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Polyamine catabolism and disease

Robert A. CASERO Jr¹ and Anthony E. PEGG²

¹ Department of Oncology, Johns Hopkins University School of Medicine and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, 21231 USA

² Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, PO Box 850, Hershey, PA 17033, USA aep1@psu.edu

Abstract

In addition to polyamine homeostasis, it has become increasingly clear that polyamine catabolism can play a dominant role in drug response, apoptosis, response to stressful stimuli, and contribute to the etiology of several pathological states, including cancer. The highly inducible enzymes spermidine/spermine N¹-acetyltransferase (SSAT) and spermine oxidase (SMO), and, the generally constitutively expressed N¹-acetylpolyamine oxidase (APAO), appear to play critical roles in many normal and disease processes. The dysregulation of polyamine catabolism frequently accompanies several disease states and suggests that such dysregulation may both provide useful insight into disease mechanism and provide unique drugable targets that can be exploited for therapeutic benefit. Each of these enzymes has the potential to alter polyamine homeostasis in response to multiple cell signals and the two oxidases produce the reactive oxygen species H₂O₂ and aldehydes, each with the potential to produce pathologies. The activity of SSAT has the potential to provide substrates for APAO or substrates for the polyamine exporter, thus reducing the intracellular polyamine concentration, the net effect of which depends on the magnitude and rate of any increase in SSAT. SSAT may also influence cellular metabolism via interaction with other proteins and by perturbing the content of acetyl CoA and ATP. The goal of this review is to cover those aspects of polyamine catabolism that have potential to impact disease etiology or treatment and to provide a solid background in this ever more exciting aspect of polyamine biology.

Keywords

Spermine; spermidine; oxidases; acetyl CoA; oxidative damage

Introduction

The recognition that polyamines are absolute requirements for mammalian cell growth has led to: 1) the study of the molecular mechanism of cell proliferation in which these naturally occurring alkylamines are involved and; 2) the targeting of their function and metabolism as a strategy for antiproliferative therapy [1,2]. Intracellular polyamine concentrations are maintained at a cell type-specific level by a highly regulated metabolic pathway, an energy dependent transport system, and an export system that leads to the efflux of both modified and unmodified polyamines. Highly regulated rate-limiting enzymes facilitate the fine control of both biosynthesis and catabolism. In biosynthesis (Fig. 1), ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) collaborate to produce the higher polyamines spermidine and spermine. Putrescine, the product of ODC is used as the substrate for the constitutive spermidine synthase that adds the aminopropyl group donated from the

decarboxylated S-adenosylmethionine provided by AdoMetDC. Spermine synthase performs a similar function on spermidine, producing spermine by the addition of another aminopropyl group. Inhibitors for each of the biosynthetic enzymes exist and have been extensively tested in multiple in vitro and in vivo systems [3]. Particular emphasis has focused on the rate-limiting enzymes, ODC and AdoMetDC. However, with respect to neoplastic disease none of these inhibitors has demonstrated clinical effectiveness in clinical trials. The reasons for these less than optimal results most probably include pharmacokinetic parameters and compensatory mechanisms within the tumor cells that allow compensation for loss of a single enzyme's activity. It should be noted however, that difluoromethylornithine (DFMO), an inhibitor of ODC, is an effective clinical agent against *Trypanosoma brucei gambiense*, one of the causative agents of African sleeping sickness [4]. Additionally, DFMO has demonstrated promising results in human trials as a chemopreventive agent [5,6].

Initially, the mammalian polyamine catabolic pathway was thought to contain two enzymes, a rate-limiting acetylase, spermidine/spermine N¹-acetyltransferase (SSAT) and a constitutively expressed N¹-acetylpolyamine oxidase (APAO) [7]. It is generally thought that the activity of APAO is rate-limited by the availability of the acetylated product produced by SSAT (Fig. 2).

During the development of alternatives to single enzyme inhibitors, the true significance of the of polyamine catabolism in polyamine homeostasis and cell survival became more clear. One of the earliest and most biochemically interesting class of polyamine analogues were the bis (alkylated)spermine analogues [8]. Besides down-regulating the activity of the biosynthetic enzymes they were found to massively increase the expression of SSAT [9,10]. It was also during the studies of the molecular regulation of this massive up-regulation that both the potential therapeutic benefits of targeted up-regulation of polyamine catabolism and the potential pathological effects of aberrant increases in polyamine catabolism began to be recognized. More recently, in an attempt to more fully understand the mechanisms of cytotoxicity mediated by the up-regulation of polyamine catabolism, a new mammalian enzyme was discovered, spermine oxidase (SMO) [11,12]. SMO is also induced by various stimuli, including polyamine analogues and pathogens and thus can have a role both as a target for antiproliferative therapy and as a potential molecular basis for specific pathologies.

The last few years has seen a steady increase in studies examining the role of intracellular polyamine catabolism in the pathologies of several human diseases as well as potential targets for chemotherapy and chemoprevention. Therefore, it is the goal of this review to summarize the most recent finding in the field related to polyamine catabolism and specifically to address those issues that are relevant to human disease.

SSAT: a highly regulated, rate-limiting step in polyamine catabolism

Since APAO has very poor activity on unacetylated polyamines, flux through this enzyme is controlled by the supply of acetylated substrates. These are produced by the action of the highly inducible enzyme SSAT. Early studies on the activity of SSAT and the regulation of its activity have been summarized in reviews [7,13]. SSAT is a primarily cytosolic enzyme although a mitochondrial form, whose function is unknown, has also been described. Its physiological substrates are spermidine and spermine [14]. It uses acetyl-CoA to acetylate primary amines. Importantly, it strongly prefers substrates of the structure $\text{RNH}(\text{CH}_2)_3\text{NH}_2$ (Fig. 2). Compounds with only two methylene groups ($\text{RNH}(\text{CH}_2)_2\text{NH}_2$) are acetylated poorly, whereas those with four methylene groups ($\text{RNH}(\text{CH}_2)_4\text{NH}_2$) are not substrates. Therefore, only the aminopropyl end of spermidine is attacked to form N¹-acetylspermidine. Spermine, which is symmetrical, can be attacked at both ends and both N¹-acetylspermine and N¹-,N¹²-diacetylspermine have been observed as products. Under normal conditions, the content of

SSAT is very low but it is rapidly and extensively induced by conditions increasing polyamines or by polyamine analogues and by a variety of pathophysiological stimuli. The detection of N¹-acetylated polyamines in urine, extracellular fluids or in cell extracts is usually highly indicative of increased SSAT activity although this accumulation obviously depends on their ability to escape degradation by APAO.

Relatively recent studies of the structure and mechanism of SSAT have shown that the enzyme is a member of the GNAT (GCN5-related N-acetyltransferase) family. It is a homodimer with a monomer of a 171 amino acids in the case of the human protein. Crystal structures of the human and mouse SSAT protein have been obtained in the presence and absence of ligands including substrates and inhibitors (Fig. 3) [15-17]. These studies provide a good understanding of the reaction mechanism and the substrate specificity. The dimeric unit contains two active sites, each located at the dimer interface and involving residues from both subunits. Tyr140, a totally conserved residue, is likely to serve as the general acid for protonation of the thiolate anion of acetyl-CoA. Glu92 interacting with water molecules acts as a general base that extracts the proton for the attacking N¹- group of the polyamine substrate. Mutation of either of these residues has a profound effect on the activity. Structures of SSAT in complex with polyamines analogues such as BENSp^m (N¹, N¹¹-bis(ethyl)norspermine) or BESpm (N¹, N¹²-bis(ethyl)spermine) show that these bind to the protein using many of the same residues as those involved in substrate binding. The strong binding of these analogues accounts for their profound effect on the protein stability described below and for the fact that they act as weak inhibitors of the acetylation reaction (Fig. 3C).

SSAT can self-acetylate at Lys26 [15]. This is an exception to the substrate specificity rules describe above. The significance of this reaction is unknown but it could regulate to the regulation of SSAT or suggest that, under some circumstances, SSAT may acetylate associated proteins.

The *Sat1* gene encodes SSAT, which is present on the X chromosome at location Xp22.1. A related gene termed *Sat2* encodes another closely related GNAT family member. The human *Sat2* gene product has a crystal structure similar overall to SSAT but with significant differences in charged surface residues and in the presumed amine substrate binding residues of the active site region [18]. The protein has 170 amino acids having 46% sequence identity and 64% sequence similarity with SSAT. Despite this similarity, it has negligible activity to acetylate polyamines. The k_{cat}/K_m specificity constant for spermidine and spermine was <0.001% of genuine SSAT and expression of the *Sat2* product did not increase cellular acetylpolyamines or reduce free polyamine content [19]. Further characterization reveals the substrate for this human protein, and for homologs from other species, is thialysine (S-(2-aminoethyl-L-cysteine) Related compounds such as 5-hydroxy-L-lysine, S-(aminoethyl)-homocysteine and O-(aminoethyl)-L-serine [19,20]. It is therefore more correctly described as thialysine-N^e-acetyltransferase.

Function of SSAT and interactions with other proteins

The primary function of SSAT is undoubtedly to maintain polyamine homeostasis. Induction of SSAT in response to a rise in polyamines leads to acetylation of spermidine and spermine, which not only changes their potential physiological activity due to alteration in charge, but also facilitates excretion and degradation via the SSAT/APAO pathway. Several other potential functions have been suggested based on the interaction of SSAT with important cellular proteins.

Current data suggest that there may be a direct connection between SSAT and polyamine efflux from the cell. The existence of a specific efflux mechanism for putrescine and acetylpolyamines has been known for some time. Recently, a transporter has been characterized and found to

consist of SLC3A2, previously known as a glycosylated heavy chain of a cationic amino acid transporter and its partner γ + LAT light chain [21]. Efflux was coupled to arginine uptake, indicating that there is a putrescine/arginine exchange reaction. SSAT was co-localized with SLC3A2 and was co-immunoprecipitated by an anti-SLC3A2 antibody suggesting that this efflux system may be closely linked to polyamine acetylation [21].

There is a specific and functional interaction between SSAT and integrin $\alpha 9 \beta 1$ [22,23]. This integrin, which mediates cell migration, binds to ligands at sites of tissue injury and inflammation. The N-terminal region of SSAT binds to the $\alpha 9$ cytoplasmic domain. This binding of SSAT is needed for $\alpha 9 \beta 1$ -mediated migration and requires the SSAT catalytic activity [22]. The integrin and the Kir4.2 K^+ ion channel co-localize in focal adhesions at the leading edge of migrating cells. SSAT-mediated catabolism of spermidine and/or spermine, which are known to mediate inward rectification of such channels [24], alters K^+ ion efflux through the Kir4.2 channel [23]. These results strongly support a novel pathway through which an anchored SSAT molecule mediates localized polyamine catabolism and subsequent biological effects.

SSAT binds to the hypoxia-inducible factor 1 (HIF-1) α subunit [25]. HIF-1 α regulates transcription at hypoxia response elements contained in genes involved in angiogenesis, glucose metabolism, erythropoiesis, inflammation, apoptosis, and cellular stress. SSAT binding stabilizes the interaction of the form of HIF-1 α present in hypoxic conditions with RACK-1 and elongin C and promotes the ubiquitination and degradation of HIF-1 α . Interestingly, thialysine- N^{ϵ} -acetyltransferase, which, as described above, has similar overall structure to SSAT, also binds to HIF-1 α and promotes degradation. However, it stabilizes the complex of hydroxylated HIF-1 α that is formed under aerobic conditions with von Hippel-Lindau protein (VHL) and elongin C [26]. Thus, these two acetyltransferases promote the O_2 -independent and O_2 -dependent degradation of HIF-1 α respectively. The mechanism of the effect is not clear but requires enzymatically active forms of the acetyltransferases and could involve protein acetylation. As described above, SSAT can acetylate an unmodified lysine in itself and thialysine- N^{ϵ} -acetyltransferase can act on 5-hydroxylysine, which might be formed in the target HIF-1 α by a lysine hydroxylase using O_2 .

Induction of SSAT

The content of SSAT is normally very low and in resting, uninduced cells much of the minimal activity that is detected in *in vitro* assays of cell extracts is not due to SSAT but to other less specific acetyltransferases and nonenzymatic reactions of acetyl-CoA. Many factors cause a large and rapid induction of SSAT activity (reviewed in: [7,13,27,28]). The increase in activity results from a rise in the amount of SSAT protein. Covalent post-translational modifications or low molecular weight effector molecules increasing activity have not been described. Stimuli elevating SSAT levels include polyamines, polyamine analogues, hormones and cytokines (corticosteroids, estradiol, progesterone, calcitriol, growth hormone, PTH, catecholamines and IGF-1), stress pathways (including ischemia-reperfusion injury), cachexia, natural products (gossypol, 7- β -hydroxycholesterol or 7- β -hydroxysitosterol, H_2O_2 , resveratrol and interferons) and drugs (non-steroidal anti-inflammatory agents (NSAIDS), cisplatin, 5-fluorouracil).

Transcriptional regulation

The *Sat1* gene encoding SSAT contains sites for the binding of transcription factors that can respond to these stimuli either directly or via the polyamine responsive element (PRE). Polyamines are powerful inducers of SSAT and some of the factors listed above may act indirectly by raising the content of free polyamines either by increasing synthesis or causing release from bound sites. The *Sat1* promoter region lacks a TATA box but has potential binding

sites for Sp-1, AP-1, C/EBP β , CREB, NF κ B and PPARs. The *Sat1* gene also contains a sequence 5'-TATGACTAA-3' which acts as a PRE. It occurs about 1500 base pairs upstream from the transcriptional start site and allows the increased transcription of SSAT mRNA when polyamine content is high [13,27]. The transcription factor, NF-E2-related factor 2 (Nrf-2) interacts constitutively with the PRE. Binding of the leucine zipper region of Nrf-2 to a 20 kDa protein termed polyamine-modulated factor-1 (PMF-1) turns on the transcription of SSAT in response to polyamines [29]. Mutations that alter the ability of these factors to interact destroy their ability to regulate the transcription of the SSAT gene [30]. Thioredoxin-1 reduces SSAT mRNA expression by decreasing PMF-1/Nrf-2 binding [31].

NSAIDs, Tumor necrosis factor- α (TNF α), and inflammatory stress increase SSAT mRNA production via nuclear factor κ B (NF κ B)

There are two NF κ B response elements in the *Sat1* gene [32] and their occupation by NF κ B is increased by NSAIDs or by TNF α [28,33]. NSAIDs also increase *Sat1* transcription via its peroxisomal proliferators response element (PPRE) and PPAR- γ [34]. The gene contains two PPREs, one of which is located at +48 bases relative to the transcription start site is specifically bound by PPAR- γ and is clearly implicated in the induction by NSAIDs. Resveratrol also acts to increase SSAT mRNA production using the PPREs [35] whereas, expression of an activated Ki-Ras oncogene gene reduces expression via this PPAR- γ mediated system [36,37].

Post-transcriptional regulation

Induction of SSAT to high levels requires a reinforcement of the effect produced by the increased transcription. Several levels of post-transcriptional regulation are documented and all respond to increased polyamine levels by increasing the amount of SSAT. These include: SSAT mRNA stability; alternative splicing of the SSAT mRNA; translational regulation of SSAT synthesis; and stabilization of SSAT protein, which normally turns over very rapidly.

The *Sat1* gene contains 6 exons: exon 1 contains the 5'UTR and the first 22 codons of the coding sequence; exons 2-5 contains parts of the coding sequence; exon 6 contains the last 56 amino acid codons, the stop codon and the 3'UTR. These exons encode mRNAs of about 1.3 and 1.5 kb. The SSAT pre-mRNA can also undergo alternative splicing by the inclusion of an additional 110bp exon between exons 3 and 4 to form SSAT-X mRNA [38]. The encoded sequence contains multiple premature termination codons thus rendering the SSAT-X variant a target for nonsense mediated mRNA decay. The inclusion of this exon is inhibited by polyamines, which therefore leads to more of the correct and stable mRNA [38]. The SSAT-X mRNA has been found to accumulate in response to X-rays, viruses and hypoxia (see [13] for references). Inhibition of host protein synthesis is known to stabilize transcripts targeted to nonsense-mediated mRNA decay.

There is also convincing evidence for translational regulation of SSAT synthesis [13,39]. The movement of SSAT mRNA into polysomes and enhanced SSAT synthesis occurs in response to increased polyamines. This translational regulation does not require either the 5' or 3' untranslated regions of the mRNA. A cytoplasmic protein, which binds to two regions (localized to nucleotides 1-45 and 492-504 of the coding region) in the mRNA, was detected and this binding was inhibited by BENSp μ m [39]. This protein may therefore repress SSAT synthesis by binding to the mRNA and blocking translation, but it has not yet been fully characterized.

SSAT turns over very rapidly with a half life of approximately 15 min. Degradation is carried out by the 26S proteasome after polyubiquitination and rapid turnover requires the -MATEE sequence located at the C-terminal end of the protein [13,40-42]. The site of ubiquitin addition is probably the surface Lys87 but this is not absolutely established. Binding of

polyamines to the protein interferes with ubiquitination and greatly increases the half-life of SSAT to >>12 h thus causing a large amplification of the effect provided by increased mRNA content and translation.

Induction of SSAT by polyamine analogues

Regulation of SSAT levels by polyamines via effects on all of the steps described above allows cells respond to respond to increased free polyamine content and thus maintain polyamine homeostasis. Some polyamine analogues with N-terminal alkyl groups such as BENSp_m or BESp_m produce an even more striking and prolonged increase in SSAT because their content does not decrease in response to the activation of the SSAT/APAO pathway and they may have a stronger effect on the regulation steps. Binding of such polyamine analogues to the SSAT protein occurs at a site overlapping the polyamine-binding portion of the active site. Therefore they act as weak inhibitors of the enzymatic reaction but the massive accumulation of SSAT protein clearly increases *in vivo* SSAT activity leading to degradation and excretion of cellular polyamines.

The increase in SSAT activity in response to polyamine analogues occurs in many cell types but varies in magnitude with some cells showing inductions of >200-fold. The cell types able to express such high amounts of SSAT have high amounts of Nrf-2 in the nucleus [27]. Increased *Sat1* gene transcription via the PRE is critical for the induction. However, even in cell lines that show large increases in SSAT protein, the increase in transcription is generally limited to less than a 10-fold increase [13,27]. The additional increase is due to the other mechanisms described above, particularly protein stabilization. The ability of polyamine analogues to enhance SSAT by acting at these post-transcriptional steps accounts for the synergism in the induction of SSAT when they are combined with the classical cytotoxins, 5-fluorouracil, cisplatin or oxaliplatin [32,43,44]. Exposure of cells to each of the cytotoxins alone results in increased SSAT mRNA, but no appreciable increase in protein or activity are observed in the absence of a polyamine analogue.

The Role of SSAT in drug response

Response to cisplatin

There was a much lower induction of SSAT by BENSp_m in human ovarian carcinoma cell lines resistant to cisplatin than cisplatin-sensitive lines. The ablated increased SSAT gene transcription was due to the presence of labile repressor proteins [45]. Transient transfection with a plasmid allowing SSAT expression despite the presence of these repressors increased SSAT levels and restored sensitivity to cisplatin [45]. A plausible explanation for these results is that activation of the SSAT/APAO pathway allows more extensive interaction of cisplatin with DNA through the removal of DNA-bound polyamines.

Response to polyamine analogues

Certain polyamine analogues such as BENSp_m and BESp_m produce a massive increase in SSAT in some tumor cells as described above. Evidence strongly supporting the hypothesis that this increase is relevant to cell killing by the analogue was obtained in experiments in which repeated exposure of CHO cells to increasing levels of BESp_m was used to derive a resistant cell line [42,46]. The resistant cells failed to increase SSAT activity in response to the BENSp_m or BESp_m and were found to contain a mutation in the *Sat1* gene that alters the coding sequence changing Leu156 to Phe. This mutation changes the binding surface for the aliphatic carbons of the substrate/inhibitor, thus preventing the analogues from protecting the mutant protein from polyubiquitination and degradation and also reduces the enzymatic activity [15,42]. Transfection of the resistant cells with a plasmid expressing wild type SSAT restored sensitivity to BENSp_m.

The massive induction of SSAT and the ability of the analogues to repress the synthesis of ODC and AdoMetDC, as well to increase production of antizyme that causes ODC degradation [47], leads to a rapid and profound drop in cellular polyamines which may account, at least in part, for the antitumor effect. Induction of high levels of SSAT also may exert antitumor effects via toxicity associated with the oxidative damage be produced by the activated SSAT/APAO pathway [27,48,49] or a reduction in acetyl-CoA and malonyl-CoA, which would decrease fatty acid synthesis [50,51]. Independent studies in which SSAT expression was increased from transfected or transduced plasmids also indicate that this is sufficient to induce cell cycle arrest in colorectal and prostate cancer cells [52,53].

As described below, the induction of SMO is also likely to be a major cause of the antitumor effect of polyamine analogues. It is possible that other effects, such as unproductive binding to critical sites normally occupied by polyamines and via targeting mTOR-mediated protein synthesis [49] or other signaling pathways may contribute. Neoplastic cells may be selectively responsive to polyamine analogues not only via the presence of high amounts of nuclear Nrf-2 leading to the high SSAT induction, but also because they are more sensitive than normal cells. Remarkably, embryonic stem cells from *Sat1* knock-out mice were actually more sensitive to BENSp^m than controls [54]. Also, apoptosis is induced in chondrocytes by BENSp^m independently of its ability to alter levels of natural polyamines by either SMO or the SSAT/APAO pathways [55]. It is possible that in some cells, the binding of the analogue by SSAT protein can protect from interaction with other sites.

It is important to note that many other polyamine analogues are also profoundly antiproliferative towards cancer cells but not all cause as a large an increase in SSAT [2, 56-61]. They may act at the other sites described above or through additional mechanisms including interfering with polyamine-mediated cell signaling or by acting on the cytoskeleton.

Useful animal models for the study of SSAT function

Inactivation of the *Sat1* gene

Mice that have the *Sat1* gene inactivated showed little change in polyamine content with only a slight increase in the spermidine:spermine ratio [54]. As expected, the back conversion of spermine and spermidine into putrescine was greatly reduced in the cells from these mice. There was some conversion of spermine into spermidine, which could be brought about by SMO. These mice were reported to have a normal life span and no major metabolic deficiencies although, at later ages, there was a development of insulin resistance [54]. Later investigations indicated that there was body fat accumulation, an increase acetyl- and malonyl-CoA pools, and a decrease in palmitate and glucose oxidation in adipose tissue [62]. Also the *Sat1* knock-out mice gained more weight and lipid when placed on a high fat diet. These results indicate that, even in the basal state where SSAT expression is low, the reactions mediated via SSAT utilize significant amounts of acetyl-CoA.

Transgenic increase of SSAT

Both transgenic mice and transgenic rats overexpressing SSAT have been obtained (reviewed in: [63-65]). The constructs for expression include: a metallothionein promoter to give general inducible expression; a keratin-6 promoter to target expression to the skin (K6/SSAT); and increased *Sat1* gene copy number that should give an enhanced response to physiological stimuli increasing SSAT.

The ubiquitous transgenic expression of high levels of SSAT led to changes consistent with the role of SSAT in regulating polyamine levels with a substantial reduction in spermidine/spermine pools with a large increase in putrescine and in N¹-acetylspermidine and excretion of acetylated polyamines including N¹, N¹²-diacetylspermine [64]. These animals also showed

a wide variety of other defects including hair loss, female infertility, weight loss, CNS effects, a tendency to develop pancreatitis and altered susceptibility to carcinogenesis. The overstimulation of the SSAT/APAO pathway leads increased oxidative damage and a large reduction in acetyl-CoA with additional metabolic consequences including profound changes in lipid metabolism (see below and [64,65]). It is therefore difficult, in some cases, to ascertain the extent to which the reduction in polyamines is directly responsible for the pathophysiology that results.

Effects on lipid and carbohydrate metabolism

Detailed studies of carbohydrate and lipid metabolism have now been carried out in mice overexpressing SSAT due to an increase in the *Sat1* gene copy number. There was a decrease in white adipose tissue, a loss of body fat with high basal metabolic rate, improved glucose tolerance and high insulin sensitivity [62,66]. A plausible explanation for these changes is that the high levels of SSAT increase metabolic flux through the polyamine pathway because ODC (and possibly other biosynthetic enzymes) are increased in response to the reduction in polyamines caused by SSAT [62,67]. This produces a futile cycle in which acetyl-CoA is depleted (Fig. 4). The decrease in acetyl-CoA, and the related reduction in malonyl-CoA, removing its inhibition of fatty acid oxidation, leads to a large increase in glucose and palmitate oxidation and a loss of fat. There is also a significant loss of ATP due to the utilization of AdoMet that is converted by the aminopropyltransferase reactions to 5'-methylthioadenosine (MTA), which is then recycled to AdoMet in ATP-dependent reactions. The futile cycle thus uses up ATP and acetyl-CoA forming adenosine, *N*-acetylaminopropanal (which is reduced to β -alanine), H_2O_2 and CO_2 . The fall in ATP increases AMPK, which activates PPAR- γ coactivator 1 α (PGC-1 α), in white adipose tissue [66]. The increase in PGC-1 α leads to the enhanced expression of the OXPHOS genes in the SSAT transgenic mice causing a reduced fat mass and improved glucose tolerance.

This mechanism is supported by experiments showing that treatment with DFMO to inhibit ODC and reduce flux through the pathway reversed the decrease in white adipose tissue and body fat and restored ATP and the levels of AMPK and PGC-1 α towards normal [66]. Other evidence for the cycle was obtained by directly measuring polyamine flux in prostate carcinoma cells transfected with a regulatable construct for SSAT expression [52,67]. When SSAT was increased, the spermidine and spermine pools were maintained by a compensatory increase in ODC and AdoMetDC activities, despite a large increase in acetylated polyamines and there was a substantial increase in flux through the pathway. This increase was only slightly affected by inhibition of APAO showing that excretion of the acetylated polyamines rather than oxidation was the primary reason for the increased flux in these cell cultures.

Hair loss

The loss of hair in SSAT transgenic mice with increased *Sat1* gene copy number is similar to that seen in mice overexpressing ODC [68]. It may therefore be due to the harmful effects of very high levels of putrescine on hair follicle development.

Pancreatitis and SSAT

Transgenic rats with overexpression of SSAT from the metallothionein promoter may be a interesting model for pancreatitis [69]. When SSAT was increased using induction with zinc, pancreatic damage occurred very rapidly. There was an increase in cathepsin B release within 2 h after induction of SSAT and acute necrotizing pancreatitis resulted. Replacement of the natural polyamines with methylated polyamine analogues that are not substrates for SSAT prevented the disease [69,70]. It is likely that loss of higher polyamines from cellular structures leads to premature zymogen activation and autodigestion of acinar cells, but is also possible

that inflammatory and oxidative stress mediators formed via the SSAT/APAO pathway may be involved since serum inflammatory cytokine levels and NFκB were activated [70].

Liver regeneration

Transgenic overexpression of SSAT blocks liver regeneration after partial hepatectomy [71]. Such regeneration is known to be accompanied by large increase in ODC and AdoMetDC resulting in a rise of putrescine and spermidine. Provision of 1-methylspermidine, which is not attacked by SSAT, overcame the effect of SSAT expression, confirming that spermidine rather than putrescine is the critical polyamine needed for liver regeneration.

As will be discussed below, ischemia-reperfusion injury (IRI) increases SSAT expression in kidney, heart and brain [72-75]. The *Sat1* knock-out mice were protected from liver or kidney damage caused by an ischemia-reperfusion model [75].

Carcinogenesis

Several studies have shown that transgenic overexpression of SSAT influences carcinogenesis in mouse models but the results of the various studies differ strikingly. When mice with increased gene copy of *Sat1* on the C57BL/6J background were bred with TRAMP mice, which develop prostate tumors due to an expression of SV40 T antigens from the probasin promoter, there was a significant reduction in tumor incidence [76]. Also, in earlier studies using these mice on a hybrid CD2F1 background, it was reported that there was a decreased tumor incidence in the skin in response to a two-stage tumorigenesis protocol [64,77].

In contrast, other studies with the same transgenic mice on the inbred C57BL/6J background showed that when they were bred with *Apc*^{Min/+} mice, which are predisposed to intestinal tumor formation, the incidence of intestinal tumors was increased [78]. Consistently, crosses of the *Apc*^{Min/+} mice with *Sat1* knock-out mice led to a 75% reduction in tumor burden [78]. Similarly, a striking increase in tumor incidence and progression to carcinomas was seen in response to the two-stage tumorigenesis protocol when K6/SSAT C57BL/6J transgenic mice in which a SSAT cDNA was driven by the K6 promoter to express only in the skin were used [79]. The increased tumor incidence was reduced by treatment with MDL72527, an inhibitor of APAO suggesting that oxidative stress or putrescine or both was responsible [80].

Several factors may be relevant to reconciling these disparate results. The mice with general SSAT overexpression have such a variety of pleiotropic phenotypes relating to the general stimulation of oxidative damage, the loss of polyamines and the profound effects on carbohydrate and lipid metabolism resulting from depletion of acetyl-CoA that they are not really suitable for detailed studies in carcinogenesis. Secondly, it is well established that elevated levels of polyamines are associated with neoplastic growth and a major loss of polyamines may account for the reduction in tumors in the TRAMP model and in the skin model with *Sat1* gene amplification, which led to a very high level of SSAT expression. In the K6/SSAT mice, there was a much lower expression of the SSAT in the skin as evidenced by only a slight reduction in hair growth [79] as opposed to the hairless phenotype of the general SSAT overexpressing mice. If a compensatory increase in polyamine synthesis is required to allow for sufficient of polyamines for tumor growth, these mice may be able to respond appropriately, whereas the higher level of SSAT would prevent such compensation. Evidence for the importance of continued polyamine synthesis in the K6/SSAT model is supported by the finding that treatment with DFMO resulted in the complete regression of established tumors. Breeding of the K6-SSAT mice with transgenic mice that express antizyme, a negative regulator of ODC, also blocked the development of tumors [80]. A similar compensatory increase in the biosynthesis of polyamines may occur in the intestinal tumor models [78]. These results focus attention on the possibility that oxidative damage caused by modest increases in

the SSAT/APAO pathway that do not also result in a major depletion of polyamines may be a particularly hazardous condition leading to tumor development.

Pathological states that maybe associated with a change in SSAT expression

Low SSAT gene expression has been linked to anxiety, depression and a propensity to suicide [81-84]. The reduced levels of SSAT mRNA correlate with the C allele of the *Sat1* 342A/C polymorphism located in the vicinity of the PRE region of the *Sat1* gene promoter [82,83]. It has been suggested that BENSpM might have a therapeutic benefit in psychiatric patients with a high risk of suicide [85], but the extent to which this charged molecule can penetrate the blood brain barrier or whether cells in the brain can induce SSAT in response to it are not known. The increase in SSAT in normal tissues was much less than in tumor tissues of patients treated with BENSpM when examined by immunohistochemistry [86].

The mechanism by which SSAT is linked to behavioral effects is unknown but may be related to effects on ion channels. Polyamines influence the activity of glutamate receptor ion channels including *N*-methyl-D-aspartate (NMDA) receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors. These channels are known to be involved in many key neurological functions including sensing pain, neuronal development, and in supporting plasticity processes that are the cellular bases of learning and memory [87]. Also, an inherited defect in spermine synthase that reduces spermine content causes Snyder-Robinson syndrome, an X-linked mental retardation condition [88,89]. Thus, SSAT-induced alterations in polyamine content could alter brain function. There are alterations in kainate receptor activity in the transgenic mice expressing high levels of SSAT from *Sat1* copy number increase [64]. Transgenic overexpression of SSAT protects from kainate related epilepsy-like seizure activity induction [90] and these mice with very high SSAT expression levels had a reduced activity and spatial learning impairment [91].

Keratosis follicularis spinulosa decalvans (KFSD) is a genodermatosis characterized by follicular hyperkeratosis, progressive cicatricial alopecia and photophobia. One form of KFSD is an X-linked recessive condition, and a patient had a gene duplication that included the *Sat1* region of the X chromosome [92]. Cultured fibroblasts from the patient had a 3-fold increase in SSAT, a reduction in spermidine and a two-fold increase in putrescine. It is therefore possible that gain-of-function mutants in the *Sat1* gene are a general cause of this form of KFSD.

Studies of molecular diagnosis for prostate cancer detection have found that an eight gene "expression signature" provides a sensitive and reliable method for early detection of disease. SSAT, along with ODC, antizyme and AdoMetDC make up half of the eight genes [93]. Additionally, the *k-Ras* oncogene negatively regulates SSAT [36], which contributes to the increase in polyamines brought about by Ras activation through increased ODC activity [94], increased polyamine uptake [95] and reduced polyamine efflux [21]. It is possible that these effects are critical in the development of tumors with Ras activation.

Thus alterations in SSAT expression can have profound effects with respect to multiple pathologies including proliferative and non-proliferative diseases.

N¹-Acetylpolyamine oxidase (APAO)

APAO is an FAD-dependent amine oxidase that was first described by Holta [96,97]. Although its activity was purified by Holta in 1977, it has only recently been cloned and more completely characterized [98,99]. This oxidase preferentially oxidizes N¹-acetylated polyamines with an affinity for N¹-acetylspermine > N¹-acetylspermidine > than N¹, N¹² diacetylspermine >>> spermine [98,99]. Consistent with the findings of Holta, the peroxisomal signaling sequence,

PRL has been identified in each of the mammalian APAOs cloned to date [98,99]. The oxidation of the acetylated polyamines produces, spermidine or putrescine, depending on the starting substrate, 3-acetoamidopropanal, and the reactive oxygen species, H_2O_2 (Fig. 1).

APAO is generally constitutively expressed in most normal tissues and its activity, as stated above, is rate-limited by the activity of SSAT that provides the acetylated substrate. However, Wallace et al. have demonstrated that APAO expression can vary considerably in human breast tumor cells, suggesting it greater regulation than is typically observed in other tissues [100]. As will be seen below, altered levels of APAO can have profound cellular effects.

The role of APAO in drug response

As described above, SSAT activity can be massively up-regulated by various stimuli. This increase in SSAT activity has the potential to result in large increases in intracellular acetylated polyamines thus providing substrate for APAO. As one mechanism for selective tumor cell toxicity that has been observed with specific polyamine analogues, we originally proposed that massive increases in acetylated polyamines produced by SSAT induced apoptosis subsequent to ROS damage down stream from H_2O_2 production by APAO [101]. Similar results from other laboratories also suggested a role of APAO in the ROS-induced response of specific cancer cells to the polyamine analogues [102]. Although the interpretation of the data was entirely consistent with what was known about SSAT and APAO at the time, this hypothesis has required modification based on more recent results that suggest SMO oxidase may be the source of H_2O_2 (see below).

Even though questions remain with respect to the direct involvement of APAO in the cellular response to the cytotoxic polyamine analogues, there is at least one other mechanism by which APAO can affect drug response. Seiler proposed that APAO could detoxify the polyamine analogue N^1 -(n-octanesulfonyl)spermine (N^1 -OSSpm) based on the increased cellular toxicity when combined with N^1,N^4 -bis(buta-2,3-dienyl)butanediamine (MDL72527), an inhibitor of APAO [103]. However, the use of the inhibitor MDL72527 is somewhat problematic as it is not specific for APAO [11,12,104,105]. Bitonti and colleagues demonstrated that bis(benzyl) polyamine analogues were also substrates for an oxidizing enzyme that was inhibited by MDL72527 and demonstrated the debenzylating activity co-eluted with the N^1 -acetylspermine oxidizing activity [106]. Lawson et al. suggested a similar mechanism for resistance to the potent antitumor polyamine analogue, N^1 -ethyl- N^{11} -[cycloheptyl)methyl]-4,8-diazaundecane (CHENSpm) [107]. In these studies, Chinese hamster ovary cells were found to be highly resistant to CHENSpm. Chromatographic results suggested that CHENSpm was metabolized, indicating that the cells were detoxifying the analogue. With the recent cloning and characterization of APAO it has been confirmed that APAO is indeed capable of oxidizing several polyamine analogues, including CHENSpm [98,99,108]. Thus APAO has the capacity to significantly alter the response to various analogues and it is possible that additional unidentified drugs are substrates for APAO.

Spermine oxidase (SMO)

The last enzyme to be cloned in the intracellular polyamine catabolic enzyme was APAO. However, in our attempt to clone APAO we made a fortunate mistake, the discovery of a FAD-dependent oxidase that oxidized spermine as its preferred substrate [12]. The products of this enzyme are spermidine, 3-aminopropanal, and H_2O_2 (Fig. 1). We initially named the gene PAOh1 as the first human polyamine oxidase to be cloned; however, Vujcic et al. subsequently confirmed the preference for spermine and renamed it spermine oxidase with the abbreviation SMO [11]. Unlike APAO, SMO is highly inducible by a variety of stimuli including the antitumor polyamine analogues [11,12,60,109]. Importantly, all of the analogues that induce SSAT also induce SMO expression; the significance of this will be discussed below.

The human gene for SMO (GenBank name SMOX) is located on chromosome 20p13 and codes for several splice variants [110,111]. Similarly, the mouse gene has been demonstrated to have multiple splice variants [112]. Active splice variants are found both in the cytoplasm and the nucleus; however, the structural requirements for nuclear translocation is controversial [111, 112]. The predominant human splice variant, SMO1 (SMO/PAOh1), codes for a 61 kD protein containing 555 amino acids. The purified, recombinant protein demonstrates a K_m for spermine of 8 μ M and K_{cat} of 7.2 s^{-1} [113].

As indicated above, SSAT expression is the result of considerable post-transcriptional regulation, with the greatest increases in protein expression being a result of enhanced translation and protein stabilization [41,114,115]. By contrast, SMO expression appears to be regulated predominantly at the level of transcription and somewhat by message stabilization [116], but there is currently no evidence of post-translation regulation in response to any of the antitumor polyamines analogues examined.

SMO activity and cellular response to polyamine analogues

As the ROS H_2O_2 is a major product of SMO activity, the rapid increase in SMO expression subsequent to analogue exposure has the potential to play a significant role in the tumor cell response to analogue treatment. We first proposed the role of H_2O_2 in the selective cytotoxic response of human non-small cell lung cancers to the bis(ethyl)polyamine analogues [101]; however, this hypothesis was formulated before the awareness of the existence of SMO. The original model proposed that the massive increase in SSAT in response to analogue produced sufficient substrate for the constitutively expressed APAO to produce cytotoxic H_2O_2 within the tumor cells. This interpretation was based on the accepted view at that time that the polyamine oxidase inhibitor, MDL72,527 was specific for APAO [117,118]. As MDL72,527 is clearly a potent inhibitor of SMO as well as APAO, a re-evaluation of the role of APAO in the production of cytotoxic H_2O_2 was necessary [113].

Although it is clear that induction of polyamine catabolism by polyamine analogues resulted in both H_2O_2 production and oxidative DNA damage, it was not clear as to the origin of the H_2O_2 . Using a stable, short hairpin RNA (shRNA) knockdown strategy it was demonstrated in a human breast cancer model that SMO was the primary source of the cytotoxic H_2O_2 produced in response to BENSp_m treatment. These data suggest that APAO plays no role in the oxidative damage produced by analogue-induced increases in polyamine catabolism in the breast model examined [48].

It is also important to note that the induction of SSAT by specific polyamine analogues leads to a reduction in intracellular polyamine pools by acetylation and export, and that this process occurs in combination with increased ROS through SMO induction. The depletion of polyamines by SSAT activity and subsequent secretion also makes cells more sensitive to ROS damage as the polyamines have been demonstrate to act as free radical scavengers that can protect DNA from H_2O_2 -induced damage [119-121]. These results indicate that the most effective polyamine analogues, with respect to tumor cell toxicity, are those that induce both SSAT and SMO.

It is not known whether APAO serves as a source for ROS in response to other stimuli; however, as APAO is a peroxisomal enzyme it is possible that any H_2O_2 produced is immediately detoxified by catalase, which is also located in the peroxisome.

Induction of SMO by various stimuli associated with human pathologies

The rapid production of H_2O_2 and/or 3-aminopropanal clearly can play a significant role in tumor cell response to the antitumor polyamine analogues. However, as ROS are thought to

play a role in the etiology of several pathologies including cancer, the potential that SMO activity has other physiological relevance must be considered.

Parchment and Pierce originally suggested that polyamine oxidation and the subsequent production of H_2O_2 was an important tissue-remodeling event in early embryogenesis [122, 123]. They postulated that polyamine oxidase-produced H_2O_2 lead to apoptosis of cells as a natural process during development. Although this process is obviously necessary for normal development it also has the potential to produce a pathological outcome if not carefully controlled.

Ischemia reperfusion injury

IRI is associated with the etiology of myocardial infarct, stroke, and acute renal tubular necrosis [124-127]. These pathologies are associated with decreased energy production and the accumulation of toxic metabolites, including ROS, which are thought to play a direct role in the injuries produced. Recent work in several laboratories indicates that the polyamine catabolic enzymes, possibly including SSAT/APAO and SMO, play a significant role in the production of ROS in myocardial infarct, kidney IRI, and in stroke. Zahedi, Solemani, and colleagues have implicated H_2O_2 production through polyamine catabolism as a source of injurious ROS in kidney IRI [128]. Using a mouse model of renal IRI, SSAT was identified as one of the genes that was significantly up-regulated and suggested that polyamine catabolism may be a source of ROS either through APAO or SMO [129]. Initial follow-up in vitro studies confirmed ROS production and injury using a Tet-inducible SSAT in kidney epithelial cells and clearly demonstrated that the addition of catalase could reduce the cytotoxic effects of SSAT expression suggesting that H_2O_2 production by polyamine catabolism was involved in the observed cytotoxicity. However, the precise source of H_2O_2 is still in question as SMO is also significantly upregulated in the Tet-inducible SSAT transfected cells (unfortunately, misidentified in the manuscript as PAO [72]). More recent in vitro studies confirm H_2O_2 production through polyamine catabolism and demonstrate DNA damage, and suggest that this leads to decreased proliferation and repair capability in injured kidneys [72]. Interestingly, the authors conclude that the H_2O_2 production by polyamine oxidation does not play a role in the observed inhibition of cell proliferation or DNA damage. However, this conclusion was solely based on the use of the polyamine oxidase inhibitor, MDL72527, at the concentration of 50 μ M, a concentration that is not sufficient to inhibit either APAO or SMO in some cell types [101]. Therefore, the role that the polyamine oxidases play in kidney IRI awaits genetic based studies; this is particularly true in light of the work of Igarashi and colleagues who have demonstrated high concentrations of polyamine catabolites in the plasma of renal failure patients [130].

It is, however, now abundantly clear that SSAT plays an important role in kidney IRI as has been confirmed, as stated above, in the SSAT knockout mouse [75]. The loss of SSAT expression was found to significantly protect both the liver and kidney from IRI. These results clearly have important implications in the treatment of IRI.

Similar to their results in patients with kidney failure, Igarashi and colleagues have also found evidence of polyamine catabolism being involved in cerebral stroke [131,132]. Although the studies do not provide data to show a mechanistic link between increased polyamine catabolism and stroke injury, the studies do suggest that the polyamine oxidases, APAO and SMO, in addition to the metabolite, acrolein provide useful biomarkers for the diagnosis of stroke. It will, however, be necessary to define any existing molecular links between ischemic stroke and polyamine catabolism to determine if it represents a useful therapeutic target for treatment or prevention of cerebral strokes.

Infection and SMO

Several recent lines of evidence suggest that SMO plays a significant role in carcinogenesis and maybe one of the direct molecular links between infection, inflammation, and carcinogenesis. The first evidence of this possibility was the observation that gut macrophages infected with *Helicobacter pylori*, the causative agent in gastric ulcers and stomach cancer, highly induced SMO and resulted in apoptosis of the affected macrophages [133]. These results suggested a mechanism by which *H. pylori* infection could escape the immune system by killing the immune cells responsible for eradicating the infection. The mechanism of apoptotic death induced by *H. pylori* in the macrophages was directly associated with the H₂O₂ produced by SMO and subsequent fatal DNA damage [133]. It is also of note that in the affected macrophages respond to *H. pylori* with a substantial increase in ODC activity thus leading to a greater production of polyamines that possibly contribute to enhanced catabolism and subsequent apoptotic cell death [134].

Although these findings alone are highly significant by indicating a means by which low level *H. pylori* infections can persist, it also suggested a direct role in which *H. pylori* infection could result in carcinogenic DNA damage. Specifically, if *H. pylori* infection leads to increased SMO expression in the gastric epithelial cells, the chronic, sub-lethal production of H₂O₂ could result in the necessary mutagenic DNA damage required for neoplastic transformation.

Similar to the observations in macrophages, when the human gastric epithelial line AGS cells are exposed to *H. pylori*, there is a significant increase in SMO mRNA, protein, and activity that results in sufficient H₂O₂ production to result in oxidative DNA damage as measure by the production of 8-hydroxy deoxyguanosine (Fig. 5) [135]. Inhibition of SMO by MDL 72527 or the reduction of its expression through the use of siRNA targeting SMO greatly reduced the DNA damage produced by *H. pylori* exposure. Most importantly, when gastric tissue of *H. pylori* infected patients was examined for SMO expression, uniformly elevated SMO mRNA levels were observed from tissues of infected individuals. However, when tissues of the same individuals were examined after eradication of *H. pylori* infection by antibiotics, the levels of SMO mRNA were uniformly and markedly reduced. When taken together, these results point directly to an association of *H. pylori* infection, SMO induction, and DNA damage and the data strongly suggest a molecular mechanism directly linking infection with the production of carcinogenic ROS. It is also important to note that the source of the ROS can be the affected epithelial cells themselves, as no immune cells are present in the in vitro cultures of AGS cells.

Inflammatory cytokines and SMO

Approximately 20-30% of epithelial cancers are thought to have an inflammatory component. The above results suggest that in specific cases, such as infection, SMO can be a source of DNA-damaging ROS. Therefore, we sought to determine if other mediators of inflammation could induce SMO and result in DNA damage, thus providing a more generalized potential for this pathway to be involved in carcinogenesis. Using TNF α , a pleiotropic inflammatory cytokine, it was possible to demonstrate that this general mediator of inflammation was capable of significant induction of SMO in two non-tumorigenic human lung epithelial cell lines [136]. The increased SMO resulted in substantial production of H₂O₂ production accompanied by oxidative DNA damage. Both the level of ROS generated and the amount of DNA damage produced could be effectively inhibited by either MDL 72,527 treatment or by specific knockdown of SMO mRNA. Additionally, it is important to note that exposure of the lung epithelial cells to the cytokine IL-6 produced similar results with respect to SMO induction, indicating that the results are not limited to a single inflammatory cytokine.

Infection, inflammation, colon cancer: a potential link to SMO

The above results indicate that inflammatory cytokines as well as infectious agents can induce SMO to sufficient levels to induce DNA damage. Another important epithelial cancer that is associated with bacterial colonization and inflammation is colon cancer. Inflammatory bowel disease and colitis, two common inflammatory conditions are associated with an increase incidence in colorectal cancer. Enterotoxigenic *Bacteroides fragilis* (ETBF) colonization is associated with active inflammatory bowel disease [137,138] and colorectal cancer (CRC) [139]. However, the molecular basis underlying the potential carcinogenic role is not known.

The only known virulence factor of ETBF is a 20 kDa zinc-dependent metalloprotease toxin termed *B. fragilis* toxin (BFT) that is secreted by ETBF in vitro and in vivo [140]. BFT acts by receptor-mediated triggering of E-cadherin cleavage and activation of cellular signal transduction proteins including receptor and nonreceptor tyrosine kinases, mitogen activated protein kinases (MAPKs), β -catenin and NF κ B, resulting in induction of c-Myc expression and intestinal epithelial cell (IEC) proliferation [141,142].

We have recently completed preliminary studies using BTF exposure to human colon epithelial cells to determine if this virulence factor can also induce ROS-induced DNA damage through SMO activity. The results of these studies indicate that exposure of colon epithelial cells to the toxin is sufficient to induce SMO resulting in DNA damage as measured by the formation of 8-oxodeoxyguanosine and this damage can be blocked by inhibition of SMO [143]. Importantly, Sears and colleagues have recently reported a murine colitis model that will be critical to evaluate the role of SMO oxidase in ETBF induced colitis and potentially, colon cancer in vivo [144].

Prostate cancer and SMO

Another human epithelial cancer in which inflammation has been strongly implicated in carcinogenesis is prostate cancer. DeMarzo, Nelson and colleagues have provided strong evidence that inflammation is intimately associated with prostate carcinogenesis [145-151]. A suspect inflammatory lesion, proliferative inflammatory atrophy (PIA) is thought to be a precursor lesion to prostatic intraepithelial neoplasia (PIN), the key precursor to prostatic adenocarcinoma. Polyamines, particularly spermine, have long been known to be present in high concentrations in the prostate [152-154]. Therefore, in light of the strong correlation with prostatic inflammation and carcinogenesis we sought to determine whether increased SMO expression was coincident with the early lesions thought to be precursors of prostate cancer.

Using our highly specific SMO antisera [136] in combination with a prostate cancer tissue microarrays (TMA) we examined a total of 879 prostate tissue cores from normal individuals, and tissue cores from prostate cancer patients where individual cores represented normal appearing prostatic tissue, PIA, PIN, and prostate adenocarcinoma [155]. Staining and analysis of the TMA revealed that expression of SMO was uniformly higher in all of the tissues of the prostate cancer patients than in the normal prostate tissues (Fig. 6). However, within the tissue cores from the cancer patients, the highest staining was found in the PIN lesions > adenocarcinoma > normal appearing prostate > PIA lesions. These data suggest that expression of SMO is an early event in the development of prostate cancer.

SMO and chemoprevention

The high expression of SMO in response to various inflammatory stimuli, its ability to produce mutagenic ROS, and its demonstrated ability to damage DNA suggest that SMO may represent a legitimate target for chemoprevention. In vitro and in vivo studies indicate that SMO activity can be inhibited or knocked down without significant deleterious results [48,136,156]. Therefore, the demonstration that SMO is one direct molecular link between inflammation and

carcinogenesis would provide an excellent starting point for chemopreventive intervention. The development of the relevant knockout animal models will be an invaluable resource to directly test the hypothesis that SMO activity is one source of carcinogenic ROS. Hopefully, these models will be available soon.

Conclusions

The role of polyamine catabolism in maintaining polyamine homeostasis is clearly just one of many important functions in which this highly regulated pathway is involved. The response of SSAT and SMO to analogue treatment likely plays a significant role in determining tumor cell sensitivity to many of the antitumor polyamine analogues. Importantly, the dysregulation of both SSAT and SMO have the potential to lead to several pathological conditions, from the potential role of SSAT in psychiatric disorders, SSAT-dependent changes in lipid and carbohydrate metabolism, to the implication that SMO plays a direct role in the etiology of inflammation-associated carcinogenesis. Each of these functions provide a better understanding for the role of the natural polyamines in cell proliferation and function, and demonstrate the critical nature of the proper regulation of this important pathway. Importantly, these functions also suggest potential targets that may be exploited for therapeutic benefit.

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Figure 1.

The polyamine metabolic pathway. ODC, ornithine decarboxylase; AdoMet, S-adenosylmethionine; AdoMetDC, S-adenosylmethionine decarboxylase, this enzyme provides the aminopropyl group for the synthesis of spermidine and spermine from putrescine and spermidine, respectively; MTA, methylthioadenosine; SMO, spermine oxidase is an inducible enzyme that produces the reactive oxygen species, H₂O₂ and the aldehyde, 3-aminopropanal; SSAT, spermidine/spermine N¹-acetyltransferase produces the acetylated polyamines that can be oxidized by APAO or excreted from the cell; APAO, N¹-acetylpolyamine oxidase is a peroxisomal enzyme that oxidizes N¹-acetylated polyamines producing 3-acetoaminopropanal and H₂O₂; DFMO, 2-difluoromethylornithine (an inhibitor of ODC); MDL 72527, N¹,N⁴-bis (buta-2,3-dienyl)butanediamine (an inhibitor of both APAO and SMO).

Figure 2.

Reactions catalyzed by SSAT and APAO. Physiological substrates for SSAT are spermidine, spermine and N¹-acetylspermine. Physiological substrates for APAO are N¹-acetylspermidine, N¹-acetylspermine and N¹,N¹²-acetylspermine.

Figure 3. Structure of SSAT. (A) Molecular surface of SSAT dimer colored according to element (nitrogen, blue; oxygen, red; carbon, grey; sulfur, yellow) with acetyl CoAs shown in green. (B) BENSpm bound to SSAT. Residues from one subunit are shown in green and from the other in purple. (C) Reaction mechanism showing role of Tyr140 and Glu92.

Figure 4. Futile cycle formed by SSAT. Elevations in SSAT aided by excretion of the acetylated products and putrescine formed by APAO cause a decrease in polyamine content. If this is compensated for by an increase in polyamine biosynthetic enzymes, a futile cycle, which depletes ATP and acetyl CoA, is created.

Figure 5.

Potential damage resulting from polyamine oxidation. Intracellular polyamines can be oxidized by both SMO and APAO. Here the potential damage resulting from spermine oxidation by SMO is illustrated. The red lightening bolts represent potentially damaging insults from reactive aldehydes and the blue lightening bolts represent potentially damaging reactive oxygen species (1) The reactive aldehyde, 3-aminopropanal, can damage (•) RNA (4), DNA (5), proteins (6), and membranes (7). Similarly, the readily diffusible H₂O₂ (2) can damage (+) RNA, DNA, membranes, and proteins, once converted to the highly reactive hydroxyl radical (3) through Fenton or Fenton-like catalysis. If oxidative damage to cells is severe enough, apoptotic cell death may ensue (8). Heritable damage to DNA may result in transformation, carcinogenesis (9) and metastatic disease. Although unexplored, H₂O₂ produced by polyamine catabolism may play a role as a signaling molecule (10).

Figure 6. Representative SMO immunohistochemical staining observed in prostate epithelial tissue microarrays. (A) normal prostate tissue, (B) benign prostate tissue from prostate cancer patient, (C) prostatic intraepithelial neoplasia, and (D) prostate adenocarcinoma.