Inflammation and polyamine catabolism: the good, the bad and the ugly

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Abstract
The induction of polyamine catabolism by specific anti-tumour polyamine analogues has increased interest in the roles polyamine catabolism play in cell growth, death and response to various anti-tumour agents. The relatively recent finding of an inducible mammalian spermine oxidase (SMO/PAOh1), in addition to the two-step spermidine/spermine N\textsuperscript{1}-acytvltransferase (SSAT)/N\textsuperscript{1}-acethylpolyamine oxidase (APAO) catabolic pathway, underscores the complexities of the regulation of polyamine catabolism by various stimuli. Furthermore, recent data indicate that infectious agents and mediators of inflammation can also up-regulate polyamine catabolism. Induction of SSAT by these agents can reduce intracellular polyamine concentrations and cell growth rate, thus providing a beneficial mechanism by which cells may adapt to inflammatory stress. However, increased polyamine catabolism can also result in substantial increases in intracellular reactive oxygen species (ROS) through the production of H\textsubscript{2}O\textsubscript{2} as a by-product of either APAO or SMO/PAOh1 activity. This increased generation of ROS can have different results, depending on the mechanism of induction and cell types involved. Targeted killing of tumour cells by agents that stimulate SSAT/APAO and/or SMO/PAOh1 is obviously a ‘good’ effect. However, induction of SMO/PAOh1 by inflammation or infectious agents has the potential to produce sufficient ROS in normal, non-tumour cells to lead to DNA damage, mutation and, potentially, carcinogenic transformation (‘bad’). The variation in the induction of these polyamine catabolic enzymes, as well as the level and timing of this induction will dictate the cellular outcome in the presence of both desirable and undesirable effects (‘ugly’). Here we discuss the relative role of each of the steps in polyamine catabolism in response to inflammatory stress.

Inflammation
Inflammation is a physiologic response to tissue damage resulting from microbial pathogen infection, chemical irritation and/or wounding. In the process of acute inflammation, various types of leukocytes, lymphocytes and other inflammatory cells are activated and attracted to the inflamed site by a signalling network involving a great number of growth factors, cytokines and chemokines, which contribute to tissue breakdown, but also strengthen and maintain the defence against infection [1,2]. Acute inflammation is usually self-limiting because the production of pro-inflammatory cytokines gives way to the anti-inflammatory cytokines as healing progresses [3,4]. However, if inflammation resolution is dysregulated, the cellular response changes to a pattern of chronic inflammation, in which the inflammatory foci are dominated by macrophages and other inflammatory cells that generate even greater amounts of growth factors and cytokines, as well as reactive oxygen and nitrogen species that may cause DNA damage. Persistent activation of macrophages can lead to continuous tissue damage [5] in a microenvironment that sustains proliferation of damaged cells, thus predisposing areas of chronic inflammation to neoplasia [6].

Inflammation, cancer and polyamines
Cancer development is a multi-step process, during which genetic alterations confer specific growth advantages, thereby driving the progressive transformation from normal cells to malignant cancer cells. Malignant growth is characterized by several key changes: self-sufficiency of growth signals, insensitivity to anti-growth signals, escape from apoptosis, unregulated proliferation potential, enhanced angiogenesis and metastasis [7]. Each of these shifts is complicated and accomplished by the combined efforts of various signalling processes.

The association between inflammation and cancer has been illustrated by numerous epidemiologic and clinical studies [1,6]. Causes of inflammation that have been linked to cancer include bacterial and viral infection (Helicobacter pylori for gastric adenocarcinoma [1,5], hepatitis virus B and C for hepatocellular carcinoma [8] and human papillomavirus for penile cancers) or non-infective physical and/or chemical irritants. The risk of developing oesophageal, pancreatic and gall bladder cancers may be increased by certain inflammatory diseases, such as oesophagitis, Barrett’s metaplasia and chronic pancreatitis [5,9]. Possible associations have also been...
described between Marjolin’s ulcer and skin carcinoma [5], asbestos and mesothelioma [5], silica, cigarette smoke, and bronchial cancer [5], chronic asthma and lung cancer [10] and pelvic inflammatory disease or ovarian epithelial inflammation and ovarian cancer [5, 11].

Polyamines are essential for cell growth and proliferation, and have also been shown to play an important role in inflammation-induced carcinogenesis. Cells have developed complex regulatory machinery to finely control intracellular polyamine pools, as dysregulation of polyamine metabolism can have serious effects on cell growth. Increased polyamine synthesis has been detected as a product of inflammation, and elevated intracellular polyamine pools are frequently observed in actively proliferating cells, including tumour cells. In one study, parasitic infection of the small intestine led to increased mucosal hyperplasia with elevated polyamine biosynthesis [12]. Additionally, H. pylori infection has been demonstrated to increase expression and activity of polyamine biosynthetic enzymes [13]. Meanwhile, intracellular levels of the polyamines themselves are capable of affecting inflammation. The polyamine spermine has been shown to inhibit pro-inflammatory cytokine synthesis in human mononuclear cells [14], as well as nitric oxide-mediated intestinal damage [15]. Furthermore, increased polyamine catabolism leading to decreased intracellular polyamine pools can lead to acute pancreatitis [16].

**Polyamine catabolism**

In mammalian cells, intracellular polyamine catabolism occurs through two distinct pathways and uses a total of three enzymes: spermidine/spermine N\(^1\)-acetyltransferase (SSAT), N\(^1\)-acetylpolyamine oxidase (APAO) and spermine oxidase (SMO/PAOh1). (NB: as the eukaryotic polyamine catabolic pathway has become more complicated with the discovery of spermine oxidase, in combination with the better recognition of the individual oxidases’ substrate specificities, it is strongly recommended that the common abbreviation for the classical FAD-dependent, N\(^1\)-acetylpolyamine oxidase involved in the back conversion of polyamines be henceforth APAO. The use of this abbreviation will more properly indicate its preferred substrate and will serve to differentiate it from the plant polyamine oxidases, the serum amine oxidases and the spermine oxidase.) SSAT acetylates both spermine and spermidine, thus providing acetylated polyamines for cellular excretion or as substrates for further back-conversion to spermidine or putrescine by APAO. Alternatively, spermine can be directly oxidized back to spermidine by SMO/PAOh1. Importantly, the oxidation reactions of both APAO and SMO/PAOh1 result in the generation of potentially toxic hydrogen peroxide (H\(_2\)O\(_2\)). This H\(_2\)O\(_2\), a reactive oxygen species (ROS), has been shown to play an essential role in the DNA damage-associated changes observed following elevated levels of polyamine catabolism. Furthermore, the depletion of intracellular polyamine pools that typically accompany induction of polyamine catabolism can itself be growth inhibitory.

**Role of SSAT (the ‘good’)**

SSAT is the rate limiting step in the SSAT/APAO polyamine catabolic pathway, and is thus one of the enzymes primarily responsible for regulation of intracellular polyamine concentrations in mammalian cells [17]. Although basal levels are generally undetectable, SSAT expression can be rapidly and highly induced by a variety of stimuli, including toxins, cellular stresses and various polyamine analogues [17–21]. The ability of these analogues to super-induce SSAT expression in a tumour-specific manner [22] has been associated with cell death [20, 23], thus affording SSAT regulation considerable attention as a target for cancer chemotherapy.

Recently, additional stimuli have been identified that result in the increased expression of SSAT at the mRNA and protein levels, including ischemia/reperfusion [24], hypoxia [25] and exposure to non-steroidal anti-inflammatory drugs (NSAIDs) [26, 27]. 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 decreases in intracellular polyamines and cell growth in a cyclo-oxygenase-independent manner [28, 29]. In select systems, this NSAID-dependent growth inhibition can be reversed by addition of polyamines, indicating involvement of the polyamine pathway [30]. Thus, one way in which the NSAIDs may act to reduce tumour growth rate is by decreasing intracellular polyamines below the optimal intracellular concentrations necessary for tumour growth and development.

We have recently shown that SSAT is one of the genes regulated by tumour necrosis factor-alpha (TNF\(\alpha\)), a pro-inflammatory cytokine produced in response to inflammation [31]. TNF\(\alpha\) is a major mediator of inflammation, with actions directed towards both tissue destruction and recovery from damage. Several receptors of the TNF family are also ‘death receptors’ that induce or modulate apoptosis in response to a variety of ligands [32]. TNF\(\alpha\) is capable of activating multiple signal transduction pathways, including that of nuclear factor \(\kappa\)B (NF-\(\kappa\)B), which plays critical roles in cell proliferation and survival [33, 34], and is pivotal for many inflammatory responses [35, 36]. Stimulation of the cell with TNF\(\alpha\) triggers a series of signalling events that ultimately lead to the phosphorylation and proteolytic degradation of \(\kappa\)B (inhibitory \(\kappa\)B), followed by activation and translocation of NF-\(\kappa\)B to the nucleus, where it regulates the expression of multiple genes. Under certain circumstances, the up-regulation of NF-\(\kappa\)B activity is responsible for the anti-apoptotic effects of TNF\(\alpha\) [37, 38]. Accordingly, inhibition of TNF\(\alpha\)-mediated NF-\(\kappa\)B activity shifts the balance of TNF\(\alpha\) stimulation towards cell death [39, 40], and in vitro studies have shown that TNF\(\alpha\) exerts anti-proliferative effects on various NSCLCs (non-small cell lung cancers) [41, 42].

In our studies, treatment of two NSCLC cell lines with TNF\(\alpha\) led to a rapid increase in SSAT expression, with a decrease in intracellular polyamine contents. A dominant negative \(\kappa\)B protein abrogated this induction, suggesting that activation of NF-\(\kappa\)B is required for the induction of SSAT.
Figure 1 | Role of polyamines in response of cells to inflammation

Changes in polyamine (PA) catabolism in response to injury, inflammation or mediators of inflammation can have a wide range of effects whose eventual outcome is dependent on the stimuli, the specific cell type and the cellular environment. This range of responses presents several attractive targets for therapeutic intervention. 3-AAP, 3-acetoaminopropanal; 3-AP, 3-aminopropanal; AcPA, N1-acetylpolyamine; 5-FU, 5-fluorouracil.

transcription by TNFα in these cells. Additionally, NF-κB response elements in the SSAT promoter were identified and characterized for their role as regulatory components responsible for this induction of SSAT expression [28]. These data suggest that the induction of SSAT by TNFα, the corresponding decreases in polyamine concentrations and the resulting slower growth rate might provide a mechanism by which cells can adapt to inflammatory stress.

Of additional significance for the induction of SSAT by TNFα is that increased SSAT activity may result in increased concentrations of acetylated spermidine and spermine, and increased polyamine oxidation via APAO. This oxidation generates H2O2, thereby leading to DNA damage and ultimate cell death, and suggesting another mechanism by which TNFα can lead to apoptosis. Further, polyamines themselves can modulate NF-κB binding in various cells. Although there has been contrasting evidence about the role of polyamines in regulating NF-κB activation and its DNA binding ability [43–46], our results suggest that a stress stimulus, like TNFα, leads to a rapid induction of SSAT expression via NF-κB activation, causing a reduction in polyamine content. It is possible that this rapid polyamine depletion inhibits NF-κB DNA binding activity, thereby reducing the induced levels of SSAT expression and suggesting a feedback mechanism in the regulation of SSAT expression.

These observations, coupled with the evidence that SSAT can be induced by various anti-inflammatory drugs [28,29], suggest a beneficiary role for SSAT induction in the maintenance of normal cellular physiology under inflammatory stress. When there is inflammation, there is recruitment of various immune cells that lead to the production of various pro-inflammatory cytokines, like TNFα. One of the ways in which this localized, high production of TNFα mediates its anti-cancer actions [47] could be through the induction of SSAT, leading to lower levels of polyamines and, potentially, increased generation of H2O2, both of which can lead to slower cell growth that allows for cellular repair and/or cell death thus preventing damaged cells from dividing (Figure 1).

Roles of polyamine oxidation (the bad and the ugly)

There are at least two oxidases involved in mammalian intracellular polyamine catabolism. Each produces H2O2 as a by-product, but differs in expression, substrate specificity and cellular localization. APAO, which is localized to the peroxisomes [48], is an FAD-dependent oxidase, which is generally constitutively expressed, and whose activity is dependent upon the availability of its substrates, the N1-acetylated polyamines. APAO is also involved in metabolizing...
specific anti-tumour polyamine analogues [48–50], thereby having a profound effect on the response of tumours to these agents.

Our previous studies using siRNA (small interfering RNA) to target the polyamine catabolic enzymes in a breast tumour model suggest that the source of cytotoxic H₂O₂ in analogue-induced polyamine catabolism is not from the SSAT/PAO two-step pathway, but rather is entirely from the recently discovered spermine oxidase (SMO/PAOh1) pathway [51–53]. Also an FAD-dependent enzyme, SMO/PAOh1 was first cloned by our laboratory based on its homology to the maize polyamine oxidase and catalyses the oxidation of spermine to spermidine. Unlike APAO, SMO/PAOh1 is typically present in the cytosol at low levels, but its activity, and subsequent production of H₂O₂, is readily induced by treatment with specific polyamine analogues, many of which are also known to induce SSAT [54].

ROS have been implicated in a variety of different pathologies [55], as their presence in the cell can oxidatively damage DNA, proteins and lipids. Elevated polyamine oxidation produces H₂O₂ that, in the presence of transition metals, generates a highly toxic ROS, the hydroxyl radical (•OH), which most commonly oxidizes DNA to produce G → T mutations that are widely found in mutated oncogenes and tumour suppressor genes [56,57]. Further, oxidative damage can lead to single- or double-strand breaks, point and frameshift mutations, chromosomal abnormalities, modified amino acid residues and inhibition of DNA repair and anti-oxidant enzymes, thereby hampering the cellular response to oxidative damage and ROS production. Additionally, ROS can act as signalling molecules and have been shown to activate various MAPKs (mitogen-activated protein kinases), NF-κB and AP-1 (activator protein 1) transcription factors [58].

The production of H₂O₂ via elevated polyamine oxidation has been implicated in the cytotoxic response of specific human tumour cell types to a variety of anti-tumour polyamine analogues, thus providing a promising target for anti-neoplastic intervention [52]. However, in normal, non-cancerous cells, up-regulation of polyamine oxidation and H₂O₂ generation can have undesirable effects. We have shown that H₂O₂ produced by macrophages in response to H. pylori (a pathogen which affects approximately 50% of the world population and is associated with peptic ulcers and gastric cancer) infection is due to the induction of macrophage SMO/PAOh1 by this pathogen [59]. 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. This possible link is supported by the observation that gastric epithelial cells exposed to H. pylori demonstrate a rapid and significant increase in SMO/PAOh1 mRNA and protein [60]. The resulting H₂O₂ was sufficient to produce DNA damage and apoptosis, as these effects 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 [60]. 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 lead to mutations necessary for the development of H. pylori-induced gastric cancer (Figure 1).

Very recent data from our laboratory [61] demonstrate that TNFα can lead to a significant increase in the production of ROS, with a concomitant increase in oxidative DNA damage, in Beas-2B immortalized non-tumorigenic bronchial epithelial cells. The source of ROS in these cells is H₂O₂ that is specifically produced from the induction of SMO/PAOh1 enzyme activity. These results suggest a general mechanism by which chronic inflammation is mechanistically linked to the DNA damage and mutations necessary for carcinogenesis through H₂O₂ produced by SMO/PAOh1. Importantly, these data also implicate SMO/PAOh1 as an important target for chemopreventive strategies.

Conclusion

Several studies have demonstrated that polyamine catabolism plays an important role in the cellular response to specific anti-tumour polyamine analogues. The studies presented here suggest additional important effects of polyamine catabolism in response to stress and inflammation. Inflammation is a physiological response to injury or infection, which can have opposing effects with respect to tumorigenesis. Acute inflammation usually counteracts cancer development, while chronic inflammation promotes cancer development. Our recent data show that TNFα, a pro-inflammatory cytokine produced during inflammation, can lead to induction of the polyamine catabolic pathways, which can reduce growth rate or lead to the death of cells prior to transformation (the ‘good’), or provide mutagenic ROS that has the potential to contribute to the transformed phenotype (the ‘bad’). These studies suggest that during acute inflammation, TNFα can lead to the induction of SSAT, which can then lead to cell growth inhibition and/or cell death by decreasing polyamine levels, increasing H₂O₂ through APAO and/or inhibiting NF-κB activity. These actions can help in limiting the carcinogenic effects of the inflammation process. By contrast, H. pylori and possibly other infectious agents have the potential to increase SMO/PAOh1, resulting in the production of ROS and DNA damage, which can give rise to a transformed phenotype. Our data, demonstrating that TNFα itself can also lead to an induction in ROS production and accompanying DNA damage by inducing SMO/PAOh1, support the linkage of spermine oxidation and inflammation-induced cancer. Consequently, the induction of polyamine catabolism can have desirable and undesirable effects on cells. Which catabolic pathways are induced, and to what extent, may be dependent on the stimuli, the cell type, cellular environment and the duration of the stimuli. The complex nature of the regulation of polyamine catabolism and its
association with disease response and aetiology underscores the importance of continued study in this challenging area.

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References