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Polyamine catabolism and oxidative damage

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Polyamines (PAs) are indispensable polycations ubiquitous to all living cells. Among their many critical functions, PAs contribute to the oxidative balance of the cell. Beginning with studies by the Tabor laboratory in bacteria and yeast, the requirement for PAs as protectors against oxygen radical–mediated damage has been well established in many organisms, including mammals. However, PAs also serve as substrates for oxidation reactions that produce hydrogen peroxide (H_2O_2) both intra- and extracellularly. As intracellular concentrations of PAs can reach millimolar concentrations, the H_2O_2 amounts produced through their catabolism, coupled with a reduction in protective PAs, are sufficient to cause the oxidative damage associated with many pathologies, including cancer. Thus, the maintenance of intracellular polyamine homeostasis may ultimately contribute to the maintenance of oxidative homeostasis. Again, pioneering studies by Tabor and colleagues led the way in first identifying spermine oxidase in *Saccharomyces cerevisiae.* They also first purified the extracellular bovine serum amine oxidase and elucidated the products of its oxidation of primary amine groups of PAs when included in culture medium. These investigations formed the foundation for many polyamine-related studies and experimental procedures still performed today. This Minireview will summarize key innovative studies regarding PAs and oxidative damage, starting with those from the Tabor laboratory and including the most recent advances, with a focus on mammalian systems.

Polyamines (PAs)[2] are naturally occurring polycationic alkylamines that are essential for growth and survival in all mammalian cells (1, 2). This absolute requirement is based on the multitude of roles PAs play, many of which relate to their positive charge at physiological pH. PAs, including putrescine (Put), spermidine (Spd), and spermine (Spm) (Fig. 1), contribute to critical cellular processes such as ion channel regulation, chromatin structure maintenance, DNA replication, transcription, and translation (3–6). They also act as free radical scavengers, and their catabolism can be a source of toxic reactive oxygen species (ROS), therefore implying their potential to affect oxidative status. The main purpose of this Minireview will be to cover the salient features of PAs and their catabolism in association with oxidative stress in both normal and disease processes.

Contributions of polyamines to cellular redox balance

Oxidative stress occurs when ROS, such as those derived from hydrogen peroxide (H_2O_2), exceed the physiological levels required for normal redox reactions and cell signaling. The resulting oxidative damage to macromolecules is associated with aging and a variety of related pathologies, including cancer (7, 8). PAs play dual roles in maintaining cellular oxidative homeostasis by both protecting against free radical–mediated damage and acting as substrates for enzymes that produce ROS.

Polyamines as protection from oxidative damage

Polyamines protect against oxidative damage in microorganisms—The natural PAs are themselves capable of acting as free radical scavengers (9–12) and protect against oxidative damage of DNA and phospholipids in cell-free systems (13–15). These properties extend to bacteria and yeast, where Tabor and colleagues first demonstrated a protective role for PAs against oxidative damage *in vivo*. Spd-deficient *Escherichia coli* cells were hypersensitive to paraquat in the presence of oxygen, suggesting that Spd reduced superoxide-associated cell death (16). In subsequent studies, *E. coli* mutants lacking PAs were extremely susceptible to ROS toxicity when grown in 95% oxygen or exposed to H_2O_2 (17). These data were among the first to demonstrate polyamine-mediated protection against oxygen radicals.

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[2] The abbreviations used are: PA, polyamine; Put, putrescine; Spd, spermidine; Spm, spermine; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; Gsp, glutathionylspermidine; GspSA, glutathionylspermidine synthetase/amidase; SSAT, spermidine/spermine N^1-acetyltransferase; PAOX, peroxisomal N^1-acetylpolyamine oxidase; SMOX, spermine oxidase; 3-AP, 3-aminopropanal; BSAO, bovine serum amine oxidase; SSAO, semicarbazide-sensitive amine oxidase; PDR, progressive diabetic retinopathy; ETBF, enterotoxigenic *Bacteroides fragilis*; CRC, colorectal carcinoma; PIN, prostatic intraepithelial neoplasia; DSS, dextran sulfate sodium; IRI, ischemia/reperfusion injury; SRS, Snyder-Robinson syndrome; AKI, acute kidney injury.

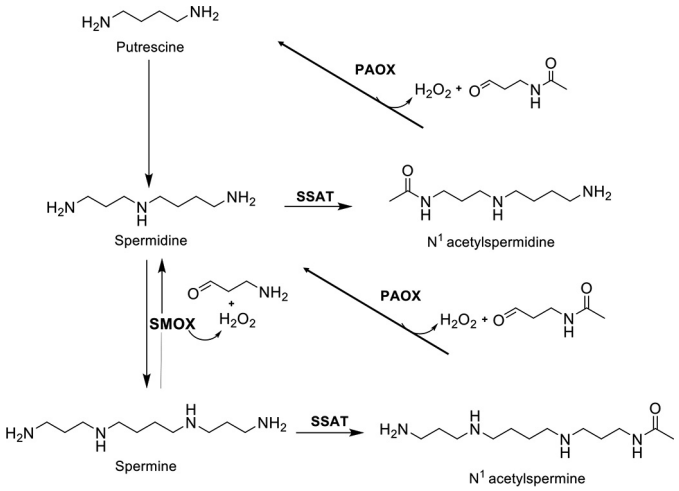

Figure 1. Polyamine catabolic pathway in mammals. SSAT catalyzes the acetyl-group transfer from acetyl-CoA to the aminopropyl end of spermidine or spermine, producing N^1-acetylspermidine or N^1-acetylspermine, respectively. These acetylated PAs are either excreted from the cell or used as substrates for PAOX, producing H_2O_2, 3-acetoamidopropanal, and either putrescine or spermidine, depending on the starting substrate. Alternatively, spermine can be directly oxidized back to spermidine by SMOX while generating H_2O_2 and 3-aminopropanal.

The polyamine modulon of *E. coli* comprises a growing collection of genes that are stimulated by PAs at the level of translation (18). Recently, this modulon was expanded to include polyamine-inducible genes specific to oxidative stress conditions, including those governing the synthesis of superoxide dismutases (SODs), glutathione (GSH), and catalases (19, 20). Thus, in addition to the direct effects of PAs on ROS in *E. coli*, PAs can stimulate the expression of proteins essential to an effective antioxidant response.

Saccharomyces cerevisiae mutants lacking Spd and Spm also require exogenous PAs for protection against ROS, even in the presence of SOD overexpression (21, 22). Microarray studies comparing Spm-deficient yeast mutants containing low levels of Spd with those supplemented with Spd revealed that Spd altered the expression of at least 500 genes greater than 2-fold, including several oxidative stress-response genes (23). Recently, the *S. cerevisiae* polyamine exporter Tpo1 was discovered to participate in the oxidative stress response by modulating intracellular Spd and Spm levels in response to H_2O_2, thereby invoking the production of proteins necessary for oxidant tolerance, including SODs and heat shock proteins, governing the duration of cell cycle arrest, and allowing adaptation to elevated H_2O_2 levels (24).

In addition to the unmodified PAs, polyamine conjugates have been implicated in protection from oxidative stress. Glutathionylspermidine (Gsp) was first identified in *E. coli* by the Tabors in 1974 (25) and was later found to be a source of Spd for the bacteria upon the induction of growth from stationary phase (26). Glutathionylspermidine synthetase/amidase (GspSA) catalyzes both the formation and removal of an amide bond between GSH and Spd to govern Gsp abundance (27). The amidase domain of GspSA is sensitive to inactivation by oxidation, resulting in Gsp-modified proteins, including Gsp disulfides and protein thiols (28). These data are consistent with the hypothesis that the formation/hydrolysis of Gsp represents an oxidative stress mechanism that contributes to the maintenance of redox homeostasis in *E. coli*.

Confirmation of a role for GSH–Spd conjugates in redox reactions came with the discovery of trypanothione (N^1,N^8-bis(glutathionyl)spermidine), a cofactor for trypanosomatid GSH reductase (29). As an important mediator of redox balance in pathogenic trypanosomes, including those responsible for human African trypanosomiasis, leishmaniasis, and Chagas' disease, the generation, use, and redox recycling of trypanothione have become targets for antitrypanosomal drug development (30). Additionally, Spd and Spm protect against free radical–mediated lipid peroxidation in *Trypanosoma cruzi* (31).

Protective effects of polyamines against oxidative stress in mammalian cells—Spm and Spd also protect mammalian cells against ROS-mediated damage, and depletion of these PAs is known to arrest cellular growth. The Gy11 embryonic fibroblast cell line is deficient in Spm due to a mutation in the Spm synthase gene (32). These cells are more sensitive to the cytotoxic effects of H_2O_2 than their normal counterparts, and further depleting their PA levels induces DNA damage and apoptosis even in the absence of H_2O_2. Using combinations of enzyme inhibitors to adjust the intracellular Spd and Spm concentrations of these cells to within normal physiological ranges protected fibroblasts from H_2O_2 exposure. Additionally, depleting cellular GSH further sensitized PA-depleted cells to H_2O_2, indicating the involvement of PAs in a protective mechanism against ROS independent of GSH (33).

Oxidative stress activates translocation of the transcription factor NRF2 (nuclear factor (erythroid-derived 2)-like 2), which then stimulates expression of genes involved in the antioxidant response. Evidence suggests that NRF2 also regulates PA biosynthesis by inducing ornithine decarboxylase activity in response to oxidative stress, thereby elevating PA levels to potentially aid in the antioxidant response (34). However, activated PA catabolism through spermidine/spermine N^1-acetyltransferase (SSAT), another transcriptional target of NRF2, also occurs in response to ROS, perhaps to limit tumor-promoting PA accumulation (34–36).

Polyamine catabolism as a source of ROS

Intracellular mammalian polyamine catabolism—Mammalian PA catabolism consists of highly regulated, inducible pathways that facilitate cellular PA homeostasis. It serves to balance PA transport and biosynthesis to maintain intracellular PAs within a cell type-specific range that is optimal for cellular function and proliferation. Mammalian PA catabolism has two distinct but interconnected pathways (Fig. 1), both of which contain oxidases that generate ROS in the form of H_2O_2.

The originally discovered catabolic mechanism is a two-step process, where Spd and Spm are acetylated in their N^1 positions by the highly-inducible SSAT (37–39). N^1-Acetylated PAs are either excreted from the cell or oxidized by peroxisomal N^1-acetylpolyamine oxidase (PAOX), resulting in H_2O_2, 3-acetoamidopropanal, and Put or Spd, depending on the starting substrate (40–43). Pharmacological superinduction of SSAT as a chemotherapeutic strategy has antitumor effects through

depletion of the natural PAs needed for basic cell functions (44, 45).

Spm can also be directly oxidized by Spm oxidase (SMOX; PAOh1) to produce H_2O_2, 3-aminopropanal (3-AP), and Spd (46, 47). Localized in the cytoplasm and nucleus of mammalian cells (48, 49), SMOX is highly inducible by many of the same stimuli that induce SSAT (46, 50). Thus, the H_2O_2 produced by SMOX has greater potential for producing genetic damage than that produced by PAOX, which, when produced in a normally functioning peroxisome, is in the presence of catalase. As Spm can exist in millimolar concentrations within the cell (1), the release of H_2O_2 via this pathway in circumstances of up-regulated SMOX activity is sufficient to evoke oxidative stress, particularly in the form of oxidative DNA damage. Furthermore, the 3-AP generated by SMOX spontaneously converts into the highly reactive and toxic unsaturated aldehyde acrolein (51, 52). Notably, the *S. cerevisiae* amine oxidase Fms1 also directly oxidizes Spm to Spd and was first described by the Tabor lab (53).

Recent studies have suggested a role for SSAT in the p53-mediated ferroptotic response to ROS stress, an iron-dependent, nonapoptotic mode of cell death characterized by accumulation of lipid ROS at the cell membrane. Activation of p53 by DNA-damaging agents induces *SAT1*, a direct target of p53, resulting in the induction of arachidonate 15-lipoxygenase, lipid peroxidation, and cell death. This *SAT1* induction ultimately sensitizes the cells to ferroptosis in the presence of ROS, manifesting as tumor suppression in xenograft models. Similarly, in embryonic fibroblasts from p53 WT or p53 acetylation-deficient mutant mice, which retain the ability to stimulate ferroptosis, *Sat1* expression is induced by p53 activation, and *Sat1* knockdown partially prevents ferroptotic cell death. The results of these studies propose a tumor-suppressive role for p53-mediated SSAT by promoting ferroptosis (54).

Extracellular polyamine oxidases—In the early 1950s, it was reported that the addition of Spm or Spd to certain culture conditions was growth inhibitory to mycobacteria (55, 56). These studies led to the discovery of the first soluble amine oxidases (57). Crude preparations of sheep serum amine oxidase allowed kinetic studies indicating oxidative deamination of Spm and Spd at rates greater than 10-fold that of other amines and concluded that this oxidative "activation" of the PAs was responsible for their antibacterial effects (58). Tabor and colleagues purified a soluble amine oxidase from bovine plasma, bovine serum amine oxidase (BSAO), that had substrate specificity for Spm and Spd and catalyzed the stoichiometric formation of their corresponding aldehydes, ammonia, and H_2O_2 (Fig. 2) (59, 60). Subsequent studies revealed that the reaction by-products of Spm or Spd with BSAO were highly toxic to *E. coli*, *Staphylococcus aureus*, bacteriophages, and mammalian spermatozoa and caused immotility in *Trypanosoma equiperdum* (61, 62).

Many studies have since concluded that adding Spd or Spm to mammalian cells in the presence of bovine serum results in extracellular oxidation of the PA and growth inhibition due to the oxidation products, not the exogenous PA (63–66). Studies testing the inhibitory effects of Spm, Spd, their aldehyde reaction products (which can convert to acrolein), and H_2O_2 in mammalian cell lines have indicated major roles for acrolein and H_2O_2 in the cytotoxic responses (51, 67–69), and in most systems, treatment with aldehyde dehydrogenase inhibitors and catalase together yielded protection from cytotoxicity (70, 71). Therefore, caution must be used when interpreting results involving PA treatment of cells in culture, particularly with regard to cellular processes involving ROS, such as autophagy. As virtually all of the mechanistic studies reported in mammalian cells regarding the role of PAs in autophagy were performed with high concentrations of PAs in medium containing bovine serum (72), the published interpretation of these studies is likely in error.

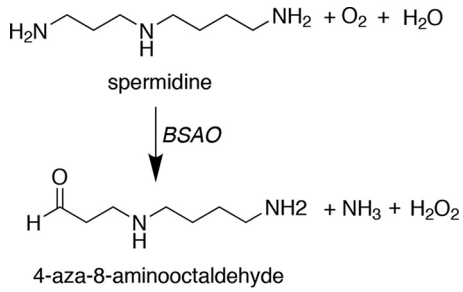

Figure 2. Extracellular polyamine oxidation. BSAO oxidizes the terminal aminopropyl nitrogens of spermine or spermidine (shown) to produce H_2O_2, ammonia, and the corresponding amino aldehydes.

Early *in vivo* pharmacological studies in the Tabor lab provided evidence of Spm degradation to Spd following intraperitoneal injections of Spm in rabbits, mice, and rats, suggesting the presence of a BSAO-like enzyme in laboratory animals (61). In humans, extracellular oxidation of PAs and acetylpolyamines has been measured in plasma from patients suffering from cerebral stroke or chronic kidney disease (73, 74). Plasma amine oxidase activity in renal failure patients correlated with the severity of disease, reduction of Spm and Spd levels, and acrolein accumulation. Oxidation of Spm was inhibited in plasma of all patients examined using a common polyamine oxidase inhibitor; however, a copper-containing oxidase inhibitor, semicarbazide, inhibited Spm degradation in half of the patients. These data suggest the presence of a human extracellular, soluble, and semicarbazide-sensitive amine oxidase (SSAO) capable of oxidizing Spm (74).

Recent ocular research implicates vascular adhesion protein (VAP1/SSAO/AOC3)–mediated oxidation of Spm in the pathology of proliferative diabetic retinopathy (PDR) (75). Chronic inflammation and oxidative stress contribute to this pathology, and like BSAO, VAP1 oxidizes primary amines to generate H_2O_2, ammonia, and aldehydes capable of forming acrolein (76). Soluble VAP1 protein levels and acrolein adducts are increased in the vitreous fluid of patients with PDR, where PA levels are also elevated (77). This potential of VAP1 as an extracellular PA oxidase has implications for pathologies and/or treatment opportunities beyond the eye that warrant further evaluation and underscore the need for cautious experimental design and interpretation when considering the administration of natural PAs.

Table 1
Cancers associated with induction of SMOX activity during chronic infection and/or inflammation

Pathogen	SMOX-associated inflammatory condition	Precursor lesion	Associated carcinoma	Refs.
H. pylori	Gastritis/peptic ulcer	Intestinal metaplasia	Gastric	84
Enterotoxigenic *B. fragilis*	Colitis, inflammatory bowel disease	Left-sided tubular adenomas and low-grade dysplasias	Colorectal	80, 92
Undetermined	Prostatitis	Prostatic intraepithelial neoplasia	Prostate	81
Hepatitis C virus	Chronic hepatitis	Undetermined	Hepatocellular	79, 93

Physiological effects of polyamine-associated oxidative stress

Conditions that cause the release of free PAs, such as changes in the macromolecules to which PAs are bound, can stimulate PA catabolism through oxidation, resulting in the generation of ROS while lowering the abundance of free PAs available to serve in an antioxidant capacity (15). Consequently, elevated levels of free PAs are associated with a number of pathologies, including cancer, neurological disorders, stroke, and kidney dysfunction.

Infection and chronic inflammation-induced spermine oxidation

SMOX is induced by a variety of stimuli, including the inflammatory cytokines tumor necrosis factor-α, interleukin-1β, and interleukin-6 (78). As chronic inflammation contributes to the carcinogenic process through the generation of ROS, evidence from multiple models suggests that increased Spm oxidation serves as a molecular mechanism linking inflammatory stimuli to cancer initiation and/or progression through increased H_2O_2 generation and reduced Spm levels (Table 1) (79–82). The accumulation of genetic and epigenetic changes is a hallmark of cancer, and unrepaired DNA damage resulting from ROS exposure can cause mutations in driver genes that contribute to carcinogenesis. H_2O_2-induced DNA damage that occurs during chronic inflammation also contributes to epigenetic changes involving DNA methylation and histone modification patterns, which reduce or silence the expression of tumor suppressor genes (83). Thus, sub-lethal, chronically elevated SMOX increases the likelihood of mutagenic and epigenetic changes associated with cancer.

Helicobacter pylori infection—H. pylori colonization of the stomach mucosa often persists for decades and causes chronic inflammation in the form of gastritis and peptic ulcers. In infected gastric epithelial cells, SMOX induction causes a chronic, low level of oxidative stress that has been directly linked to H_2O_2-dependent DNA damage without the induction of apoptosis or cell death (82, 84). SMOX expression is increased in gastric tissues from all stages of gastritis through carcinoma, relative to normal gastric mucosa, but is most highly expressed in the high-grade precursor lesion intestinal metaplasia (84). In *H. pylori*-positive gastritis patients living in geographically isolated high-risk *versus* low-risk regions of Colombia, SMOX was identified as the key factor influencing the progression to gastric cancer in high-risk regions (85). Further studies of these populations revealed differential expression of microRNA-124 that targets the 3′-UTR of SMOX and limits its translation. Analysis of DNA from the gastric mucosae of the Colombian patients revealed significantly higher levels of *miR-124* gene methylation in those patients considered at high risk for progression to gastric cancer, consistent with the low expression levels of the mature miR-124 and unregulated production of SMOX activity in response to *H. pylori* infection (86). This uncontrolled production of ROS from SMOX combined with decreased levels of Spm for protection increases the likelihood of additional genetic and epigenetic changes in a potential feed-forward loop.

Enterotoxigenic Bacteroides fragilis infection—The induction of SMOX has also been observed following infection with Enterotoxigenic *B. fragilis* (ETBF), a colitis-inducing bacterium that is positively correlated with the development of colorectal cancer (CRC). Detection of the secreted virulence factor of ETBF, *B. fragilis* toxin, is an early marker for colon carcinogenesis that has been positively associated with early colonic neoplasms, particularly tubular adenomas and low-grade dysplasias biopsied from the left side of the colon (87). ETBF has been referred to as an "α-bug" or "driver" bacteria in CRC. While the toxin itself induces DNA damage, the host responds to infection with the production of ROS, cytokines, and chemokines, thereby producing an environment with an altered mucosal immune response and bacterial community that further potentiates oncogenesis (88–90). Using the multiple intestinal neoplasia mouse model of ETBF-induced colitis, a role for SMOX was identified in the accumulation of ROS-mediated DNA damage and subsequent development of colon carcinogenesis (80). Pharmacological inhibition of PA oxidation in ETBF-infected mice decreased intestinal inflammation, aberrant proliferation, and tumor number. In this same model, ROS resulting from ETBF infection caused recruitment of DNA-modifying enzymes to regions of DNA damage, resulting in epigenetic changes associated with aberrant tumor suppressor gene silencing (83).

More recently, levels of ETBF in paired biopsies of human primary CRCs and adjacent normal tissues were correlated with expression levels of PA metabolism genes. Both c-MYC and SMOX expression levels were increased in 80% of CRC tissues, with the greatest expression of SMOX observed in stage I and II cancers. Although the majority of patients were colonized with ETBF, the level of colonization was generally low, with the highest levels in earlier disease stages (92). These results are in line with those suggesting important roles for SMOX and ETBF in early stages of neoplasia and indicate that SMOX may remain elevated in the absence of ETBF.

Other inflammation-associated cancers—Elevated SMOX expression has also been documented in precancerous inflammatory conditions in the absence of infection. A tissue microarray of human prostate biopsies revealed the highest SMOX immunostaining in precursor prostatic intraepithelial neoplasia (PIN) lesions. This study also concluded that men who have developed PIN or prostate cancer have higher SMOX expres-

sion levels in benign prostate epithelium than men who have not had these lesions, suggesting increased SMOX as a risk factor for prostate carcinogenesis (81). Similarly, a recent study demonstrated increased SMOX expression in hepatic tissue from patients with chronic hepatitis. SMOX staining was further increased in hepatocellular carcinoma tissues and was positively correlated with poorer overall survival and relapse-free survival (93). Hepatitis is typically associated with hepatitis B or C virus infection and fibrosis, and recent *in vitro* studies have indicated that hepatitis C virus induces SMOX activity in hepatoma cells (79), suggesting a role for SMOX in hepatocellular carcinogenesis.

Immunomodulation through polyamine oxidation

In vitro studies have suggested that one way *H. pylori* infection alters the immune response is by highly inducing SMOX in macrophages, leading to their dysfunction and death and creating a permissive environment that allows for chronic infection (94). Recently, the effect of SMOX activity on immunomodulation was studied in mouse models of colitis due to pathogenic infection or epithelial injury-associated inflammation. SMOX is up-regulated in patients with ulcerative colitis and inflammatory bowel disease due to its high expression in infiltrating mononuclear cells, rather than colonic epithelial cells (95). In WT mice, infection with *Citrobacter rodentium* increased cytokine and chemokine levels and inflammatory cell infiltration in association with histological injury and mucosal hyperplasia, and these changes were diminished in SMOX knockout mice. Conversely, when colon inflammation resulted from administration of dextran sulfate sodium (DSS), SMOX knockout mice displayed increased histological damage and cytokine expression beyond that of DSS-treated WT mice, with more frequent colitis-associated mortality. These data suggest that in the context of infection, the regulation of intracellular polyamine levels by SMOX serves an immunomodulatory function, while in the setting of colitis associated with epithelial injury, SMOX-generated Spd may serve a protective role (96).

Ischemia/reperfusion injury

Ischemia reperfusion injury (IRI), physical trauma, and toxins induce PA catabolism through SSAT and SMOX in multiple organs, leading to tissue damage (73, 97–101). SSAT, in particular, plays a significant role in promoting kidney and liver damage in IRI (102), and conditional knockout of SSAT in proximal tubule epithelial cells, where the primary effects of IRI manifest, decreases renal damage severity via reductions in both PAOX and SMOX activities. Increasing SSAT expression in kidney cells caused increased mitochondrial damage, stimulating apoptosis and suggesting PA oxidation as a source of oxidative stress (103). Knockout of SSAT in mice or pharmacological inhibition of PA oxidation during IRI demonstrated that PA catabolism contributes to activation of the innate immune response, increasing inflammation and apoptosis at the site of injury. It therefore appears that PA catabolism functions in the initial injury as well as furthering damage via immunomodulation (103).

Snyder-Robinson syndrome

Snyder-Robinson syndrome (SRS), an X-linked mental retardation syndrome resulting from loss-of-function mutations in the Spm synthase gene, biochemically results in accumulation of high intracellular Spd levels and a near-complete lack of Spm (104). A recently developed *Drosophila* model of SRS suggests a role for increased Spd catabolism and the toxic metabolites it produces in establishing oxidative stress and lysosomal defects in the *Drosophila* nervous system, resulting in altered mitochondrial function and impaired autophagy that are also observed in affected SRS patient cells. ROS were highly elevated in the brains of mutant flies, and antioxidant therapies partially restored mitochondrial function, suggesting that increasing antioxidant capacity may be beneficial for SRS patients (105).

Therapeutic opportunities

Inhibition of polyamine oxidation

SMOX shares significant homology with PAOX as well as the histone-modifying enzyme lysine-specific demethylase-1 (106). As the crystallization of SMOX has eluded many attempts, the development of a specific inhibitor for SMOX alone has been challenging. However, studies with existing inhibitors and genetic manipulation of SMOX indicate it is an attractive therapeutic target for multiple pathologies. Most notably, the apparent role for SMOX induction in the development of epithelial cancers suggests the inhibition of SMOX as a potential target for prevention in individuals at risk for carcinogenesis, particularly in association with infection and chronic inflammation.

Of relevance to chemotherapy, a limitation of the common chemotherapeutic agent cisplatin is acute kidney injury (AKI). In mice with cisplatin-induced AKI, SSAT and SMOX levels increase, stimulating endoplasmic reticulum stress response genes that culminate in apoptosis and kidney damage (107). Knockout of SSAT or SMOX or neutralization of the by-products of PA oxidation reduces the severity of damage, implicating a role for PA catabolism in AKI. Thus, incorporation of an inhibitor of PA oxidation into a cisplatin treatment regimen could prevent kidney injury that might otherwise limit treatment. Furthermore, as organ damage due to insults such as ischemia/reperfusion, toxins, and trauma appears to have a PA catabolic regulatory component, suppression of these components is a promising strategy for the protection of tissues in a variety of contexts.

During stroke, PA catabolism is induced due to the release of free PAs from damaged RNA (73, 108), and the extent of this induction was recently correlated with aging (109). Acrolein, spontaneously formed from SMOX-generated 3-AP, is the metabolite most responsible for neuronal damage (110). Masuko *et al.* (111) designed inhibitors of the PA oxidases and investigated their effects on brain infarct sizes in a photochemically-induced thrombosis model. Inhibitor C9-4 (N^1-nonyl-1,4-diaminobutane) most potently decreased brain infarct volume with a therapeutic window longer than 12 h and is thus a potential drug candidate for the treatment of brain ischemia.

Therapeutic induction of polyamine oxidation

In certain circumstances, the production of potentially harmful oxidative stress may have therapeutic potential. A major intracellular source of ROS is metabolic activity, and rapidly proliferating cells require a compensatory increase in metabolism. Therefore, populations such as cancer cells or pathogenic microorganisms may have increased sensitivity to treatments that produce additional oxidants.

BSAO as a mediator of polyamines and oxidative stress—The ability of BSAO to convert PAs into toxic aldehydes and H_2O_2, thus producing oxidative stress, has suggested its potential use in a therapeutic setting due to the abundance of PAs in proliferating cells, including tumor cells. Immobilized BSAO injected directly into B16 melanoma tumor xenografts in mice decreased tumor growth by ~70% through the induction of apoptosis (112). Experiments in multidrug-resistant tumor cell lines have indicated their increased sensitivity to the oxidation products of BSAO and Spm (113–116). Recent advances in drug delivery technology have led to the incorporation of BSAO into nanoparticle formulations, thereby enhancing the stability of its catalytic activity and improving its potential as an *in situ* treatment strategy to reduce intratumoral PA levels while generating tumor-toxic oxidative stress (117–119).

Induction of SMOX by polyamine analogues—The use of PA analogues to exploit the self-regulatory nature of PA metabolism is a well-studied strategy for inhibiting growth of cancer cells. Members of the bis(ethyl) group of analogues strongly induce PA catabolism through large inductions of both SSAT and SMOX. In certain cancer cell types, cytotoxicity from these analogues is attributed to the production of H_2O_2 via SMOX simultaneously with depletion of the natural PAs (50, 121, 122). Curcumin, a natural polyphenol and popular dietary supplement, was recently shown to increase uptake of these PA analogues, resulting in enhanced polyamine depletion and growth inhibition and allowing a substantial reduction in the required effective analogue dose (123). As curcumin possesses multiple antitumor properties, this combination with a PA analogue would target multiple anticancer pathways (124). Finally, two PA analogues that have been studied clinically as single agents, bis(ethyl)norspermine and PG-11047 (45), have been incorporated into self-immolative nanoparticles capable of packaging and delivering therapeutic nucleic acids in addition to the PA analogue (91, 125). These prodrugs may allow for controlled drug release as well as the ability to simultaneously target additional antitumor pathways via specific cargo. These parent compounds and their prodrug derivatives were recently also shown to have an antiviral effect on Zika virus replication through the induction of SSAT and SMOX (120).

Conclusion

The multiple mechanisms through which PAs and their catabolism can affect oxidative homeostasis in organisms, including humans, allow for a wide array of possible outcomes in both normal and pathological states. Although a prime function of the PA catabolic pathways may be to maintain PA homeostasis at a set point, the potential to exploit the pathway for therapeutic benefits is great. This is especially true for the oxidation of Spm by SMOX in response to infection and inflammation. All data currently point to this enzyme as a rational target for chemoprevention strategies. Additionally, the targeted, tumor-specific super-induction of PA catabolism by specific PA analogues continues to hold promise for future anticancer therapies. Finally, in addition to PA catabolism being a homeostatic mechanism, there are other possibilities that should be explored. For instance, PA catabolism might provide important signaling molecules, like H_2O_2, at levels that are not injurious. Similarly, the recent linkage between PA catabolism and immune modulation opens new avenues for investigation and possible treatments. Although the catabolism of PAs has long been studied, with the Tabor laboratory leading the way in many aspects, many avenues remain to be explored.

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