CANCER THERAPY

In alternative to interfere with PA metabolism, PUT, SPD and SPM were tested as cancer treatments *per se*. Background information on this specific topic that is mainly out of the scope of the present manuscript, can be found with more details in the reference [44]. Briefly, ^{3}[H]-PUT has been used as vehicle molecule to deliver *in situ* tumour irradiation, taking advance of selective recruitment from circulating fluids; SPM and SPD conjugated with acridine, anthracene and 7-chloroquinoline have been exploited as DNA intercalating agents; finally, PA have been conjugated with specific drugs, as series of nitroimidazoles and bifunctional alkylating agent or chlorambucil [44].

Polyamine analogues share most of the natural PA cellular functions. They compete for uptake with the active channels and, inside the cellular moiety; they are able to affect the transcription of several important genes, both related or not with PA metabolism. PA analogues inhibit PA biosynthesis reducing ODC activity, by enhanced antizyme (AZ) production, that in turn halts also the recruitment of circulating PA [79, 80]. Consistently, PA analogues enhance PA catabolism blocking the ubiquitination and degradation of SSAT [81] and provoking an augmented level of SMO enzyme [6]. Furthermore, PA analogues increase the efflux of both the polyamines and their acetyl derivatives through the membrane [82]. Each of the above described processes leads to a strong PA intracellular depletion interfering negatively with the cellular growth. PA analogues were developed to overcome the concerted action of increasing activities of non-targeted enzymes and augmented PA uptake (PA homoeostasis) that represents the "Achille's heel" of the direct enzyme inhibition. On the other hand, it should be taken into account that some PA analogues have been demonstrated to interact with specific enzymes (*e.g.* SMO) being neither exclusively PA mimetic, nor exclusively PA antagonists. PA analogues possess both characteristics to different levels, and it is still problematic to define a precise mechanism of their action. A difficulty in the elucidation of PA analogues cytotoxicity is also due to the high variability of different cell lines to a given drug administration [44]. Notably, as summarized in Table (**2**), the cytotoxicity of homologous tetramines are not purely correlated with the SSAT induction and the depletion of PA pools [44]. Under a prospective point of view, PA analogues of SPD and SPM, should inhibit cell proliferation both altering PA metabolism, thus depleting the PA pools, and by competing the natural PA from functioning, even though the mechanisms of these actions are not completely clarified.

PA analogues can be sub-divided into four groups on the basis of their structure (symmetric, asymmetric, conformationally restricted analogues, and macrocyclic polyamines) [22], and in particular, SPM analogues were recognized to produce a higher inhibitory effect on cellular growth [83]. Herein after, SPM analogues will be focused (for a more exhaustive reviews on several PA analogues, see [22, 44]).

Symmetrically Substituted Bis(alkyl)spermine Analogues

The rational to design alkyl derivatives of PA came up from the need to mask amino groups recognized by endogenous amine oxidises and then metabolized to toxic by-products [44, 84]. Alkyl groups were symmetrically added to the primary amino groups of the SPM chain structure. First consequence of the SPM analogues treatment showed a clear cytotoxicity resembling that exerted by DFMO but, notably, cell type dependent. The resistant to the toxic action of DFMO small-cell lung-cancer NCI H157 cell line, was more prone to induce SSAT activity and to die as compared to the lung-cancer NCI H82 cell line, sensitive to DFMO. Interestingly, first evidences were collected of the higher toxic activity exerted by bis(ethyl)SPM (BESPM) *versus* SPD analogues [85]. This phenomenon was corroborated by further experimental evidences, where bis(alkyl)SPM analogues induced apoptotic cell death linked to SSAT activation and hampered biosynthetic PA metabolism [22, 44].

Table 2. Summary of Mechanistic and Biochemical Properties of the Main Class of SPM Analogues Described in the Text: DENSPM as Symmetrical Analogue; IPENSPM as Asymmetrical Analogue; CGC-11047 as Conformationally Restricted Analogue

SPM Analogues	Mechanistic and Biochemical Hypothetical Properties
DENSPM [86] (symmetrically substituted SPM analogue)	Competes with natural PA for uptake; stabilize SSAT at RNA and protein levels; activates SSAT and SMO, inhibits ODC; down regulates cell growth and induces apoptotic cell death in tumour cells. Depletes intracellular PA content.
IPENSPM [103] (asymmetrically substituted SPM analogue)	Competes with natural PA for uptake; induces both SMO and SSAT, contrarily to CHENSPM. Blocks cell cycle at G2/M cell with altered tubulin polymerization; cytotoxic in tumour cell lines.
CGC-11047 [106] (conformationally restricted SPM analogue)	Competes with natural PA for uptake; induces both SMO and SSAT; positive outcome in several human tumour models *in vivo*. Depletes intracellular PA content. Anti-angiogenic activity in a macular degeneration in *in vivo* model

The scenario of the cellular effects driven by BESPM was further exerted and amplified by the most potent SSAT inducers among symmetrically substituted SPM analogues, that is N^1,N^{11}-di(ethyl)norspermine (DENSPM; or BENSPM or BE333) (herein after DENSPM), Table (**2**) and Fig. (**4A**) [86]. Mechanistic interactions between DENSPM and PA metabolism are related mainly to the inhibition of the natural PA uptake [22] and to induce SSAT stability, both at transcriptional [87] and translational levels [84]. Recently, the crystal resolution of human SSAT provided the evidence that SSAT can self-stabilize its structure by functional different states of its symmetrical *vs* asymmetrical homo-dimers. As the consequence of analogues treatment is the SSAT stabilization, has been seen that SSAT residues responsible for the analogues links, correlate better for DENSPM instead of BESPM [88], thus mechanistically justify the higher SSAT induction of DENSPM. Biochemical effects of these interactions lead to PA depletion, induction of SSAT and SMO activities, cell growth arrest and apoptosis, Table (**2**) [22]. DENSPM represents the ideal prototype of the analogue compounds, strongly negatively affecting PA biosynthesis, forcing PA catabolism by both SSAT and SMO activation and competing with natural PA for cellular uptake. Although experimental preclinical protocols were promising on the clinical use [22, 89] DENSPM Phase I and Phase II clinical trials gave poor positive outcomes, associated with unbearable toxic side effects. Notably, the three Phase I trials demonstrated a clear cut off on the administration protocol, being higher dosage with multiple treatments (twice or three-times-day) not compatible with patient tolerances [90, 91]. Unfortunately, when the protocol was a single day dosage, with low negative side effects, the clinical outcome was insufficient to cure the non-small-cell lung cancer [92]. A unique Phase II protocol was achieved on pre-treated breast cancer patients with presence of metastasis, by a single day dosage. The clinical outcome was again negative, with abdominal pain, nausea, digestive disorder and central nervous system toxicity as side effects [93, 44]. It is still unclear the biochemical reasons why DENSPM treatments produce scarce or inefficacy clinical outcomes. In spite of a likely systemic DENSPM concentration that was adequate to reach tumor tissues, actual DENSPM tumours uptake was not assessed as well as the expected PA metabolism alteration [22].

Trying to correlate the increase of SSAT activity with the chemotherapeutic success, a growing interest has been posed in evaluating SSAT activity as a consequence of drugs administration. Effectively, SSAT increased during the administration of different chemotherapeutic drugs in several tumor cell lines [22, 94, 95], thus underlying the principle of testing combined treatments with standard chemotherapeutics and DENSPM. In particular, combined treatments with DENSPM and oxaliplatin (Eloxatin, Sanofi-Aventis) enhanced SSAT accumulation and activity [96]. It should be noted that SSAT activation is strongly related to a multitude of cellular stress conditions, along with chemotherapeutic treatments, tissues injures, and differentiation pathway activation [97]. Transgenic mice over expressing SSAT do not show extensive mortality at any embryonic and cellular compartments, but, controversially, in conditionally genetic modified mice, where SSAT is over expressed in the intestinal epithelium and/or keratinocytes, carcinogenesis was clearly reported [98, 99]. Thus, it could be of relevance to gain a better understanding if SSAT activation should be considered as a chemotherapeutic process (trigger) or a physiological stress response (target). Symmetrically substituted bis(alkyl)spermine analogues were not able to answer to this crucial question; their use could contrast cancer growth selectively, alone or in combined protocols, in the former hypothesis, but they could implement the intolerable toxic side effects on healthy tissues, if the latter event is preponderant.

Asymmetrically Substituted Bis(alkyl)spermine Analogues

Looking for additional PA analogues with less systemic and central nervous system toxicity, rise up the hypothesis to design PA derivatives with a more complex structure. To this end, asymmetrical groups were added to the SPM and norspermine (norSPM) molecules, which resulted the most potent growth inhibitors [100, 48]. Although two members of the asymmetrical SPM substituted, N^1-propargyl-N^{11}-ethylnorSPM (PENSPM) and N^1-cyclopropyl-methyl-N^{11}-ethylnorSPM (CPENSPM), produced overlapping results with the DENSPM treatments [101], two other members, N^1-cycloheptylmethyl-N^{11}-ethylnorspermine (CHENSPM) and (*S*)-N^1-(2-methyl-1-butyl)-N^{11}-ethyl-4,8-diazaundecane (IPENSPM), Table (**2**) and Fig. (**4B**), produced significant contradictory effects, when compared each other. In spite of small structural variation, CHENSPM does not activate SSAT, but drives cells to enter the apoptotic pathway, through a G2/M cell cycle block [102]. On the contrary, IPENSPM blocks cells at G2/M of the proliferation cycle, but does induce SSAT [103]. In spite of a mechanistic interpretation of the way of action of these asymmetrical SPM analogues is still unclear, these results represented a first evidence of the SPM analogues chemotherapeutic effects not

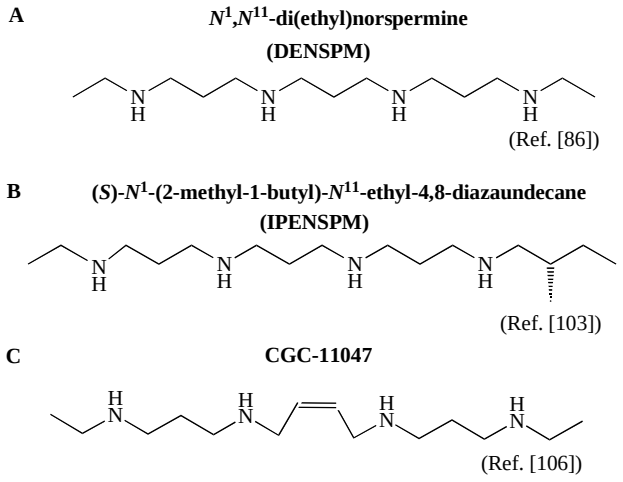

Fig. (4). Chemical structures of some SPM-derived analogues. (**A**) Structure of DENSPM as an example of a symmetrically substituted SPM analogue. (**B**) Structure of IPENSPM as an example of an asymmetrically substituted SPM analogue. (**C**) Structure of CCG-11047 as an example of a conformationally restricted SPM analogue. (GChemPaint Chemical Structure Editor, GNU software). Abbreviation used in the figure are listed in the text of the manuscript.

necessarily linked to SSAT activation, thus indicating SSAT as trigger of cell damage, at least in a cell type dependent manner. Both CHENSPM and IPENSPM enhance SMO expression, probably by the augmented SPM uptake, due to analogues antagonism to be recruited from the extra-cellular compartment. As a net consequence, both SSAT, indirectly via acetylated PA further metabolised by APAO, and SMO activities can contribute to the over production of metabolic toxic side-products.

Conformationally Restricted SPM Analogues

A further hypothesis to gain a therapeutic advantage modifying SPM backbone was to restrict the free rotation around the carbon-to-carbon bonds [104, 105]. Unsaturated double bond (inserted to the BESPM backbone) to obtain the CGC-11047 analogue, Table (**2**) and Fig. (**4C**) [106], and a cyclopropyl bond in the central 4-carbon methylene bridge (inserted in the BEHSPM backbone) to obtain the CGC-11093 analogue [107], gave rise to a new class of compounds with elevated proliferation inhibitory effect and low general toxicity [22, 44]. Both CGC-11047 and CGC-11093 are under clinically evaluation in Phase I and Phase II trials, alone or in combination with several other chemotherapeutic drugs [22]. Interestingly, resembling the uncertain biochemical property of the asymmetrical SPM analogues, only CGC-11047 is able to induce SSAT and SMO activities. Never less, others conformationally restricted SPM analogues still maintain their anticancer activities, albeit they do not enhance neither SSAT or SMO enzymes. [22].

Finally, studies performed on a mouse model of age-related macular degeneration, showed a novel application of conformationally restricted SPM analogues, that is related to an anti-angiogenesis activity [108]. This application is still not clearly associated with the alteration of PA metabolism but it is obviously well related to fight cancer.

SPERMINE METABOLISM, INFLAMMATION AND CANCER

A large number of human cancers have been directly associated to chronic inflammation [109], since first evidences by Rudolf Virchow in 1863 [110]. Inflammation should acts as temporally limited adaptive response, although insufficient curative response to injury often guides to various diseases, comprising cancer [111]. As an example, inflammatory bowel disorders, such as ulcerative colitis and Crohn's disease, lead to colorectal cancer [112], while the administration of anti-inflammatory drugs reduces the cancer incidence [113]. During inflammation and in most degenerative diseases, oxidant by-products of cellular metabolism cause an oxidative stress, which is an unbalance between reactive oxygen species (ROS) production and detoxification. Augmented levels of ROS by promotion of the inflammatory stimuli are believed to be chemical effectors in inflammation-driven carcinogenesis [114]. However, aside inflammation response, balanced ROS level induces the positives outcome of *hormesis* [115] as demonstrated in human hyper baric oxygen therapy [116] and in the oxidative damage after γ-irradiation in rats [117]. On the contrary, high ROS levels, overwhelming detoxifying mechanisms have been described as mutagenic, and to cause cell death and apoptosis. Thus, both exogenous and endogenous exposure to ROS, plays a fundamental pivotal role in cell behavior. Mechanistically, oxidative stress promotes tumor necrosis factor (TNFα) production and its binding to tumour necrosis factor receptor 1 (TNFR1) by Jun N-terminal kinase (JNK) activation [118]. The cytokine TNF-α, plays a fundamental role as inflammation promoter after cellular stress [119], and has been recently found to enhance SMO expression, in human lung epithelial cell line [120]. Interestingly, TNF-α affects PA metabolizm, enhancing the transcription level of APAO, SSAT and SMO, but only SMO activity increases. Since SMO metabolizm produces H_2O_2 as by-product, which is a DNA damaging agent, it has been postulated that the link between inflammation and cancer could be inherent in ROS over production by SMO over activity. These findings were sharply demonstrated by selective inhibition of APAO, SSAT and SMO enzymes by means of RNA-interference experiments, where only the SMO ablation leads to the inhibition of oxidative DNA damage induced by TNF-α. Consistently, identical results have been obtained after treatment with the inflammation responsive cytokine interleukin-6, thus depicting a pattern that directly links inflammation and DNA damage through the production of the H_2O_2 by the oxidation of SPM by SMO [120], although it could be of much interest to confirm this mechanistic link in *in vivo* experimental models. Additionally, damaged DNA by oxidative species causes genomic instability and replication errors, both markers of early stages of tumor transformation [121]. As matter of fact, the biochemical marker of oxidative DNA damage, 8-Oxo-7,8-dihydro-2-deoxyguanosine (8-oxo-dG) has been found augmented in *Helicobacter pylori*-induced gastric tumor [122], TNF-α-induced pulmonary carcinogenesis [120], and in mouse neuroblastoma cell line where SMO activity was ectopically forced [43].

CONCLUSION AND PERSPECTIVES

In the last decade the knowledge on the PA metabolism advanced consistently, particularly in relation to cancer and other hyper-proliferative disorders. The increased sensitivity of the analysis on the PA metabolizm and the possibility to dissect the metabolic pathways increased also the relevance to cancer cell biology. Accordingly, our knowledge of the effects of agents that interfere with PA metabolism advanced, and the goal to develop tumor-selective polyamine-related agents is becoming realistic. Molecular and biochemical tools, crystal structures for several of the polyamine metabolic enzymes, *in silico* conformationally crystal structure simulations and the availability of engineered genetically modified mice models have confirmed the mandatory roles of PA in cell physiology and tumors growth. The absolute requirement for PA in cell function and viability implies that analogues must be different enough from the natural PA to be able to act as metabolic modulators, but not so different as to be excessively toxic to normal tissues. One obstacle to drug development is the different physiological effects on animal cells displayed by PA analogues with the same high charge at physiological pH, but with slightly different backbones. On the light of this overall complexity, the study of PA metabolism is not at the point to face changeover from basic research to clinical utility, but necessarily, it

has to represent the two sides of the same coin. Beside the obvious effort to create novel and more selective PA analogues in order to effectively halt tumor cell proliferation by means of PA metabolizm alteration, some basic knowledge on the action of the analogues still does need to be investigated. Within this frame, the source of ROS driving DNA damage, the analogues-enzymes interactions, and, more in general, the specific cellular targeting, are issues that definitely deserve more attention. Meantime, it should be considered that SPM analogues are easily synthesized, extremely water-soluble, stable at room temperature for a long time, and thus can be characterized both in *in vitro* and *in vivo* experimental models. The possibility to express *in vitro* the recombinant SMO enzyme, allows to improve to a deeper and deeper level the knowledge of the structures and functional interactions of synthetically derived SPM compounds with SMO enzyme. These studies will determine a panel of newly synthesized SMO specific inhibitors in order to design potentially anticancer drugs to be used alone or in combinatorial therapeutic protocols.

ABBREVIATIONS

AcCoA = Acetyl-coenzyme A

AcSPD = N^1-acetylspermidine

AcSPM = N^1-acetylspermine

AdoDATAD = S-adenosyl-1,12-diamino-3-thio-9-azadodecane

AdoDATO = S-adenosyl-1,8-diamino-3-thiooctane

AdoMetDC = S-adenosylmethioninedecarboxylase enzyme

APAO = N^1-acetylpolyamine oxidase

APE1 or REF1 = apurinic/apyrimidinic endonuclease

AZ = antizyme

BESPD = N^1,N^8bis(ethyl)SPD

BESPM = bis(ethyl)SPM

CPENSPM = *N*1-cyclopropyl-methyl-*N*11-ethylnorSPM

CuAO = cupper amino oxidase

DENSPM = N^1,N^{11}-di(ethyl)norspermine

= or BENSPM or BE333

CHENSPM = *N*1-cycloheptylmethyl-*N*11-ethylnorspermine

dcSAM = S-adenosylmethioninedecarboxylated

DFMO = 2-fluoromethylornithine

1,12-ddd = 1,12 diaminododecane

EGBG = ethylglyoxalbis(guanylhydrazone)

FAD = flavin-adenine-dinucleotide

G3 = N^3 -prenylagmatine

H_2O_2 = hydrogen peroxide

IPENSPM = (*S*)-*N*1-(2-methyl-1-butyl)-*N*11-ethyl-4,8-diazaundecane

JNK = Jun N-terminal kinase

MDL 72527 = N^1,N^4-bis(2,3-butadienyl)-1,4butanediamine

MGBG = methylglyoxal bis(guanylhydrazone)

MTA = 5'-deoxy-5'-(methylthio)adenosine

norSPM = norsperpime

ODC = ornithine decarboxylase

ORN = ornhitine

8-oxo-dG = 8-Oxo-7,8-dihydro-2′-deoxyguanosine

PA = polyamines

PAO-h1 = human polyamine oxidase

PENSPM = N1-propargyl-N11-ethylnorSPM

Phex = phosphate-regulating gene with homologies with endopeptidases on the X chromosome

PUT = putrescine

ROS = reactive oxygen species

SAO = Serum amino oxidase

SAM = S-adenosyl-methionine

SAM486A = 4-(aminoiminomethyl)-2,3-dihydro-1H-inden-1-one-diaminomethylenehydrazone

SMO = spermine oxidase

SPM = spermine

SMS = spermine synthase

SPD = spermidine

SPDS = spermidine synthase

SSAT = spermidine/spermine N^1-acetyltransferase

TNFα = tumour necrosis factor

TPQ = 2,4,5-trihydroxyphenylalanine quinone

ZmPAO = *Zea mays* polyamine oxidase

REFERENCES

[1] Cohen, S. S. *Guide to the polyamines;* Oxford University Press: NY, **1998**.

[2] Wallace, H. M.; Fraser, A. V.; and Hughes, A. A perspective of polyamine metabolism. *Biochem. J.* **2003**, *376*, 1-14.

[3] Schipper, R. G.; Penning, L. C.; Verhofstad, A. A. Involvement of polyamines in apoptosis. Facts and controversies: effectors or protectors? *Semin. Cancer Biol.* **2000**, *10*, 55-68.

[4] Thomas, T.; Thomas, T. J. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cell. Mol. Life Sci.* **2001**, *2*, 244-258.

[5] Wang, Y.; Devereux. W.; Woster, P. M.; Stewart, T. M.; Hacker, A.; Casero, R. A. Jr. Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analog exposure. *Cancer Res.* **2001**, *61*, 5370-5373.

[6] Vujcic, S.; Diegelman; P.; Bacchi, C. J.; Kramer, D. L.; Porter, C. W. Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. *Biochem. J.* **2002**, *367*, 665-675.

[7] Cervelli, M.; Polticelli, F.; Federico, F.; Mariottini, P. Heterologous expression and characterization of mouse spermine oxidase . *J. Biol. Chem.* **2003**, *278*, 5271-5276.

[8] Soulet, D.; Gagnon, B.; Rivest, S.; Audette, M.; Poulin, R. A. A fluorescente probe of polyamine transport accumulates into intracellular acidic vesicles *via* a two-step mechanism. *J. Biol. Chem.* **2004**, *279*, 49355-49366.

[9] Belting, M.; Persson, S.; Fransson, L. A. Proteoglycan involvement in polyamine uptake. *Biochem. J.* **1999**, *338*, 317-323.

[10] Gerner, E. W.; Meyskens, F. L. Jr. Polyamines and cancer: old molecules, new understanding. *Nat. Rev. Cancer* **2004**, *4*, 781-792.

[11] Thomas, T.; Thomas, T. J. Polyamine metabolism and cancer. *J. Cell. Mol. Med.* **2003**, *7*, 113-126.

[12] Seiler, N. Thirty Years of Polyamine-Related Approaches to Cancer Therapy. Retrospect and Prospect. Part 1. Selective Enzyme Inhibitors. *Curr. Drug Targets* **2003**, *4*, 537-564.

[13] McCann, P. P.; Pegg, A. E. Ornithine decarboxylase as an enzyme target for therapy. *Pharmacol. Ther.* **1992**, *54*, 195-215.

[14] Wallace, H. M.; Fraser, A. V. Inhibitors of polyamine metabolism: review article. *Amino Acids* **2004**, *26*, 353-365.

[15] Herr, H. W.; Warrel, R. P.; Burchenal, J. H. Phase I trial of -difluoromethylornithine (DFMO) and methylglyoxal bis(guanylhydrazone) (MGBG) in patients with advanced prostatic cancer. *Urology* **1986**, *28*, 508-511.

[16] Meyskens, F. L.; Kingsley, E. M.; Glattke, T.; Loescher, L.; Booth, A. A Phase II study of -difluoromethylornithine (DFMO) for the treatment of metastatic melanoma. *Invest. New Drugs* **1986**, *4*, 257-262.

[17] Horn, Y.; Schechter, P. J.; Marton, L. J. Phase I–II clinical trial with -difluoromethylornithine — an inhibitor of polyamine biosynthesis. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1103-1107.

[18] Ganju, V.; Edmonson, J. H.; Buckner, J. C. Phase I study of combined interferon, -difluoromethylornithine (DFMO), and doxorubicin in advanced malignancy. *Invest. New Drugs* **1994**, *12*, 25-27.

[19] Mitchell, M. F.; Tortolero-Luna, G.; Lee, J. J.; Hittelman, W. N.; Lotan, R.; Wharton, J. T.; Hong, W. K.; Nishioka, K. Phase I dose de-escalation trial of -difluoromethylornithine in patients with grade 3 cervical intraepithelial neoplasia. *Clin. Cancer Res.* **1998**, *4*, 303-310.

[20] Prados, M. D.; Wara, W. M.; Sneed, P. K.; McDermott, M.; Chang, S. M.; Rabbitt, J.; Page, M.; Malec, M.; Davis, R. L.; Gutin, P. H.; Lamborn, K.; Wilson, C. B.; Phillips, T. L.; Larson, D. A. Phase III trial of accelerated hyperfractionation with or without difluromethylornithine (DFMO) versus standard fractionated radiotherapy with or without DFMO for newly diagnosed patients with glioblastoma multiforme. *Int. J. Radiat. Oncol. Biol. Phys.* **2001**, *49*, 71-77.

[21] Levin, V. A.; Hess, K. R.; Choucair, A.; Flynn, P. J.; Jaeckle, K. A.; Kyritsis, A. P.; Yung, W. K.; Prados, M. D.; Bruner, J. M.; Ictech, S.; Gleason, M. J.; Kim, H. W. Phase III randomized study of postradiotherapy chemotherapy with combination -difluoromethylornithine-PCV versus PCV for anaplastic gliomas. *Clin. Cancer Res.* **2003**, *9*, 981-990.

[22] Casero, R. A. Jr.; Marton, L. J. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. *Nature Rev. Drug Discov.* **2007**, *6*, 373-390.

[23] Chen, Y.; Weeks, R. S.; Burns, M. R.; Boorman, D. W.; Klein-Szanto, A.; O'Brien, T. G. Combination therapy with 2-difluoromethylornithine and a polyamone transport inhibitor against murine squamous cell carcinoma. *Int. J. Cancer* **2006**, *118*, 2344-2349.

[24] Meyskens, F. L. Jr.; Gerner, E. W. Development of 2-difluoromethylornithine (DFMO) as a chemoprevention agent. *Clin. Cancer Res.* **1999**, *5*, 945-951.

[25] Simoneau, A. R.; Gerner, E. W.; Nagle, R.; Ziogas, A.; Fujikawa-Brooks, S.; Yerushalmi, H.; Ahlering, T. E.; Lieberman, R.; McLaren, C. E.; Anton-Culver, H.; Meyskens, F. L. Jr. 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 Epidemiol. Biomarkers Prev.* **2008**, *17*, 292-299.

[26] Meyskens, F. L. Jr.; McLaren, C. E.; Pelot, D.; Fujikawa-Brooks, S.; Carpenter, P. M.; Hawk, E.; Kelloff, G.; Lawson, M. J.; Kidao, J.; McCracken, J.; Albers, C. G.; Ahnen, D. J.; Turgeon, D. K.; Goldschmid, S.; Lance, P.; Hagedorn, C. H.; Gillen, D. L.; Gerner, E. W. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: A randomized placebo-controlled, double-blind trial. *Cancer Prev. Res.* **2008**, *1*, 32-38.

[27] Sporn, M. B.; Hong, W. K. Clinical prevention of recurrence of colorectal adenomas by the combination of difluoromethylornithine and sulindac: An important milestone. *Cancer Prev. Res.* **2008**, *1*, 9-11.

[28] Freedlander, B. L.; French, F. A. Carcinostatic action of polycarbonyl compounds and their derivatives. II. Glyoxal bis(guanylhydrazone) and derivatives. *Cancer Res.* **1958**, *18*, 360-363.

[29] Porter, C. W.; Dworaczyk, D.; Ganis, B.; Weiser, M. M. Polyamines and biosynthetic enzymes in the rat intestinal mucosa and the influence of methylglyoxal bis(guanylhydrazone). *Cancer Res.* **1980**, *40*, 2330-2335.

[30] Pegg, A. E. Inhibition of spermidine formation in rat liver and kidney by methylglyoxal bis(guanylhydrazone). *Biochem. J.* **1973**, *132*, 537-540.

[31] Pleshkewych, A.; Kramer, D. L.; Kelly, E.; Porter, C. W. Independence of drug action on mitochondria and polyamines in L1210 leukemia cells treated with methylglyoxal-bis(guanylhydrazone). *Cancer Res.* **1980**, *40*, 4533-4540.

[32] Igarashi, K.; Porter, C. W.; Morris, D. R. Comparison of specificity of inhibition of polyamine synthesis in bovine lymphocytes by ethyl-glyoxal bis(guanylhydrazone) and methylglyoxal bis(guanylhydrazone). *Cancer Res.* **1984**, *4*, 5326-5331.

[33] Stanek, J.; Caravatti, G.; Frei, J.; Furet, P.; Mett, H.; Schneider, P.; Regenass, U. 4-Amidinoindan-1-one 20-amidinohydrazone: a new potent and selective inhibitor of S-adenosylmethionine decarboxylase. *J. Med. Chem.* **1993**, *36*, 2168-2171.

[34] Siu, L. L.; Rowinsky, E. K.; Hammond, L. A; Weiss, G. R; Hidalgo, M.; Clark, G. M.; Moczygemba, J.; Choi, L.; Linnartz, R.; Barbet, N. C.; Sklenar, I. T.; Capdeville, R.; Gan, G.; Porter, C. W.; Von Hoff, D. D.; Eckhardt, S. G. A Phase I and pharmacokinetic study of SAM486A, a novel polyamine biosynthesis inhibitor, administered on a daily-times-five every-three-week schedule in patients with advanced solid malignancies. *Clin. Cancer Res.* **2002**, *8*, 2157-2166.

[35] Hibasami, H.; Pegg, A. E. Differential inhibition of mammalian amynopropyltransferase activities. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 1398-1405.

[36] Pegg, A. E.; Tang, K. C.; Coward, J. K. Effects of S-adenosyl-1,8-diamino-3-thiooctane on polyamine metabolism. *Biochemistry* **1982**, *21*, 5082-5089.

[37] Pegg, A. E.; Wechter, R.; Poulin, R.; Woster, P. M.; Coward, J. K. Effects 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*, 8446-8453.

[38] Flayeh, K. A.; Wallace, H. M. Polyamine oxidase activity in a human colonic carcinoma cell line. *Biochem. Soc. Trans.* **1990**, *18*, 1225.

[39] Lindsay, G. S.; Wallace, H. M. Changes in polyamine catabolism in HL-60 human promyelogenous leukaemic cells in response to etoposide-induced apoptosis. *Biochem. J.* **1999**, *337*, 83-87.

[40] Quash, G.; Keolouangkhot, T.; Gazzolo, L.; Ripoll, H.; Saez, S. Diamine oxidase and polyamine oxidase activities in normal and transformed cells. *Biochem. J.* **1979**, *177*, 275-282.

[41] Bey, P.; Bolkenius, F. N.; Seiler, N.; Casara, P. N-2,3-butadienyl-1,4butanediamine derivatives: potent irreversible inactivators of mammalian polyamine oxidase. *J. Med. Chem.* **1985**, *28*, 1-2.

[42] Dai, H.; Kramer, D. L.; Yang, C.; Murti, K. G.; Porter, C. W.; Cleveland, J. L. The polyamine oxidase inhibitor MDL 72527 selectively induces apoptosis of transformed hematopoietic cells through lysosomotropic effects. *Cancer Res.* **1999**, *59*, 4944-4954.

[43] Amendola, R.; Bellini, A.; Cervelli, M.; Degan, P.; Marcocci, L.; Martini, F.; Mariottini, P. Direct oxidative DNA damage, apoptosis and radio sensitivity by spermine oxidase activities in mouse neuroblastoma cells. *Biochim. Biophys. Acta Rev. Cancer* **2005**, *1775*, 15-24.

[44] Seiler, N. Thirty Years of Polyamine-Related Approaches to Cancer Therapy. Retrospect and Prospect. Part 2. Structural Analogs and Derivatives. *Curr. Drug Targets* **2003**, *4*, 565-585.

[45] Byers, T. L.; Kameji, R.; Rannels, D. E.; Pegg, A. E. Multiple pathways for uptake of paraquat, methylglyoxal bis(guanylhydrazone) and polyamines. *Am. J. Physiol.* **1987**, *252*, 663-669.

[46] Persson, L.; Holm, I.; Ask, A.; Heby, O. Curative effect of DL-2-difluoromethylornithine on mice bearing mutant L1210 leukemia cells deficient in polyamine uptake. *Cancer Res.* **1988**, *48*, 4807-4811.

[47] Porter, C. W.; Miller, J.; Bergeron, R. J. Aliphatic chain length specificity of the polyamine transport system in ascites L1210 leukemia cells. *Cancer Res.* **1984**, *44*, 126-128.

[48] Fraser, A. V.; Woster, P. M.; Wallace, H. M. Induction of apoptosis in human leukaemic cells by IPENSpm, a novel polyamine analog and anti-metabolite. *Biochem. J.* **2002**, *367*, 307-312.

[49] Aziz, S. M.; Gosland, M. P.; Crooks, P. A.; Olson, J. W.; Gillespie, M. NA novel polymeric spermine conjugate inhibits polyamine transport in pulmolary artery smooth muscle cells. *J. Pharmacol. Exp. Ther* . **1995**, *274*, 181-186.

[50] Weeks, R. S.; Vanderwerf, S. M.; Carlson, C. L.; Burns, M. R.; O'Day, C. L.; Cai, F.; Devens, B. H.; Webb, H. K. Novel lysine-spermine conjugate inhibits polyamine transport and inhibits cell growth when given with DFMO. *Exp. Cell Res.* **2000**, *261*, 293-302

[51] Porter, C. W.; Bergeron, R. J. Spermidine requirement for cell proliferation in eukaryotic cells: structural specificity and quantitation. *Science* **1983**, *219*, 1083-1085.

[52] Porter, C. W.; Sufrin, J. R. 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.

[53] Seiler, N. How important is the oxidative degradation of spermine?: Minireview article. *Amino Acids* **2004**, *26*, 317-319.

[54] Leeuwenhoek, A. van in Observationes D. Anthonii Leeuwenhoek, de natis e semine genitali animalculis. Letter dated November. *Philos. Trans. R. Soc. Lond.* **1677**, *12*, 1040-1043.

[55] Ladenburg, A.; Abelm J. Über das Aethylenimin (Spermin?). *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 758-766.

[56] Wrede, F. Über die aus menschlichem Sperma isolierte Base Spermin. *Dtsch. Med. Wochenschr.* **1925**, 51-24.

[57] Dudley, H. W.; Rosenheim, O.; Starling, W. W. The chemical constitution of spermine. III. Structure and synthesis. *Biochem. J.* **1926**, *20*, 1082-1094.

[58] Feuerstein, B. G.; Pattabiraman, N.; Marton, L. J. Spermine-DNA interactions: a theoretical study. *Proc. Natl. Acad. Sci. USA* **1986**, *16*, 5948-5952.

[59] Seiler, N.; Atanassov, C. L. The natural polyamines and the immune system. *Prog. Drug Res.* **1994**, *43*, 87-141.

[60] Nilsson, J.; Gritli-Linde A.; Heby, O. Skin fibroblasts from spermine synthase-deficient hemizygous gyro male (*Gy*/Y) mice overproduce spermidine and exhibit increased resistance to oxidative stress but decreased resistance to UV irradiation. *Biochem. J.* **2000**, *352*, 381-387.

[61] Stefanelli, C.; Pignatti, C.; Tantini, B.; Fattori, M.; Stanic, I.; Mackintosh, C. A.; Flamigni, F.; Guarnieri, C.; Caldarera, C. M.; Pegg, A. E. Effect of polyamine depletion on caspase activation: a study with spermine synthase-deficient cells. *Biochem. J.* **2001**, *355*, 199-206.

[62] Agostinelli, E.; Arancia, G.; Dalla Vedova, L.; Belli, F.; Marra, M.; Salvi, M.; Toninello, A. The biological functions of polyamine oxidation products by amine oxidises: Perspectives of clinical applications. *Amino Acids* **2004**, *27*, 347-358.

[63] Cervelli, M.; Bellini, A.; Bianchi, M.; Marcocci, L.; Nocera, S.; Ponticelli, F.; Federico, R.; Amendola, R.; Mariottini, P. Mouse spermine oxidase gene splice variants: Nuclear subcellular localization of a novel active isoform. *Eur. J. Biochem.* **2004**, *271*, 760-770.

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

[65] Schiller, M.; Blank, N.; Heyeder, P.; Herrmann, M.; Gaipl, U. S.; Kalden, J. R.; Lorenz, H-M. Induction of apoptosis by spermine-metabolites in primary human blood cells and various tumor cell lines. *Apoptosis* **2005**, *10*, 1151-1162.

[66] Calcabrini, A.; Arancia, G.; Marra, M.; Crateri, P.; Befani, O.; Martone, A.; Agostinelli, E. Enzymatic oxidation products of spermine induce greater cytotoxic effects on human multidrug-resistant colon carcinoma cells [LoVo] than on their wild-type counterparts. *Int. J. Cancer* **2002**, *99*, 43-52.

[67] Pledgie, A.; Huang, Y.; Hacker, A.; Zhang, Z.; Woster, P. M.; Davidson, N. E.; Casero, R. A. Jr. Spermine oxidase SMO(PAOh1), Not N1-acetylpolyamine oxidase PAO, is the primary source of cytotoxic H_2O_2 in polyamine analog-treated human breast cancer cell lines. *J. Biol. Chem.* **2005**, *280*, 39843-39851.

[68] Bianchi, M.; Bellini, A.; Cervelli, M.; Degan, P.; Marcocci, L.; Martini, F.; Scatteia, M.; Mariottini, P.; Amendola, R. Chronic sublethal oxidative stress by spermine oxidase over activity induces continuous DNA repair and hypersensitivity to radiation exposure. *Biochim. Biophys. Acta* **2007**, *1773*, 774-783.

[69] Bellelli, A.; Cavallo, S.; Nicolini, L.; Cervelli, M.; Bianchi, M.; Mariottini, P.; Zelli, M.; Federico, R. Mouse spermine oxidase: a model of the catalytic cycle and its inhibition by N,N^1-bis(2,3-butadienyl)-1,4-butanediamine. *Biochem. Biophys. Res. Commun.* **2004**, *322*, 1-8.

[70] Bianchi, M.; Polticelli, F.; Ascenzi, P.; Botta, M.; Federico, R.; Mariottini, P.; Cona, A. Inhibition of polyamine and spermine oxidases by polyamine analogs. *FEBS J.* **2006**, *273*, 1115-1123.

[71] Binda, C.; Angelini, R.; Federico, R.; Ascenzi, P.; Mattevi, A. Structural bases for inhibitor binding and catalysis in polyamine oxidase. *Biochemistry* **2001**, *40*, 2766-2776.

[72] Bolkenius, F. N.; Bey, P.; Seiler, N. Specific inhibition of polyamine oxidase *in vivo* is a method for the elucidation of its physiological role. *Biochim. Biophys. Acta* **1985**, *838*, 69-76.

[73] Delle Monache, G.; Volpe, A. R.; Delle Monache, F.; Vitali, A.; Botta, B.; De Bonnevaux, S. C.; De Luca, C.; Botta, M.; Corelli, F.; Carmignani, M. Further hypotensive metabolites from *Verbesina caracasana*. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3249-3254.

[74] Corelli, F.; Federico, R.; Cona, A.; Venturini, G.; Schenone, S.; Botta, M. Solution and solid-phase synthesis of aminoalkylguanidines inhibiting polyamine oxidase and nitric oxide synthase. *Med. Chem. Res.* **2002**, *11*, 309-321.

[75] Wu, T.; Yankovskaya, V.; McIntire, W. S. Cloning, sequencing, and heterologous expression of the murine peroxisomal flavoprotein, N^1-acetylated polyamine oxidase. *J. Biol. Chem.* **2003**, *278*, 20514-20525.

[76] Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comput. Chem.* **1983**, *4*, 187-217.

[77] Binda, C.; Coda, A.; Angelini, R.; Federico, R.; Ascenzi, P.; Mattevi, A. A 30 Å long U-shaped catalytic tunnel in the crystal structure of polyamine oxidase. *Structure* **1999**, *7*, 265-276.

[78] Huang, Q.; Liu, Q.; Hao, Q. Crystal structures of Fms1 and its complex with spermine reveal substrate specificity. *J. Mol. Biol.* **2005**, *348*, 951-959.

[79] Mitchell, J. L.; Leyser, A.; Holtorff, M. S.; Bates, J. S.; Frydman, B.; Valasinas, A. L.; Reddy, V. K.; Marton, L. J. Antizyme induction by polyamine analogs as a factor in cell growth inhibition. *Biochem. J.* **2002**, *366*, 663-671.

[80] Zhu, C.; Lang, D. W.; Coffino, P. Antizyme2 is a negative regulator of ornithine decarboxylase and polyamine transport. *J. Biol. Chem.* **1999**, *274*, 26425-26430.

[81] Coleman, C. S.; Pegg, A. E. Polyamine analogs inhibit the ubiquitination of spermidine/spermine N1-acetyltransferase and prevent its targeting to the proteasome for degradation. *Biochem. J.* **2001**, *358*, 137-145.

[82] Xie, X.; Gillies, R. J.; Gerner, E. W. Characterization of a diamine exporter in Chinese hamster ovary cells and identification of specific polyamine substrates. *J. Biol. Chem.* **1997**, *272*, 20484-20489.

[83] Porter, C. W.; McManis, J.; Casero, R. A.; Bergeron, R. J. Relative abilities of bis(ethyl) derivatives of putrescine, spermidine, and spermine to regulate polyamine biosynthesis and inhibit L1210 leukemia cell growth. *Cancer Res.* **1987**, *47*, 2821-2825.

[84] Brunton, V. G.; Grant, M. H.; Wallace, H. M. Mechanisms of spermine toxicity in baby hamster kidney (BHK) cells. The role of amine oxidases and oxidative stress. *Biochem. J.* **1991**, *280*, 193-198.

[85] Casero, R. A. Jr.; Celano, P.; Ervin, S. J.; Porter, C. W.; Bergeron, R. J.; Libby, P. R. Differential induction of spermidine/spermine N1-acetyltransferase in human lung cancer cells by the bis(ethyl)polyamine analogs. *Cancer Res.* **1989**, *49*, 3829-3833.

[86] Bergeron, R. J.; Hawthorne, T. R.; Vinson, J. R.; Beck, D. E. Jr.; Ingeno, M. J. Role of the methylene backbone in the antiproliferative activity of polyamine analogs on L1210 cells. *Cancer Res.* **1989**, *49*, 2959-2964.

[87] Fogel-Petrovic, M.; Shappell, N. W.; Bergeron, R. J.; Porter, C. W. Polyamine and polyamine analog regulation of sper-

midine/spermine N1-acetyltransferase in MALME-3M human melanoma cells. *J. Biol. Chem.* **1993**, *268*, 19118-19125.

[88] Bewley, M. C.; Graziano, V.; Jiang, J.; Matz, E.; Studier, F. W.; Pegg, A. E.; Coleman, C. S.; Flanagan, J. M. Structures of wild-type and mutant human spermidine/spermine N1-acetyltransferase, a potential therapeutic drug target. *Proc. Natl Acad. Sci. USA* **2006**, *103*, 2063-2068.

[89] Bernacki, R. J.; Bergeron, R. J.; Porter, C. W. Antitumor activity of N,N'-bis(ethyl)spermine homologues against human MALME-3 melanoma xenografts. *Cancer Res.* **1992**, *52*, 2424-2430.

[90] Creaven, P. J.; Perez, R..; Pendyala, L.; Meropol, N. J.; Loewen, G.; Levine, E.; Berghorn, E.; Raghavan, D. Unusual central nervous system toxicity in a Phase I study of N^1N^{11} diethylnorspermine in patients with advanced malignancy. *Invest. New Drugs* **1997**, *15*, 227-234.

[91] Streiff, R. R.; Bender, J. F. Phase 1 study of N1-N11-diethylnorspermine (DENSPM) administered TID for 6 days in patients with advanced malignancies. *Invest. New Drugs* **2001**, *19*, 29-39.

[92] Hahm, H. A.; Ettinger, D. S.; Bowling, K.; Hoker, B.; Chen, T. L.; Zabelina, Y.; Casero, R. A. Jr. Phase I study of n(1), n(11)-diethylnorspermine in patients with non-small cell lung cancer. *Clin. Cancer Res.* **2002**, *8*, 684-690.

[93] Wolff, A. C.; Armstrong, D. K.; Fetting, J. H.; Carducci, M. K.; Riley, C. D.; Bender, J. F.; Casero, R. A. Jr.; Davidson, N. E. 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. *Clin. Cancer Res.* **2003**, *9*, 5922-5928.

[94] Boyer, J.; Allen, W. L.; McLean, E. G.; Wilson, P. M.; McCulla, A.; Moore, S.; Longley, D. B.; Caldas, C.; Johnston, P. G. Pharmacogenomic identification of novel determinants of response to chemotherapy in colon cancer. *Cancer Res.* **2006**, *66*, 2765-2777.

[95] Maxwell, P. J.; Longley, D. B.; Latif, T.; Boyer, J.; Allen, W. L.; Lynch, M.; McDermott, U.; Harkin, D. P.; Allegra, C. J.; Johnston, P. G. Identification of 5-fluorouracil-inducible target genes using cDNA microarray profiling. *Cancer Res.* **2003**, *63*, 4602-4606.

[96] Choi, W.; Gerner, E. W.; Ramdas, L.; Dupart, J.; Carew, J.; Proctor, L.; Huang, P.; Zhang, W.; Hamilton, S. R. Combination of 5-fluorouracil and N^1,N^{11}-diethylnorspermine markedly activates spermidine/spermine N^1-acetyltransferase expression, depletes polyamines, and synergistically induces apoptosis in colon carcinoma cells. *J. Biol. Chem.* **2005**, *280*, 3295-3304.

[97] Pegg, A. E. Spermidine/spermine-N1-acetyltransferase; a key metabolic regulator. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *283*, 885-1010.

[98] Tucker, J. M.; Murphy, J. T.; Kisiel, N.; Diegelman, P.; Barbour, K. W.; Davis, C.; Medda, M.; Alhonen, L.; Jänne, J.; Kramer, D. L.; Porter, C. W.; Berger, F. G. Potent modulation of intestinal tumorigenesis in Apcmin/+ mice by the polyamine catabolic enzyme spermidine/spermine N1-acetyltransferase. *Cancer Res.* **2005**, *65*, 5390-5398.

[99] Wang, X.; Feith, D. J.; Welsh, P.; Coleman, C. S.; Lopez, C.; Woster, P. M.; O'Brien, T. G.; Pegg, A. E. Studies of the mechanism by which increased spermidine/spermine N1-acetyltransferase activity increases susceptibility to skin carcinogenesis. *Carcinogenesis* **2007**, *28*, 2404-2411.

[100] Saab, N. H.; West, E. E.; Bieszk, N. C.; Preuss, C. V.; Mank, A. R.; Casero, R. A. Jr.; Woster, P. M. Synthesis and evaluation of unsymmetrically substituted polyamine analogs as modulators of human spermidine/spermine-N^1-acetyltransferase (SSAT) and as potential antitumor agents *J. Med. Chem.* **1993**, *36*, 2998-3004.

[101] Casero, R. A. Jr.; Woster, P. M. Terminally alkylated polyamine analogs as chemotherapeutic agents. *J. Med. Chem.* **2001**, *44*, 1-26.

[102] Ha, H. C.; Woster, P. M.; Casero, R. A. Jr. Unsymmetrically substituted polyamine analog induces caspase-independent programmed cell death in BCL-2-overexpressing cells. *Cancer Res.* **1998**, *58*, 2711-2714.

[103] Ha, H. C.; Woster, P. M.; Yager, J. D.; Casero, R. A. Jr. The role of polyamine catabolism in polyamine analog-induced programmed cell death. *Proc. Natl Acad. Sci. USA* **1997**, *94*, 11557-11562.

[104] Reddy, V. K.; Valasinas, A.; Sarkar, A.; Basu, H. S.; Marton, L. J.; Frydman, B. Conformationally restricted analogs of ^1N,^{12}N-bisethylspermine: synthesis and growth inhibitory effects on human tumor cell lines. *J. Med. Chem.* **1998**, *41*, 4723-4732.

[105] Loussouarn, G.; Marton, L. J.; Nichols, C. G. Molecular basis of inward rectification: structural features of the blocker defined by extended polyamine analogs. *Mol. Pharmacol.* **2005**, *68*, 298-304.

[106] Waters, W. R.; Frydman, B.; Marton L. J.; Valasinas, A.; Reddy, V. K.; Harp, J. A.; Wannemuehler, M. J.; Yarlett, N. (1)*N*, (12)*N*]bis(ethyl)-*cis*-6, 7-dehydrospermine: a new drug for treatment and prevention of *Cryptosporidium parvum* infection of mice deficient in T-cell receptor α. *Antimicrob. Agents Chemother.* **2000**, *44*, 2891-2894.

[107] Frydman, B.; Porter, C. W.; Maxuitenko, Y.; Sarkar, A.; Bhattacharya, S.; Valasinas, A.; Reddy, V. K.; Kisiel, N.; Marton, L. J.; Basu, H. S. A novel polyamine analog (SL-11093) inhibits growth of human prostate tumor xenografts in nude mice. *Cancer Chemother. Pharmacol.* **2003**, *51*, 488-492.

[108] Lima e Silva, R.; Kachi, S.; Akiyama, H.; Shen, J.; Hatara, M. C.; Aslam, S.; Gong, Y. Y.; Khu, N. H.; Lauer, T. W.; Hackett, S. F.; Marton, L. J.; Campochiaro, P. A. Trans-scleral delivery of polyamine analogs for ocular neovascularization. *Exp. Eye Res.* **2006**, *83*, 1260-1267.

[109] Mueller, M. M.; Fusenig, N. E. Friends or foes—bipolar effects of the tumour stroma in cancer. *Nature Rev. Cancer* **2004**, *4*, 839-849.

[110] Balkwill, F.; Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* **2001**, *357*, 539-545.

[111] Schottenfeld, D.; Beebe-Dimmer, J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA. Cancer J. Clin.* **2006**, *56*, 69-83.

[112] Seril, D. N.; Liao, J.; Yang, G. Y.; Yang, C. S. Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* **2003**, *24*, 353-362.

[113] Eaden, J.; Abrams, K.; Ekbom, A.; Jackson, E.; Mayberry, J. Colorectal cancer prevention in ulcerative colitis: a case–control study. *Aliment Pharmacol. Ther.* **2000**, *14*, 145-153.

[114] Kundu, J. K.; Surh, Y. -J. Inflammation: Gearing the journey to cancer. *Mutat. Res.* **2008**, *659*(1-2), 15-30.

[115] Goldman, M. Cancer risk of low-level exposure. *Science* **1996**, *271*, 1821-1822.

[116] Dennog, C.; Hartmann, A.; Frey, G.; Speit, G. Detection of DNA damage after hyperbaric oxygen (HBO) therapy. *Mutagenesis* **1996**, *11*, 605-609.

[117] Zastawny, T. H.; Czerwinska, B.; Drzewiecka, B.; Olinski, R. Radiation-induced oxidative DNA base damage and its repair in nuclear matrix-associated DNA and in bulk DNA in hepatic chromatin of rat upon whole-body gamma-irradiation. *Free Radic. Biol. Med.* **1997**, *22*, 101-107.

[118] Kamata, H.; Honda, S.; Maeda, S.; Chang, L.; Hirata, H.; Karin, M. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **2005**, *120*, 649-661.

[119] Beutler, B. A. The role of tumor necrosis factor in health and disease. *J. Rheumatol. Suppl.* **1999**, *57*, 16-21.

[120] Babbar, N.; Casero, R. A. Jr. Tumor Necrosis Factor-A Increases Reactive Oxygen Species by Inducing Spermine Oxidase in Human Lung Epithelial Cells: A Potential Mechanism for Inflammation-Induced Carcinogenesis. *Cancer Res.* **2006**, *66*, 11125-11130.

[121] Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB. J.* **2003**, *17*, 1195-1214.

[122] Xu, H.; Chaturvedi, R.; Cheng, Y.; Bussiere, F. I.; Asim, M.; Yao, M. D.; Potosky, D.; Meltzer, S. J.; Rhee, J. G.; Kim, S. S.; Moss, S. F.; Hacker, A.; Wang, Y.; Casero, R. A. Jr.; Wilson, K. T. Spermine oxidation induced by Helicobacter pylori results in apoptosis and DNA damage: implications for gastric carcinogenesis. *Cancer Res.* **2004**, *64*, 8521-8525.

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