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Mammalian Polyamine Catabolism: A Therapeutic Target, a Pathological Problem, or Both?

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With the recent discovery of the polyamine catabolic enzyme spermine oxidase (SMO/PAOh1), the apparent complexity of the polyamine metabolic pathway has increased considerably. Alone or in combination with the two other known members of human polyamine catabolism, spermidine/spermine N^1-acetyltransferase, and N^1-acetylpolyamine oxidase (PAO), SMO/PAOh1 expression has the potential to alter polyamine homeostasis in response to normal cellular signals, drug treatment and environmental and/or cellular stressors. The activity of the oxidases producing toxic aldehydes and the reactive oxygen species (ROS) H_2O_2, suggest a mechanism by which these oxidases can be exploited as an antineoplastic drug target. However, inappropriate activation of the pathways may also lead to pathological outcomes, including DNA damage that can lead to cellular transformation. The most recent data suggest that the two polyamine catabolic pathways exhibit distinct properties and understanding these properties should aid in their exploitation for therapeutic and/or chemopreventive strategies.

Key words: acetyltransferase, oxidase, reactive oxygen species, spermine, spermidine.

The metabolism of the polyamines spermidine, spermine, and their diamine precursor, putrescine have been a target for antineoplastic therapy since these naturally occurring alkyl amines were found to be essential for normal mammalian cell growth. Intracellular polyamine concentrations are maintained at a cell type–specific set point through the coordinated and highly regulated interplay between biosynthesis, transport, and catabolism. Biosynthesis of polyamines is predominantly regulated by the activities of ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) (Fig. 1) and considerable attention has been paid to polyamine biosynthesis as a target for antiproliferative therapy. However, as more details have been discovered about the important roles that intracellular polyamine catabolism plays in polyamine homeostasis, drug response, and cell survival, focus on polyamine metabolism is now more balanced between biosynthesis and catabolism. Massive upregulation of polyamine catabolism after cellular exposure to the antitumor polyamine analogues and the recent recognition that mammalian polyamine catabolism is a combination of the classical spermidine/spermine N^1-acetyltransferase (SSAT)/N^1-acetylapolymine oxidase (PAO) two-step catalysis (*1*) and the newly discovered spermine oxidase (SMO/PAOh1) (*2*, *3*) has forced a rethinking as to the potential ways in which polyamine catabolism can affect multiple cellular processes including drug response and cellular damage. As recent reviews have covered the general topic of polyamine function and polyamine metabolism as a target for therapy (*4*, *5*), this review will focus primarily on the recent findings related to polyamine catabolism.

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Spermidine/spermine N^1-acetyltransferase (SSAT)

Cloning and characterization of SSAT. Early studies of polyamine catabolism suggested that the polyamines themselves were substrates for an intracellular polyamine catabolizing oxidase (*6*). However, it was also noted that the velocity of the oxidase reaction was significantly increased in the presence of benzaldehyde. The reason for this did not become clear until the discovery of SSAT (*7*) and the preferred substrates of the reported oxidase were determined to be N^1-acetylspermine and N^1-acetylspermidine. It was soon confirmed that SSAT was the rate-limiting step in the two-step back conversion pathway from spermine to spermidine and spermidine to putrescine (*1*).

The cloning of the human SSAT gene by our laboratory enabled the first studies of a mammalian polyamine catabolic enzymes at the molecular level (*8*, *9*). The human gene is located at the Xp22.1 locus and consists of at least 6 coding exons (*9*). Recent reports indicate an alternative splice variant exists and codes for a truncated protein that may play a role in the survival of tumor cells existing in less than optimal conditions (*10*, *11*). Subsequent cloning of SSAT genes from other species demonstrate a high homologies with the human gene (*12*, *13*). In each case the SSAT gene codes for a 20 kDa cytosolic enzyme that as a homodimer, catalyzes the transfer of the acetyl group from acetyl coenzyme A to the N^1 position of either spermidine (K_m 55–140 μM) or spermine (K_m 5–60 μM) (*1*). Although the basal levels of SSAT are generally undetectable, the expression of the gene can be rapidly induced by a variety of stimuli including toxins, cellular stresses, and various drugs (*1*, *14–17*).

It was the discovery that specific antitumor polyamine analogues highly induce SSAT activity in a cell type–specific manner that led to an increased interest in polyamine catabolism as a drug target (see below) (*18*, *19*).

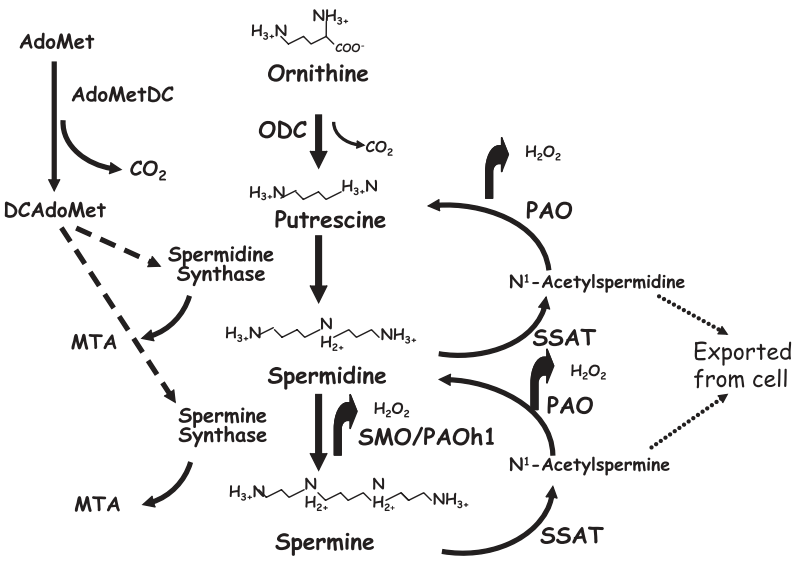

Fig. 1. **The polyamine metabolic pathway.** Abbreviations used: AdoMet, *S*-adenosylmethionine; DCAdoMet, decarboxylated *S*-adenosylmethionine; MTA, methyl-thioadenosine; ODC, ornithine decarboxylase; PAO, N^1-acetylpolyamine oxidase; SMO/PAOh1, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase.

These systems, initially useful in describing the SSAT induction response to the polyamine analogues, also provided an excellent system within which the regulation of SSAT expression could be studied.

Transcriptional Regulation of SSAT expression. The first level of regulation of human SSAT occurs at the level of transcription. In the most responsive of human tumor types, induction of enzyme activity can be as much as 10,000-fold. Initially, transcription was thought to pay a large part in this induction. However, even in cell types demonstrating the highest analogue-induced SSAT activity, the human non-small cell lung cancer and human melanoma cell lines, transcription was found to only be induced in the range of 3- to 7-fold (*20*, *21*). Although transcription only accounts for a small portion of the observed huge induction of SSAT by specific polyamine analogues in specific cell types, it appears to be an absolutely necessary step in its expression.

The analogue-inducible expression of SSAT was demonstrated to be under the control of a polyamine responsive element (PRE)—1,492 bases upstream of the transcriptional start site (*22*). This element appears to be constitutively bound by the transcription factor NF-E2–related factor-2 (Nrf-2) in responsive cells, regardless of whether or not the cells have been exposed to an inducing agent. Through the use of a yeast two-hybrid system the analogue modulated transcription cofactor, polyamine-modulated factor-1 (PMF-1) was identified (*23*). PMF-1 does not have a DNA-binding domain, thus can only activate transcription after binding to another factor. Consequently, the current model for analogue-induced transcription of SSAT consists of the PRE being constitutively bound by Nrf-2, and in the presence of inducing analogues, or excess natural polyamines, PMF-1 binds to Nrf-2 and activates transcription. It is important to note that the current data do not preclude the possibility of additional factors modulating SSAT transcription in response to polyamine analogues or other stimuli.

Posttranscriptional regulation of SSAT. Although it appears that increased transcription is absolutely required for the observed high induction of SSAT in response to specific analogues, transcription alone cannot account for the observed several thousand fold induction observed in some tumor cell types. Such results clearly implicate the potential for extensive posttranscriptional regulation. It is now known that SSAT expression is modulated at every step, including transcription, message stabilization, increased translational efficiency, and protein stabilization. Exposure of responsive cells to specific polyamine analogues leads to an increase in steady-state SSAT mRNA that is a combination of increased transcription and stabilization of mRNA (*20*, *21*, *24*, *25*). However, even though the mRNA resulting from stabilization can be increased over 100-fold, this increase is still not sufficient for the observed maximal increases in SSAT protein that can approach 1% total cellular protein in specific cell types (*21*, *24*). Although some of the difference between the increased levels of mRNA and protein could be accounted for by increased translational efficiency (*20*, *25*), the most likely level of control is stabilization of the protein. SSAT has been demonstrated to have an extremely short half-life (<30 min) (*26*). However, in the presence of analogues, the half-life of SSAT protein increases to over 3 hours, even in the absence of new protein synthesis (*26*). The mechanism by which this increase in stability occurs appears to be through the ability of the polyamine analogue to block the efficient polyubiquitination of SSAT, mediated by the carboxy terminal MATEE amino acid sequence of the SSAT protein (*27*, *28*).

Potential role of SSAT activity in cellular response to the antitumor polyamine analogues. Much of what is now known about the regulation of SSAT expression is a direct result of the interest produced by the observations
linking high SSAT activity and the response to specific polyamine analogues. Although the cell type–specific response of SSAT superinduction was first discovered in non–small cell lung cancers (*18*) several other cellular and *in vivo* tumor model systems have demonstrated significant antitumor responses to the analogues that is associated with the induction of SSAT (*29*, *30*). Most importantly, this response has been demonstrated to be a tumor

cell–specific response (*31*), indicating that the superinduction is limited to the exposed tumor cells and not adjacent normal cells.

Although the induction of SSAT has been implicated in cellular response to specific polyamine analogues, the most direct evidence was provided by the studies of Vujcic *et al*. using a Tet-repressible expression system for SSAT in the MCF-7 human breast cancer cell line (*32*). Expression of the transfected SSAT gene led to increased basal SSAT expression (~10-fold) and a decrease in growth rate, in untreated cells. However, the cells transfected with the SSAT gene were significantly more sensitive to the antitumor polyamine analogue N^1,N^{11}-bis(ethyl)norspermine (BENSpm) than the wild type MCF-7 cells. Similar results have also been reported using non-inducible systems and are entirely consistent with high expression of SSAT being one mechanism by which polyamine analogues inhibit tumor cell growth (*33*). It is important to note that the superinduction of SSAT protein is only seen in cells that are treated with analogue, regardless of whether they are expressing transfected or endogenous SSAT. This finding is consistent with the hypothesis that the major mechanism leading to extreme levels of SSAT protein is analogue-mediated stabilization of the protein.

Although BENSpm demonstrated impressive antitumor activity in preclinical systems, indicating that its activity was in part dependent on SSAT, clinical trials completed thus far have not demonstrated significant activity when BENSpm is used as a single agent. BENSpm was found to be well tolerated in clinical trials and data continue to indicate tumor selectivity. Therefore, attempts are being made to improve the clinical response to BENSpm through the use of combinations with standard chemotherapeutic drugs.

Combination trials were first attempted in breast cancer models and showed considerable promise demonstrating synergy with 5-FU and BENSpm (*34*). 5-FU was used in an MCF-7 breast tumor cell model for gene array analysis that demonstrated that 5-FU treatment alone could lead to an increase in SSAT mRNA (*14*). Using a HCT 116 colon cancer model Choi *et al.* demonstrated that the combination of 5-FU and BENSpm led to a synergistic expression of SSAT mRNA and increased apoptosis compared to either treatment alone (*16*). Unfortunately, none of the above studies provided data demonstrating actual increases in SSAT protein or activity. Hector, et al. have recently reported that the combination of oxaliplatin and BENSpm led to a synergistic antitumor effect in both melanoma and ovarian cancer, and that this effect was associated with a synergistic increase in SSAT mRNA and protein expression. These studies also strongly suggest a mechanism for the observed synergy that may be clinically relevant. Specifically, exposure to the standard cytotoxic agents produces a significant increase in steady-state SSAT mRNA. However, in the absence of BENSpm, little or no increase in SSAT protein is observed. When BENSpm is combined with the cytotoxic agent, the result is a substantial increase in protein, potentially resulting from the same post-transcriptional regulatory mechanisms observed when BENSpm is used alone; specifically, increased translational efficiency and protein stabilization. These results have important clinical implications in that, when compared to BENSpm treatment alone, reduced doses of BENSpm are required to achieve the same SSAT induction when used in combination with the standard cytotoxic agents. Such combinations therefore hold great promise and may overcome the clinical limitations observed thus far when polyamine analogues have been used as single agents.

Additional stimuli leading to increased SSAT expression. The induction of SSAT expression by multiple agents has now been well established. Recently, some additional and interesting stimuli have also been identified that result in the increased expression of SSAT at the mRNA and protein levels. That SSAT is a stress response gene is not surprising, considering its original description was from carbon tetrachloride–exposed rat livers (*7*). Several recent studies have demonstrated that SSAT expression can also be modulated by various other stresses including ischemia/reperfusion in multiple organs (*35–38*). Although the role of this induction is not completely clear, the induction of SSAT appears to be associated with ischemic injury.

Agents unrelated to the polyamines and general toxins have also been demonstrated to induce the expression of SSAT, possibly through increased transcription. Multiple non-steroidal anti-inflammatory drugs (NSAIDs) that are being considered for use as chemopreventive agents, including aspirin and sulindac sulfone, have demonstrated the ability to increase SSAT activity, leading to a decrease in intracellular polyamines and decreased cell growth in a cyclooxygynase-independent manner (*39–41*). In some cases, this NSAIDs-dependent growth inhibition can be reversed by addition of polyamines, indicating that at least in these select systems, NSAID growth inhibition may involve the polyamine pathway (*40*). The mechanism by which some NSAIDs induce SSAT expression in colon cancer cells appears to be through increased transcription mediated by a peroxisomal proliferators response element (PPRE) and the peroxisome proliferators–activated receptor-γ (PPAR-γ) (*41*). The current results suggest that one way in which the NSAIDs may act to reduce tumor growth rate is by decreasing intracellular polyamines below the optimal intracellular concentrations necessary for tumor growth and development. However, continued study will be necessary to determine the relative importance of this activity in the observed antitumor effects of the NSAIDs.

Transgenic models altering the expression of SSAT. Multiple transgenic models have now been established to aid in the understanding of how SSAT activity can modulate polyamine homeostasis, cell growth, and response to antitumor polyamine analogues. The first SSAT transgenic mouse lines harbored multiple copies (>20) of the SSAT gene and produced significant alterations in polyamine pools in specific tissues (*42*). Using this transgenic strategy, Jänne and co-workers has published extensively on the effects of over expression of SSAT on multiple organ systems. The results of these studies have recently been thoroughly (*43*) reviewed and thus will not be repeated in detail here.

The major findings of these transgenic studies can be summarized as follows: increased SSAT activity has a profound effect on many processes, ranging from polyamine homeostasis and skin abnormalities to decreases in fertility and central nervous system effects (*43*). The skin

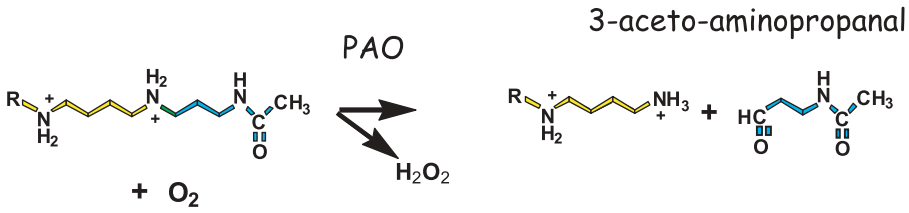

Fig. 2. **Catalysis by the N^1-acetylpolyamine oxidase, PAO**. PAO oxidizes the N^1-acetylated substarte provided by the activity of SSAT. R represents H in N^1-acetylspermidine or $NH_{3+}(CH_2)_3$ in N^1-acetylspermine.

abnormalities observed in SSAT overexpressing mice bear a striking resemblance to the skin phenotype observed in ODC transgenic mice (*42*, *44*). Specifically, the mice lose their hair at a very early age, resulting from loss of the hair follicles to dermal cysts that led to extensive wrinkling of the skin. This and other phenotypic abnormalities appear to be associated with the high intracellular putrescine levels that result from both ODC overexpression and SSAT overexpression.

Interestingly, the transgene studies confirm that the major point of regulation leading to massive increases in SSAT protein is at the message level, as observed in the *in vitro* systems detailed above. In the absence of analogue exposure, the mice expressing the SSAT transgene, or fibroblasts from the transgenic mice, only express high levels of SSAT mRNA. Only after analogue exposure do they demonstrate an extremely high expression of SSAT protein and activity (*42*, *45*).

Skin targeted expression studies with SSAT by Coleman, et al. provided additional insight into the effects of SSAT over expression (*46*). Using a K6 promoter, epidermal keratinocytes in the hair follicles of transgenic mice expressed high levels of SSAT. Unlike the transgenic mice expressing multiple copies, these mice did not lose their hair or develop an abnormal phenotype. However, the mice were found to have increased tumor susceptibility when challenged using a two-stage tumorigenesis protocol with DMBA/TPA, and produced malignant carcinomas at a rate of 31% compared to no malignant tumors in identically challenged wild type littermates.

The increased tumor incidence in the K6 promoter model is in contrast with 2 other SSAT transgenic models. In the mouse models expressing multiple systemic copies of SSAT and exhibiting the hairless phenotype, the transgenic animals were resistant to the DMBA/TPA tumorigenesis protocol and produced significantly less papillomas than animals that did not express the transgene (*47*). Similarly, Kee *et al.* demonstrated in the a double transgenic TRAMP (transgenic adenocarcinoma of mouse prostate)/SSAT prostate cancer model that the expression of SSAT reduced prostate tumor growth as compared to the single transgene bearing TRAMP mice (*48*). The authors attributed this decrease in prostate tumor growth to a depletion of acetyl CoA pools resulting from increased flux through the polyamine biosynthetic/catabolic pathways in mice overexpressing SSAT.

Interestingly, the same group has recently reported an increase in tumor number and size in a double transgenic model of the $Apc^{Min/+}$ (MIN) mouse (*49*). Here the authors found that, similar to results reported Coleman *et al.* (*46*), expression of SSAT in the MIN mouse led to the production of more tumors in the MIN mouse. When the Min mice were crossed with SSAT knockout mice, fewer tumors were observed, suggesting that SSAT may play a role in the MIN phenotype. Rather than increased SSAT activity and attendant flux inhibiting tumor growth as they observed in their prostate model, here the authors invoke increased flux and potential metabolic imbalance to account for the increase in tumor formation in the SSAT/$Apc^{Min/+}$ mice. The complexity of the results of these systems suggest that the ultimate effects of SSAT overexpression are very system dependent and clearly more experimentation will be necessary to determine what the flux is going on.

N^1-Acetylpolyamine oxidase (PAO)

Specificity and function. The initial description of intracellular polyamine catabolism indicated that a peroxisomal oxidase was responsible for the oxidation of both spermine and spermidine (*6*). However, the rate of the oxidation reaction was found to be greatly enhanced by the presence of benzaldehyde, leading to the discovery that acetylated polyamines were the preferred substrates of the oxidase. As Seiler's recent review of polyamines extensively covers PAO (*50*), the goal here will be to focus on the newest findings regarding mammalian PAO, since its recent cloning and characterization (*51*, *52*).

Polyamine oxidase is generally a constitutively expressed, FAD-dependent oxidase that preferentially oxidizes N^1-acetylated polyamines (Fig. 2). The enzyme-bound FAD cofactor facilitates the electron cycling that results, in the presence of molecular oxygen, in the production of H_2O_2, 3-acetoamidopropanal, and either spermidine or putrescine, depending on the starting substrate. Each of the cloned mammalian PAO's codes for a terminal peroxisomal localization sequence (-PRL) consistent with the original findings of Hollta (*6*, *51*, *52*). The purified mouse and human PAOs each have high catalytic activity for the N^1-acetylated polyamines (K_{cat} = 4.5–32 s^{-1}) and relatively high affinities for each substrate (K_m = 0.85–1.8 μM). PAO has the highest affinity for N^1-acetylspermine (>N^1-acetylspermidine > N^1,N^{12}-diacetylspermine >>>> spermine) (*52*, *53*).

We had originally proposed that the sensitivity of various non-small cell lung cancers to the antitumor polyamine analogues was due to H_2O_2 produced by PAO subsequent to the high induction of the rate-limiting SSAT activity (*54*). However, these studies were interpreted with the assumption that *N*,*N*′-(butadienyl)-1,4-butanediamine (MDL72,527) (*55*) was a specific inhibitor that solely inhibited PAO, an assumption that later proved to be incorrect (see below). Our more recent results using an siRNA strategy, rather than enzyme inhibition, suggest it may be necessary to seriously reconsider the role of PAO in polyamine homeostasis and back conversion (*56*). Specifically, our studies in a breast tumor model suggest that the source of H_2O_2 in analogue-induced polyamine catabolism is not from the SSAT/PAO two-step pathway, but rather is

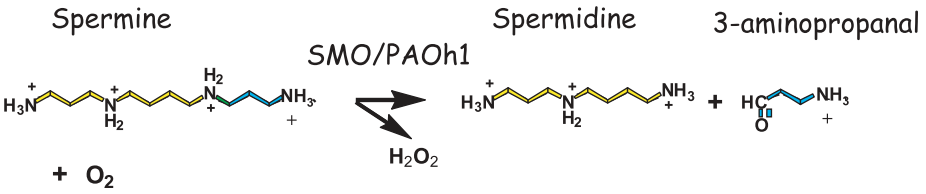

Fig. 3. **Catalysis of spermine by the spermine oxidase SMO/PAOh1**.

entirely from the newly discovered spermine oxidase (SMO/PAOh1, see below) (*2*, *3*, *56*). If this finding is determined to be a general response and not limited to specific systems, it will have considerable implications in the true role of PAO.

Inhibitors of PAO. As indicated above, MDL 72,527 was designed specifically to inhibit the FAD-dependent PAO (*55*). Wu, et al. have recently provided evidence that this inhibition is a result of covalent modification of the PAO-bound FAD that produces an irreversibly inhibited PAO containing a flavocyanine (*57*). Although MDL 72,527 is clearly an effective inhibitor of PAO, it is not entirely specific for this flavin containing enzyme. As implicated above, it also effectively inhibits the newly discovered spermine oxidase, SMO/PAOh1 (*2*, *3*, *58*). However, MDL 72,527 has a much lower affinity for the purified spermine oxidase than it does for the purified PAO (K_i 63 μM *versus* 1 μM) (*57*, *58*). Whether this 60-fold difference is relevant in situ is yet to be determined.

Other compounds have recently been determined to effectively inhibit both PAO and SMO/PAOh1. Several oligoamine polyamine analogues were found to inhibit both purified recombinant oxidases when used in the micromolar range (*53*, *59*). It is currently not known whether this inhibition plays any role in the antiproliferative effects of these agents (*60*).

Potential role for PAO in tumor response to the antitumor polyamine analogues. Although our initial hypothesis implicated H_2O_2 production by PAO having a direct role in the response of human tumors to specific antitumor polyamine analogues (*54*), our most recent results in human breast tumor lines strongly suggest this is not the case (*56*). However, another possible role for PAO in analogue response was implicated by the results of Lawson *et al*., where Chinese hamster ovary (CHO) cells were demonstrated to be resistant to the cytotoxic effects of N^1-ethyl-N^{11}-[(cycloheptyl)methyl]-4,8-diazaundecane (CHENSpm) (*61*). Chromatographic analysis of CHENSpm-treated CHO cells indicated that CHENSpm was being metabolized in the treated cells. If the CHO cells were treated with the combination of CHENSpm and the PAO inhibitor, MDL 72,527, the metabolism of CHENSpm was inhibited and the CHO cells became significantly more sensitive to the analogue.

The above data implicate the potential of PAO to detoxify specific analogues. However, since MDL 72,527 is also capable of inhibiting SMO/PAOh1, the above study was not conclusive. With the cloning and characterization of mammalian PAO it has now been convincingly demonstrated that PAO is capable of metabolizing multiple symmetrically substituted polyamine analogues (*51*, *52*, *62*). Our recent results with purified, recombinant human PAO demonstrate that this enzyme is also capable of oxidizing unsymmetrically substituted polyamine analogues with aminopropylimine termini (*53*). Although our results were consistent with those of Vujcic *et al*. (*51*) with respect to analogue cleavage at the interior imine nitrogen, we did not find that N^1,N^{14}-bis(ethyl)homospermine (BEHSpm) or any analogue with terminal aminobutyl termini were oxidized by the purified human PAO. Consistent with the finding of Lawson *et al*. (*61*), when the human lung adenocarcinoma line, A549, was transfected with PAO, the resulting high expressing PAO clones were entirely resistant to the analogues demonstrated to be substrates for PAO.

Taken together, the above findings indicate that although PAO activity may or may not be responsible for producing the H_2O_2 leading to the cytotoxic response of tumor cells to specific polyamine analogues, the ability of PAO to oxidize some cytotoxic polyamine analogues may have a profound effect on the response of tumors to these agents.

Spermine oxidase (SMO/PAOh1)

Cloning and characterization of the mammalian spermine oxidase (SMO/PAOh1). The mammalian spermine oxidase is the most recent of the FAD-dependent polyamine oxidases to be cloned. This enzyme, which oxidizes spermine to produce spermidine, H_2O_2, and 3-aminopropanal (Fig. 3) was first cloned by our laboratory based on its homology to the maize polyamine oxidase (*2*, *63*). We originally named this new gene/protein PAOh1 for the first human polyamine oxidase to be cloned. The structural features of the coded proteins resembled what would be expected for the N^1-acetylpolyamine oxidase, which was the original target of our cloning strategies. However, unlike the constitutively expressed PAO, the resulting gene was found to be highly inducible by many of the same analogues that are known to induce SSAT and efficiently oxidized spermine. Vujcic, et al. confirmed the substrate specificity of spermine oxidase that they called SMO (*3*). SMO/PAOh1 is located at the 20p13 locus, and codes for a 61 kDa protein. Although the major isoform is coded by 7 exons and contains 555 amino acids, multiple splice variants of both the mouse and human have been identified (*2*, *3*, *64–66*). One of the active mouse splice variants has been localized to the nucleus (*65*, *67*); however, the significance of this finding remains to be determined.

As stated above, unlike PAO that is generally expressed at constitutive levels and regulated by the availability of the acetylated substrate, SMO/PAOh1 is generally present at low levels and is readily inducible by several polyamine analogues (*2*, *3*, *68*). The majority of inducible human SMO/PAOh1 expression is a result of increased mRNA subsequent to increased transcription and message stabilization, and is not a product of post translational stabilization (*69*). The *cis*-elements and *trans*-acting factors controlling SMO/PAOh1 transcription have yet to be identified.

The recombinant human SMO/PAOh1 has a high affinity and catalytic activity for spermine ($K_m \sim 8$ μM with a

k_{cat} = 7.2 s^{-1}) (*59*). Although the kinetic constants of the purified mouse protein are similar to those of the human enzyme, the reported K_m for spermine is at least 10 times higher for the mouse protein (*70*). Unlike human PAO, SMO/PAOh1 does not effectively oxidize any of the polyamine analogues examined thus far, suggesting greater substrate specificity than is observed for PAO (*59*).

Role of SMO/PAOh1 activity in cellular response to antitumor polyamine analogues. The production of H_2O_2 has been implicated in the cytotoxic response of specific human tumor cell types to a variety of antitumor polyamine analogues. Our initial hypothesis that the H_2O_2 was produced by the SSAT/PAO pathway (*54*) has been called into question by results of our more recent studies (*56*). One complicating factor in the interpretation of results indicating the role of one pathway or the other is the fact that most analogues that induce SSAT also induce SMO/PAOh1. Therefore, since specific inhibitors of SSAT and/or SMO/PAOh1 are not currently available, we chose to use a stabile siRNA approach targeting each of the pathways alone and in combination. Using human breast cancer cell lines, we were able to demonstrate that both SSAT and SMO/PAOh1 contribute to the antiproliferative effects of BENSpm (*56*). However, the sole source of H_2O_2 produced by polyamine catabolism in the MDA MB 231 cell line was SMO/PAOh1 and not the SSAT/PAO pathway. In the breast cancer cell lines used, it appears that the fate of the acetylated polyamines is excretion rather than back conversion through oxidation by PAO. Although these data strongly suggest that PAO plays little or no role in analogue-induced polyamine catabolism and homeostasis in the breast cancer lines used, additional trials in other systems will be necessary to determine the universality of this finding. These data also emphasize the potential of targeting both SSAT and SMO/PAOh1 for induction with the goal of producing the greatest growth inhibition.

Physiologic implications of SMO/PAOh1-produced H_2O_2. The catalytic activity of SMO/PAOh1 produces two toxins in equimolar amounts: the aldehyde, 3-aminopropanal, and the reactive oxygen species (ROS), H_2O_2 in addition to the polyamine spermidine. Clearly, both the aldehyde and the ROS can produce cellular damage, and both may be involved in the damage reported to result from increased polyamine catabolism in response to ischemia/reperfusion (*37*, *38*). It is also clear from the analogue studies presented above that increased polyamine catabolism and specifically H_2O_2 production by SMO/PAOh1 can lead to cytotoxic events. The question that arises is what other physiological consequences of polyamine catabolism-produced H_2O_2 may occur?

Parchment and Pierce first suggested that the H_2O_2 produced by polyamine oxidation was critical for embryonic development by leading to apoptosis of unnecessary cells (*71*). Although such a process is clearly advantageous to the developing embryo it is also possible that inappropriate activation of polyamine oxidation could lead to serious deleterious effects.

Recently we have examined the effects of pathogen-induced SMO/PAOh1 on macrophages. *Helicobacter pylori* affects approximately 50% of the world population and is associated with peptic ulcers and gastric cancer. How *H. pylori* infection escapes immune detection and elimination by the gut macrophages is not entirely understood. Our studies indicate that *H. pylori* infection produces a rapid induction of macrophage SMO/PAOh1, producing sufficient H_2O_2 within the macrophage to lead to apoptotic cell death, and thus presenting a plausible mechanism by which *H. pylori* avoids eradication by the affected macrophages (*72*).

The results of these studies not only provided insight as to how *H. pylori* might escape macrophage-mediated cell death, but also suggested a possible direct link between *H. pylori* infection and gastric cancer. Oxidative damage of DNA is known to be directly linked to carcinogenesis. Therefore, we hypothesized that if *H. pylori* infection produced a similar increase in SMO/PAOh1 in infected gastric epithelial cells, the resulting H_2O_2 may be sufficient to damage DNA thus suggesting a causal link between infection and a mechanism to produce some of the necessary genetic changes that are required for the initiation of carcinogenesis.

It was found that when gastric epithelial cells are exposed to *H. pylori* there is a rapid and significant increase in SMO/PAOh1 mRNA and protein (*73*). The H_2O_2 resulting from *H. pylori*–induced SMO/PAOh1 activity is sufficient to produce DNA damage and apoptosis. Importantly, the DNA damage observed, 8-OH deoxyguanosine is a frequently occurring mutation associated with the carcinogenic process. It is important to note that the DNA damage and apoptotic effects of *H. pylori* could be significantly attenuated through the use of siRNA specifically targeting SMO/PAOh1. The association between *H. pylori* infection and SMO/PAOh1 expression was further confirmed in gastric epithelial cells from patients with diagnosed *H. pylori*–induced gastritis. Specifically, using laser capture microdissection material from affected individuals, it was found that patients with *H. pylori*–induced gastritis had uniformly high expression of SMO/PAOh1 mRNA that was uniformly reduced in each patient when the infection was eradicated with antibiotic treatment (*73*).

These results taken together demonstrate a mechanism by which *H. pylori* infection can lead directly to DNA damage in gastric epithelial cells through induction of SMO/PAOh1 and further suggest the possibility that DNA damage produced in this manner may be causally linked to *H. pylori*–induced gastric cancer.

It has also been recently reported that over expression of SMO/PAOh1 produces oxidative damage of DNA with or without exposure to ionizing radiation (*74*). These results are entirely consistent with the potential of inappropriate expression of SMO/PAOh1 to produce the genetic changes necessary for the initiation of carcinogenic transformation.

Many questions remain regarding the possible causal link between infectious agents and polyamine catabolism in carcinogenesis. A critical question to be answered is whether or not the induction of SMO/PAOh1 and the subsequent DNA damage is unique to *H. pylori* infection or is a general response to multiple infectious agents in different tissues. Chu, et al., using a glutathione 1 and 2 double knockout mouse, have demonstrated an increased incidence of bacteria-associated gastrointestinal cancers correlated with infection-associated inflammation and oxidative damage (*75*). If SMO/PAOh1 is found to be directly linked to the etiology of inflammation-induced cancers, it immediately suggests a chemopreventive strategy whereby the activity of the spermine oxidase could be targeted.

Conclusions

In the last 15 years a considerable wealth of information has become available to increase our understanding of the importance of polyamine catabolism in polyamine homeostasis and drug response. The most recent studies of polyamine catabolism in response to various pathogens opens a new and exciting field of study that may have repercussions well beyond what has already been recognized. Such studies will be helpful in the arena of antineoplastic chemotherapy and may have great potential in the prevention of specific cancers. Many questions still remain to be answered; among them what role, if any, does the classical PAO plays in polyamine homeostasis? Is the major fate of N^1-acetylated polyamines to be excreted from the cell? Is the H_2O_2 produced by polyamine catabolism always pathologic or does it have a role in cell signaling? What is the relationship between the family of FAD-dependent polyamine oxidases, their splice variants, and the newly discovered FAD-dependent lysine demethylase (*76*)? As more is learned about the regulation and role of the polyamine oxidases in cellular function it is hoped that those data will lead to the answers of these important questions.

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