HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2023 August 01.

Published in final edited form as:

Nat Rev Cancer. 2022 August ; 22(8): 467–480. doi:10.1038/s41568-022-00473-2.

Polyamines in cancer: integrating organismal metabolism and antitumour immunity

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Abstract

The natural mammalian polyamines putrescine, spermidine and spermine are essential for both normal and neoplastic cell function and replication. Dysregulation of metabolism of polyamines and their requirements is common in many cancers. Both clinical and experimental depletion of polyamines have demonstrated their metabolism to be a rational target for therapy; however, the mechanisms through which polyamines can establish a tumour-permissive microenvironment are only now emerging. Recent data indicate that polyamines can play a major role in regulating the antitumour immune response, thus likely contributing to the existence of immunologically ‘cold’ tumours that do not respond to immune checkpoint blockade. Additionally, the interplay between the microbiota and associated tissues creates a tumour microenvironment in which polyamine metabolism, content and function can all be dramatically altered on the basis of microbiota composition, dietary polyamine availability and tissue response to its surrounding microenvironment. The goal of this Perspective is to introduce the reader to the many ways in which polyamines, polyamine metabolism, the microbiota and the diet interconnect to establish a tumour microenvironment that facilitates the initiation and progression of cancer. It also details ways in which polyamine metabolism and function can be successfully targeted for therapeutic benefit, including specifically enhancing the antitumour immune response.

The naturally occurring polyamines putrescine, spermidine and spermine are small polycationic alkylamines that exist at millimolar concentrations inside eukaryotic cells. These compounds have protonated amino groups at physiological pH levels (structures provided in Supplementary Table 1), allowing them to interact with negatively charged macromolecules[1], thereby involving them in a variety of cellular processes, including

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The authors contributed equally to all aspects of the article.

Competing interests

The Casero and Stewart laboratory and Johns Hopkins University receive research funding from Panbela Therapeutics Inc., of which M.T.C. is an employee.

Peer review information

Nature Reviews Cancer thanks Susan Gilmour, Chaim Kahana and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41568-022-00473-2.

chromatin organization, gene regulation, cellular proliferation and differentiation, cell death and immune system function[2–6].

Through coordinated biosynthesis, catabolism and transport, polyamine homeostasis is a tightly regulated process (FIG. 1a). The biosynthetic and catabolic enzymes have been well characterized biochemically and structurally, with the exception of difficulties in obtaining a crystal structure of spermine oxidase (SMOX)[7]. The polyamine transport system in prokaryotes, yeast and trypanosomatids is also well defined: however, the molecular players in metazoan polyamine transport have proven more elusive[8–11]. Models have been derived from biochemical studies of the mammalian polyamine transporter, which is energy dependent and saturable, with high affinity for its substrates[9] (BOX 1). Evidence indicates a fundamental role for an active transporter that is dependent on membrane potential[9,12]. None of the current models for mammalian transport encompasses all of the available biochemical data[12–15], indicating the existence of multiple mechanisms that may be context dependent. Recent discoveries have identified important components of and roles for the transport of polyamines into and out of various vesicles within the intracellular transport system that contribute to the regulation of extracellular polyamine uptake, overall polyamine content and organellar health[16–18]. Conversely, certain cell types transport polyamines into secretory vesicles, thereby contributing to the availability of polyamines in the microenvironment[12]. Details of the most recently described transport proteins are provided in BOX 1 along with suggestions for their incorporation into the current transport models.

Cancer cells require sustained, elevated intracellular polyamine pools to maintain continual proliferation[19]. These elevated levels are maintained through a combination of increased biosynthesis, increased transport and decreased catabolism, with numerous oncogenes, including *MYC, JUN, FOS, KRAS* and *BRAF,* contributing to this maintenance[20–25] (FIG. 1a). Most notably, the genes encoding the two rate-limiting enzymes of polyamine biosynthesis, ornithine decarboxylase (ODC; encoded by *ODC1*) and S-adenosylmethionine decarboxylase (AMD1), are both direct transcriptional targets of MYC[20,26]. With few exceptions, cancers of nearly every type have demonstrated marked increases in *MYC* expression, through either gene duplication or gene mutation, that is positively associated with increased polyamine biosynthesis through ODC[19,26–28]. Details of oncogenic signalling and its crosstalk with polyamine metabolism have been covered elsewhere[29].

Due to its direct link with oncogenes, polyamine metabolism has long been a target for potential cancer therapeutic agents. While the effects of modulating polyamine homeostasis in tumour cells have been well studied, less is known regarding the effects of polyamine-modulating agents on non-tumour cells that constitute the tumour microenvironment (TME), including the functioning of immune cells and cancer-associated immunity. However, evidence that polyamines have anti-inflammatory, immunosuppressive properties supports the use of strategies reducing the levels of polyamines to improve the antitumour immune response[19]. Recent advances in immunotherapy have brought to light the importance of studying cancer in the context of its true macroenvironments and microenvironments, and the proliferation of untargeted metabolomics studies has provided data on polyamine levels and metabolism under a wide range of conditions. This Perspective aims to consolidate current knowledge of the effects of the dysregulation of polyamine metabolism on the

recruitment and function of various cell types in the TME, with particular emphasis on immune cells. Changes in polyamine homeostasis in response to microenvironmental factors, including hypoxia, the microbiota and dietary polyamines, are also discussed. Finally, we examine the potential for polyamine-blocking therapies (PBTs) in targeting dysregulated polyamine metabolism in tumour cells directly as well as in reducing the immunosuppressive TME.

Extracellular sources of polyamines

In addition to their high concentrations within cells, polyamines are abundant components of the extracellular environment, particularly in the gastrointestinal tract, where polyamines present in its lumen are derived from the diet, the microbiota, gastrointestinal secretions and the shedding of epithelial cells. Luminal polyamines are present in millimolar concentrations[30] and have been shown to be utilized by cells throughout the body in support of growth, leaving micromolar polyamine concentrations in systemic circulation[31,32] (FIG. 1b).

Dietary polyamines and their effects.

Plantand animal-derived foods are significant sources of polyamines. Spermidine and putrescine are generally most abundant in plant-based foods and cheeses, while levels of spermine tend to be highest in fresh meat, including red meat, pork and poultry meat[33]. Spermidine and spermine occur naturally in foods, while high putrescine content in food may also result from microbial fermentation processes or contamination[33]. Dietary polyamine intake differs among countries, generally ranging between 140 and 390 μmol per day, with putrescine accounting for the greatest proportion[33]. Long-term intake of polyamine-rich foods has been shown to increase blood polyamine concentrations in healthy human volunteers and mice[34]. As intracellular polyamine biosynthesis decreases with age[35], reliance on extracellular polyamines increases, and dietary supplementation with spermidine and spermine has been shown to increase longevity and reduce age-related diseases in a variety of model systems[35–39]. Dietary spermidine, in particular, is believed to have multiple lifespan-extending properties, including effects on autophagy, senescence and inflammation, although the precise mechanisms responsible for these effects are not well understood[40–43]. Details of these studies were reviewed recently[38].

Dietary polyamines are fully absorbed in the proximal portion of the small intestine and have been detected unmodified in the inferior vena cava and portal vein[31,44]. Polyamines originating from the alimentary tract can be accumulated by enterocytes as well as other proliferating cells throughout the body that may be incapable of sufficient polyamine biosynthesis, including aging, tumour and immune cells[31,45] (FIG. 1b). Therefore, dietary polyamines can interfere with antitumour strategies involving inhibitors of polyamine biosynthesis, particularly those targeting cells of the gastrointestinal epithelium, which may directly interact with dietary or microbiota-derived components[46]. Awareness of this compensatory uptake of polyamines has been essential in designing more efficacious treatment strategies, including the incorporation of low-polyamine diets[46,47] and combination strategies using polyamine transport inhibitors (PTIs). Supporting early

polyamine-limiting studies in mice[48], a phase 3 clinical trial demonstrated that a low-polyamine diet can complement polyamine-targeting therapies in preventing metachronous colorectal adenomas[46]. A recent study by Corral and Wallace examined the effects of α-difluoromethylornithine (DFMO), an inhibitor of ODC that is clinically approved for the treatment of African trypanosomiasis, on uptake of putrescine and spermidine in colorectal cancer cells[49]. Although basal affinity for spermidine was approximately ten times greater than that for putrescine, DFMO increased the affinity for putrescine but not for spermidine, while increasing V_{max} for both compounds. Polarity studies revealed uptake of both polyamines via both apical and basolateral surfaces, with faster uptake of spermidine from the apical side (FIG. 1b), emphasizing the potential importance of luminal polyamines in supporting the maintenance of polyamine homeostasis.

The interplay between the microbiota and polyamines.

The commensal bacteria and other microorganisms that colonize and interact with specific niches of the human body are collectively known as the human microbiota[50]. Included in this definition are the compounds and metabolites associated with the microbial population, including polyamines and their derivatives. The precise composition of the local microbiota and the interplay between the species involved can result in either beneficial or pathological effects, including cancer. Microbial dysbiosis, resulting from dietary or specific disease states that alter the fine balance established in the healthy gastrointestinal system, is often associated with inflammation and breakdown of the commensal bacterial barrier. This facilitates access of microbial molecules to the circulation, where they can promote tumour initiation and progression as well as response to certain treatments, including immunotherapies[51], at distant sites in addition to those with which they are in direct contact. Importantly, the microbiota can affect the TME at both the local level and the systemic level.

Polyamines are easily detected in the intestinal lumen even under fasting conditions, as most polyamines in the lower intestine are generated by the gastrointestinal microbiota[30–32,52]. Dietary supplementation with probiotics increased longevity in mice through increasing the production of polyamines by the microbiota, resulting in reduced expression of pro-inflammatory genes and improved intestinal barrier function[53]. In another study, spermine accumulation was found to be increased in the colonic lumen of mice with dysbiosis compared with healthy mice. This increase was associated with increased abundance of the commensal microbiota expressing high levels of polyamine biosynthetic and transport proteins. Similarly, providing spermine in the drinking water of wild type mice noticeably altered the intestinal microbiota profile as well as that of the microbiota attached to the colonic epithelium. These data support a role for microenvironment-derived spermine in maintaining host microbiota composition[54].

A recent study measuring faecal metabolites in healthy patients whose composition of the gut microbiota was considered ‘elderly-type’, as determined by principal coordinate analysis clustering, indicated increased levels of N^8-acetylated spermidine compared with the levels in patients of the same age whose microbiota composition was considered “adult-type”[55]. Increasing age is associated with low-level, chronic inflammation, raising the risk of many age-related diseases, including cancer, and changes in the gut microbiota

and intestinal permeability are contributing factors. Treatment of colon cancer cells with N^8-acetylspermidine concentrations correlating with those that may be encountered physiologically in the intestinal lumen induced the expression of EGF signalling genes and pro-inflammatory cytokines, stimulated proliferation and rescued oxaliplatin-induced cell death, suggesting that extracellular N^8-acetylspermidine in the TME might promote progression of colon cancer and alter treatment response[55].

The microbiota can also influence the TME through biofilm formation. Implicated in the aetiology and maintenance of inflammatory conditions in the colon, such communities of organisms profoundly affect the types of and locations in which tumours develop. Johnson et al. demonstrated that the presence of colonic biofilm in patients with cancer was associated with increased levels of N^1,N^{12}-diacetylspermine, a polyamine metabolite that may affect tumour growth and biofilm formation, within both normal and cancer tissues, compared with the absence of colonic biofilm. Importantly, although the presence of biofilms is predominately associated with right-sided colon tumours, left-sided, biofilm-positive colon tumours also demonstrated significantly higher levels of N^1,N^{12}-diacetylspermine than biofilm-negative tumours[56]. These studies suggested that microbial polyamine metabolism may contribute to this increase in N^1,N^{12}-diacetylspermine levels[51], indicating significant interplay between the microbiota, its associated epithelial tissue and the regulation of polyamine metabolism, resulting in a microenvironment that affects both the bacterial community and epithelial tissue. Subsequent immunohistochemistry studies demonstrated the localization of N^1,N^{12}-diacetylspermine within colon tumour cells, with only weak staining of surrounding non-transformed cells. However, as the authors of the study also demonstrated that tumour cells actively take up N^1,N^{12}-diacetylspermine from the TME, these results do not exclude a microbial contribution[57]. Biofilms from both healthy individuals and patients with cancer have shown carcinogenic potential[58], and those from patients with familial adenomatous polyposis, an inherited predisposition to colorectal cancer, contain elevated levels of secreted bacterial oncotoxins, compounds that promote tumorigenesis through direct interactions with colonic epithelial cells[59]. This is interesting considering that normal colonic mucosa of patients with familial adenomatous polyposis contains elevated polyamine levels that can be indicative of polyp development and cancer risk status[60], although a direct connection between biofilm status and mucosal polyamine levels in patients with familial adenomatous polyposis has not been made. It remains to be determined what the effects of increased levels of acetylated polyamines may be on the associated immune microenvironment, warranting further studies. However, N^1,N^{12}-diacetylspermine has been proposed as a non-invasive prognostic biomarker for non-small-cell lung cancer, triple-negative breast cancer and ovarian cancer, and thus may be an important metabolite associated with multiple epithelial cancers[61–64].

The gut microflora can have a profound effect on the gastrointestinal epithelium, and a recent mouse study confirmed that changes in gut microbiota composition affect the chemistry of every organ in the animal[65,66]. A recent report from Parida et al. demonstrated how the pathogenic bacterium enterotoxigenic *Bacteroides fragilis* (ETBF), which is normally found in the gut and associated with colorectal cancer, can colonize other sites, including the breast, and that both gut colonization and breast colonization can induce carcinogenic changes in the breast tissue[67]. The only known virulence factor of ETBF

is *B. fragilis* toxin (BFT), which was found in the breast tissue of gut-infected mice, indicating that circulating BFT from the gut microorganism was directly affecting breast tissue. In this mouse system, gut or ductal colonization by ETBF accelerated breast cancer growth and metastases, likely through activation of the ß-catenin and NOTCH1 pathways. As induction of polyamine catabolism through SMOX is implicated in ETBF-induced epigenetic changes and tumorigenesis in colon epithelial cells[68], it will be interesting to learn whether dysregulated polyamine metabolism also contributes to BFT-mediated carcinogenesis in the breast.

Roles of polyamines in the TME

Lymphocyte function.

Polyamines have been implicated in the functioning of the adaptive immune system, including B cell lymphopoiesis and activation as well as T cell activation. B cell activity can inhibit tumour development in many ways, including the production of tumour-reactive antibodies and the priming of $CD4^+$ T cells and $CD8^+$ T cells[69]. The expression of MYC is required throughout B cell lymphopoiesis and activation[70]. As MYC is a direct inducer of *ODC1* expression[20], increased expression of MYC, and subsequently the polyamine biosynthetic enzymes, occurs during development and following B cell receptor activation[71]. Additionally, supplementation of spermine can limit apoptosis of activated B cells in vitro, suggesting that polyamines play a role in repressing the clonal deletion of B cells following activation[71]. While there are limited reports directly linking polyamines to B cell development and activation, the importance of sustained elevated MYC expression suggests a link to polyamine metabolism. The activation of B cells, potentially through polyamine upregulation, can aid in antitumour immunity by increasing tumour antigen presentation by B cells and the subsequent T cell proliferation.

Following T cell activation, ODC enzymatic activity is increased, as polyamine production is an important part of normal T cell function[72,73]. Arginine is the amino acid precursor for ornithine and is required for T cell activation and T cell receptor (TCR) signalling events[74,75] (FIG. 2). TCR activation in $CD4^+$ T cells triggers the conversion of arginine into ornithine and agmatine, thus promoting the production of putrescine[76]. Exposure of T cells to the oncometabolite (*R*)-2-hydroxyglutarate results in inhibition of ODC. This inhibition and subsequent downregulation of polyamine biosynthesis is sufficient to suppress early TCR signalling activities[77]. The proliferation of T cells after TCR stimulation and optimal cytolytic T lymphocyte induction are fully dependent on an increased polyamine pool[78,79]. Additionally, polyamines regulate T cell differentiation. Spermidine promotes the differentiation of T cells into regulatory phenotypes through the induction of FOXP3, reducing overall inflammation[80]. Through interplay with the epigenome and the tricarboxylic acid cycle, polyamine metabolism is also centrally responsible for the ability of helper $CD4^+$ T cells to differentiate into their functional subsets, including T helper 1 (T_H1), T_H2 and T_H17 cells and regulatory T (T_{reg}) cells)[81,82]. Owing to its competing roles in activating TCR signalling and promoting T_{reg} cell phenotypes, polyamine biosynthesis has the potential to both positively and negatively influence inflammation and tumour immunogenicity.

Immunosuppressive microenvironments.

The TME is often immunosuppressive, which allows malignant cells to evade immune surveillance[83]. As stated earlier herein, polyamines are necessary for normal functioning of both B and T cells; however, the increased expression of polyamine biosynthetic enzymes and the elevated levels of spermine and spermidine in malignant tumours compared with non-malignant tissues have been implicated in an immunosuppressive phenotype. Increased ODC expression in keratinocytes suppresses contact hypersensitivity, a T cell-driven inflammatory response, and promotes carcinogenesis in the epidermis[84]. Specialized cell populations that contribute an immunosuppressive phenotype require high levels of polyamines to support their growth and metabolism[76,85,86] (FIG. 2). Increased consumption of L-arginine, a precursor for polyamine metabolism, by both tumour cells and suppressive myeloid cells reduces its availability in support of cytotoxic T cell proliferation and functioning[87]. Immunosuppressive myeloid-derived suppressor cells (MDSCs), dendritic cells and monocyte-derived M2 macrophages are often abundant in the immunosuppressive microenvironment of tumours, and these cell types all rely on polyamine metabolism for their function in dampening the immune system[88] (detailed in FIG. 2).

Putrescine, the product of the action of ODC, has been directly implicated in macrophage modulation in a myeloid-specific *Odc1*-knockout model. Putrescine reduced M1 macrophage polarization in response to infection with *Helicobacter pylori* or *Citrobacter rodentium*[89]. Importantly, these data are consistent with the authors' hypothesis that macrophage-specific loss of putrescine can result in chromatin remodelling and enhanced M1 gene expression, directly linking suppression of polyamine biosynthesis with an increase in the 'immune-friendly', antitumour immune cell population. Similarly, increased macrophage-specific ODC expression was observed in human colon tissues from patients with active ulcerative colitis, Crohn's disease, colitis-associated dysplasia and carcinogenesis compared with tissues from unaffected individuals or those with inactive ulcerative colitis. The myeloid cell-specific knockout of *Odc1* in an azoxymethane–dextran sodium sulfate model of colitis-associated carcinogenesis reduced tumour burden and number, while the number of M1 macrophages in the tumours increased, compared with the wild-type control[90].

Interestingly, there have been reports suggesting that spermidine is an antitumour immune activator. One study suggested that abhydrolase domain-containing protein 5 (ABHD5), a co-activator of adipose triglyceride lipase, reduces the biosynthesis of spermidine in tumour-associated macrophages[91]. This reduction in spermidine biosynthesis was proposed as a possible mechanism leading to increased growth of colorectal cancer cells. Unfortunately, the mechanistic studies performed to validate this hypothesis were not conclusive because of the likely production of toxic metabolites resulting from high concentrations of spermidine used in the presence of serum containing amine oxidases, a mechanism unrelated to actual immune modulation[42].

Recently, Miska and colleagues suggested a novel mechanism for polyamine promotion of immunosuppression in glioblastoma, notably in the suppressive tumour-associated myeloid cell (TAMC) population, which includes tumour-associated macrophages and MDSCs[92]. In a glioblastoma model, their data suggest that putrescine acts as a pH buffer against

the acidic TME, promoting the survival and metabolic functions of TAMCs. Survival was dramatically increased in their immunocompetent mouse model upon reduction of the levels of polyamines; however, this survival benefit was abrogated in immunodeficient mice, indicating antitumour activity through promotion of an adaptive immune response. Studies in mouse models of breast cancer and melanoma showed that reduced tumour polyamine content partially alleviated immunosuppression by reducing the survival of TAMCs[93]. The reduction in tumour polyamine content specifically decreased cytoprotective autophagy in immunosuppressive leukocytes, implying that the high polyamine levels in tumours were driving the immunosuppressive phenotype in part due to an upregulation of protective autophagy in TAMCs. Studies in non-cancer models further provide evidence for the potential polyamine-mediated regulation of the innate immune response in cancer. Spermine has been shown to inhibit the innate immune response by significantly attenuating the levels of inducible nitric oxide synthase (NOS2) in macrophages responding to *H. pylori* infection, apparently through a post-transcriptional mechanism[94]. Furthermore, spermidine alleviated autoimmune encephalomyelitis in an experimental model through regulation of infiltrating $CD4^+$ T cells and macrophages within the central nervous system[95].

Dendritic cells also utilize arginine to increase production of polyamines, which subsequently induce expression of indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that metabolizes tryptophan into immunoregulatory kynurenines, thereby contributing to an immunosuppressive phenotype[96]. MDSCs also export polyamines into the microenvironment, thereby providing dendritic cells with additional polyamines to exacerbate IDO1 expression[96]. A recent review by Proietti and colleagues details the mechanistic interactions between polyamines and kynurenines that form the basis of an immunomodulatory circuit that may be amenable to cancer immunotherapy[97].

Hypoxia.

A hypoxic TME influences polyamine homeostasis in tumour cells in several ways (FIG. 3a). Hypoxia-inducible factor 1α (HIF1α) is a master transcription factor regulator of the hypoxic rescue programme. The activation of this HIF1α transcriptional programme is a hallmark of proliferating, aggressive tumours that protects cells from acute cell death by restoring nutrient and oxygen supply to the TME. Hypoxic conditions stimulate both uptake of exogenous polyamines and intracellular polyamine biosynthesis through ODC induction, resulting in increased levels of putrescine and spermidine[98]. Exposure of HT-29 colon cancer cells to extracellular spermine augmented the hypoxia-initiated reduction in mRNA and protein expression levels of CD44, a cell adhesion molecule, and increased invasion through Matrigel in a dose-dependent manner, supporting a potential role for polyamines in facilitating tumour cell migration, invasion and metastases[99]. Importantly, depleting polyamines with DFMO during hypoxia increased apoptosis in multiple cancer cell lines[98], suggesting that polyamines are essential for cancer cell adaptation to hypoxic stress. These data indicate that hypoxic tumour cells have increased polyamine requirements, thereby conferring vulnerability to PBT.

The polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase (SSAT, also known as SAT1) plays an important role in regulating the ubiquitination and degradation

of HIF1α under aerobic conditions by stabilizing the interaction of HIF1α with RACK1 (REF. [100]). Mutagenesis of the catalytic arginine[101] residue of SSAT reduced its negative regulatory effect on HIF1α degradation, suggesting a requirement for the acetyltransferase function of SSAT in oxygen-independent HIF1α degradation. Bis(ethyl)polyamine analogues, such as PG-11047, can induce SSAT hyperactivation, suggesting the potential to decrease the tumour-protective hypoxic response by increasing HIF1α ubiquitination and degradation. Data supporting this mechanism have not been reported. However, combining PG-11047 with the VEGF inhibitor bevacizumab has provided additive tumour-inhibitory growth effects in prostate cancer mouse xenografts[101] and resulted in partial responses in patients with advanced solid tumours[102]. Beyond induction of SSAT, bis(ethyl)polyamine analogues also induce SMOX, downregulate ODC and compete for uptake with the natural polyamines, all of which are important in the antitumour response and may be amplified in the context of hypoxia.

HIF1α has been shown to directly stimulate transcription and expression of SMOX in rat glial cells[103]. Acrolein, a SMOX reaction by-product, facilitates glial cell migration through the autocrine generation of the pro-inflammatory chemokine CXC motif ligand 1 (CXCL1)[104]. As SMOX activity can contribute to carcinogenesis, the results of this study may have important correlatives in the hypoxic TME. CXCL1 promotes migration of tumour-associated neutrophils and MDSCs, as well as tumour cells themselves[105]. Furthermore, SMOX is negatively regulated by miR-124 (REF. [106]), a tumour-suppressive microRNA with reduced expression levels in a variety of hypoxic and ischaemic tissues. Expression of miR-124 has been negatively correlated with a hypoxic gene signature in tumour tissue samples from patients with glioblastoma[107,108].

Aside from affecting polyamine metabolism in tumour cells, a hypoxic TME is also an immunosuppressive TME, characterized by the presence of suppressive immune cell populations, including MDSCs and T_{reg} cells[109]. The effect of hypoxia on polyamine metabolism in these populations has not been studied, and may have important implications for polyamine-targeting strategies and tumour immunotherapy, particularly considering that the immune checkpoint blockade protein PDL1 is an HIF1α target that is upregulated by hypoxia in MDSCs, thereby increasing tumour immune tolerance[109].

Inflammation.

Inflammation is a predisposing factor supporting the development and progression of cancer. Epithelial cells exposed to inflammatory conditions, including pathogenic infection, physical irritants, hypoxia and intestinal barrier failure, are at enhanced risk of carcinogenic transformation[110]. Modulators of inflammation create a more tumour-permissive TME, characterized by infiltration of immunosuppressive cells, as described in the previous section, while also providing growth-promoting signals to epithelial and cancer cells[111]. Oxidative stress resulting in damage to DNA is a major procarcinogenic factor driving inflammation-associated carcinogenesis[112]. While polyamines, particularly spermine, have antioxidant properties and can act as free radical scavengers[113], increased polyamine metabolic flux in epithelial cells in response to inflammation also contributes to the production of damaging hydrogen peroxide and aldehydes.

Evidence that the natural polyamines have anti-inflammatory properties has led to the suggested use of dietary polyamine supplementation as a treatment for chronic inflammatory and autoimmune conditions. In mouse colitis models, orally administered spermidine provided protection against disease severity as measured by multiple markers of intestinal inflammation[114,115]. In addition to reducing the infiltration of neutrophils[114,115], spermidine reduced the accumulation of colonic macrophages, with the population of pro-inflammatory M1 macrophages reduced, while expression levels of M2 macrophage markers were increased. Furthermore, the large influx of T cells in response to dextran sodium sulfate was reduced when mice received spermidine either before or after treatment with dextran sodium sulfate, with levels of T_H1 cell and T_H17 cell markers reduced, while T_H2 cell and T_{reg} cell marker levels were increased, consistent with the ability of polyamines to contribute to an anti-inflammatory, but immunosuppressive, microenvironment[115]. In dendritic cells, spermidine was shown to induce a transcription factor FOXO3–mediated decrease in the expression of inflammatory cytokines[116]. Several mechanistic studies have suggested that spermidine induces expression of protein tyrosine phosphatase non-receptor (PTPN) genes, particularly the gene encoding PTPN2, a negative regulator of inflammatory cascades. However, many of these studies are confounded by high concentrations of spermidine administered in the presence of bovine serum amine oxidases[42,68]. As extracellular spermidine oxidation is likely in these experiments, and a major by-product is hydrogen peroxide, which also induces PTPN2, the contributions of these mechanisms to the immunosuppressive microenvironment and potential carcinogenic sequelae await validation.

The anti-inflammatory effects of spermidine and spermine described above may be beneficial in the treatment of chronic inflammation and in wound repair. However, it is important to acknowledge the potential danger of establishing an immunosuppressive microenvironment by elevating polyamine levels in conditions also known to contribute to carcinogenesis. Many chronic infection and/or inflammatory conditions, including *H. pylori*-associated gastritis and colitis, have established disease progression cascades leading to neoplastic transformation of epithelial cells in which the metabolism of spermine plays a role (FIG. 3b). In addition to being a rich source of polyamines and their metabolites, certain pathogenic components of the gastrointestinal microbiota can affect polyamine levels within the epithelial tissue with which they are associated by inducing alterations in epithelial cell polyamine metabolism. In particular, the chronic induction of spermine oxidation via pro-inflammatory cytokines and pathogenic infection generates DNA-damaging hydrogen peroxide and reactive aldehydes believed to be an early event contributing to inflammation-associated carcinogenesis in gastric, colon and prostate epithelial tissue[68,117]. Additionally, spermine oxidation reduces the intracellular concentration of spermine, which also functions as a free radical scavenger[68], while increasing the pool of free spermidine. Considering that this chronic, low-grade induction of SMOX activity may occur for years without causing overt symptoms, it is likely that carcinogenic changes may have already occurred that would be exacerbated by increasing levels of polyamines in the microenvironment. As transformed cells generally have upregulated polyamine transport, the idea of adding polyamines as an anti-inflammatory strategy would create the potential for selectively ‘feeding’ any transformed cells present in the population while establishing an immunosuppressive microenvironment conducive for tumour growth. Inhibitors of SMOX are an ongoing area

of investigation as a means of reducing the potential for epigenetic or genetic changes in response to a chronic inflammatory environment[68,118,119] (TABLE 1).

Synergies in targeting polyamine metabolism and immune checkpoint blockade

The relatively increased dependency of tumour cells on polyamines as well as the critical physiological roles of polyamines in various immune cell types makes targeting the polyamine metabolic pathway a feasible treatment strategy. While inhibitors have been designed for all the polyamine biosynthetic enzymes, the most successful inhibitor to date is DFMO. DFMO irreversibly inhibits ODC by covalently binding to its active site, generally resulting in cytostasis through depletion of putrescine and spermidine[19,120,121]. Although highly successful in the treatment of African sleeping sickness, DFMO has shown only minor success as a single cancer agent[19,122]. DFMO is, however, exceedingly well tolerated and has demonstrated impressive results in chemoprevention trials[32,123–125]. Its cytostatic properties have successfully prevented tumour formation in numerous in vivo models, and clinical trials have shown that low-dose DFMO is safe and sufficient to reduce polyamine levels[126,127].

DFMO is also being assessed as a potential drug for combination cancer therapies. The in vivo effects of DFMO are strongest when its use is combined with a polyamine-free diet, indicating the utility of combining the use of DFMO with the use of PTIs as a means of ‘polyamine-blocking therapy’ (PBT)[128,129]. Early PTIs were either extremely toxic or unable to fully prevent polyamine transport; however, advances in PTI chemistry produced inhibitors that are effective in their inhibition of polyamine transport and have minimal toxicity[130–133]. Of these, AMXT 1501 and Trimer44NMe are the most well studied PTIs (TABLE 1). In combination with DFMO, these PTIs have resulted in tumour growth inhibition in mouse models of colon cancer, melanoma, breast cancer, neuroblastoma, glioma and chemotherapy-resistant pancreatic cancer[93,134–138]. A phase 1 AMXT 1501 and DFMO combination trial is currently ongoing for patients with advanced solid tumours (NCT03536728).

Immunotherapies are the fastest-growing anticancer drug class and have led to major advances in the treatment of multiple cancers. Although there has been marked success with immune checkpoint blockade in melanoma, renal cell carcinoma and non-small-cell lung cancer, many solid tumours fail to respond to immune checkpoint inhibitors. These immunologically ‘cold’ tumours have limited immunogenicity due to a lack of infiltrating cytotoxic T lymphocytes and an increase in abundance of immunosuppressive cells such as T_{reg} cells or MDSCs. The success of immune checkpoint blockade therapy is dependent on reactivation of antitumour T cells present within the TME[139]. Because the low level of T cells present in the microenvironment of cold tumour types limits the efficacy of immunotherapy, a major focal point to significantly advance immunotherapy is the reprogramming of cold tumours into hot tumours.

Global sequencing projects, such as The Cancer Genome Atlas, have catalogued the mutation and/or amplification of oncogenes across nearly all tumour types, allowing the

prediction of polyamine-dependent tumour types with exploitable genetic changes. Notably, MYC is amplified in approximately a quarter of all breast cancers and in more than half of the highly aggressive basal breast cancer subtype[140,141]. Similarly, 31% of ovarian cancers and nearly 10% of prostate cancers harbour *MYC* amplification[142,143]. In a study of more than 500 pancreatic ductal adenocarcinoma samples, *MYC* amplification was seen in 14% and was an independent marker of poor prognosis[144–146]. All four of these tumour types are traditionally considered 'immunologically cold' and show limited response to immune checkpoint blockade. Because of the direct effect of MYC on polyamine biosynthesis, many of these cancers, in particular prostate cancer, have elevated polyamine levels, and their survival is highly dependent on maintaining an increased polyamine pool. Alterations in *KRAS* have been detected in 25% of all cancers, many of which have poor prognosis[147]. Up to 95% of all pancreatic ductal adenocarcinoma cases contain a mutation in *KRAS,* while more than a third of all lung cancers have *KRAS* mutations[148]. Approximately 12% of gynaecological cancers have *KRAS* mutations, while RAS proteins are known to be upregulated in breast tumours despite infrequent mutation[149,150]. KRAS and MYC are both known to drive polyamine biosynthesis and tumour growth, potentially explaining the heavy reliance of these tumours on polyamines[20,23,121].

The high dependence of many immunologically cold cancers on polyamines makes them strong candidates for polyamine-targeting therapy. While there are countless efforts to achieve immunological reprogramming of cold tumours, the strategy of reducing the levels of polyamines through PBT as an immunomodulator is a new but rapidly evolving field. A major benefit of PBT is that most cancers are dependent on elevated concentrations of polyamines for sustained growth, so the reduction of intracellular polyamine concentrations can have a multifaceted effect. Numerous studies have shown that reduction of the levels of available polyamines can block tumour proliferation, while simultaneously increasing the immunogenicity of cold tumours. The combination of DFMO and the PTI AMXT 1501 blocks tumour growth but only in immunocompetent mouse models with functional T cells[135]. Similarly, treatment with the PTI Trimer44NMe in combination with DFMO successfully activated antitumour immune responses in the immunologically cold CT26.CL25 colon cancer model[137]. PTI and DFMO combination treatment significantly decreased intratumoural levels of immunosuppressive cell types, including granulocytic MDSCs, T_{reg} cells and M2 macrophages, compared with vehicle treatment. Additionally, treatment increased secretion of the pro-inflammatory cytokine interferon-γ (IFNγ) and the percentage of $CD8^+$ cytotoxic T cells[137]. The response was fully T cell dependent, as mice with depleted T lymphocytes showed no increase in survival. Also, this PBT combination effectively prevented tumour growth in breast cancer and melanoma models that were resistant to anti-PD1 monotherapy. The antitumour effect of PD1 blockade was stimulated by PBT in both models, with increased survival over that observed with either PBT or PD1 blockade alone[93]. A recent preclinical study showed that DFMO co-treatment enhanced PD1 blockade in both a partially anti-PD1-responsive Lewis lung carcinoma model and an anti-PD1-non-responsive melanoma model[151]. The synergy seen between DFMO and anti-PD1 treatment in these two model systems was primarily mediated by increased survival and activity of intratumoural $CD8^+$ T cells. It remains to be determined whether the immune effects of DFMO are directly attributable to polyamine depletion or whether the effects

stem from modulation of metabolic pathways, such as arginine and thymidine metabolism, directly influenced by polyamine biosynthesis.

A main goal in reprogramming a cold tumour is to increase the number of antitumour T cells in the TME and reduce the number or effectiveness of immunosuppressive cells. The inhibition of polyamine accumulation, through ODC inhibition and polyamine transport inhibition, leads to an antitumour response linked to increased T cell-mediated antitumour activity[87,93,135,152]. One working hypothesis is that because ODC inhibition through DFMO treatment reduces intratumoural MDSCs, there is increased availability of extracellular arginine for T cells[75,76] (FIG. 2). Arginine is produced by the urea cycle, enzymes of which, including arginase 1 (ARG1), are repressed by the tumour suppressor gene *TP53*. Indeed, p53-mediated regulation of the urea cycle can control polyamine biosynthesis. In colon tumour cells lacking *TP53*, the urea cycle was upregulated, leading to increased arginine and polyamine production[153]. The implications of these results for the TME have not yet been described, but one can speculate that the increased ability of a tumour cell to synthesize arginine, rather than acquire it from the TME, could increase arginine availability to immune cells in the TME, perhaps contributing to tumour immunogenicity. Clinically, both mutated *TP53* and dysregulation of the urea cycle have been correlated with increased immune infiltration and response to immune checkpoint inhibitors[154–156].

While not strictly PBT, co-treatment with DFMO and the epigenetic modifier 5-azacytidine in an ovarian cancer mouse model reversed the immunosuppressive TME and increased infiltration of pro-inflammatory cells beyond that observed in untreated mice or mice treated with a single agent[157]. Because DFMO treatment can reduce intracellular levels of folate-dependent metabolites, including S-adenosylmethionine[158], co-treatment with DFMO and 5-azacytidine may affect DNA methylation on two fronts: DFMO can reduce intracellular levels of S-adenosylmethionine, the methyl donor for various methyltransferases, including DNMT1, which in turn can be inhibited by 5-azacytidine. This combination treatment significantly increased survival and intratumoural recruitment of $IFN\gamma^{+}CD4^{+}$ T cells, $CD8^{+}$ T cells and natural killer cells. Most notably, the response appeared to be most dependent on the change in macrophage populations following co-treatment. Combination therapy led to a repolarization of macrophages from an M2 tumour-permissive phenotype to a predominately M1 pro-inflammatory phenotype[157].

While most currently available immunological data were obtained using PTIs in conjunction with DFMO-mediated ODC inhibition as PBT, it is possible that substituting certain polyamine analogues for DFMO would also prove effective in reducing the abundance of immunosuppressive cells in the TME. SBP-101 (diethyldihydroxyhomospermine), a symmetrically substituted spermine analogue, has shown efficacy against pancreatic ductal adenocarcinoma models and has been safely administered in phase 1 clinical trials in patients with pancreatic cancer[159–161]. Bis(alkyl)spermine analogues compete with natural polyamines for cellular uptake, upregulate polyamine catabolism and inhibit polyamine biosynthesis[19,118]. Through product inhibition and a decrease in the accumulation of natural polyamines, these analogues have the potential to increase the availability of arginine in the TME. Because increased availability of arginine availability promotes T cell function and can recruit new T cells into the TME[72,75,76,87], the study of these analogues as components

of PBT is warranted. Furthermore, as the TME of many solid tumours is high in M2 macrophages and low in effector T cells, a decrease in the levels of available polyamines could lead to a switch from the M2 macrophage to M1 macrophage phenotype, concurrent with an increase in the recruitment of effector T cells. Thus, PBT-mediated reprogramming of the TME would be expected to increase the efficacy of immune checkpoint blockade in immunologically cold cancers and provide new therapeutic avenues in fatal diseases. Compounds of interest for use in PBT to potentially modulate the TME as well as tumour cells are listed in TABLE 1 (structures are provided in Supplementary Table 2).

Conclusions and future perspectives

The antitumour effect of polyamine-targeting therapies on cancer cells is well established. However, emerging data demonstrating their influence on cancer immunity and other factors of the TME indicate that their anticancer mechanisms extend beyond direct manipulation of polyamine levels in cancer cells. While polyamine function in cancer-related immune cell populations is an area of active investigation, effects of their modulation in other cell types in the TME remain to be adequately studied. For example, basic fibroblast growth factor (bFGF), which is produced by cancer-associated fibroblasts (CAFs) and plays an important role in CAF-mediated tumour cell migration and invasion, was shown to regulate *ODC1* expression years ago; however, the polyamine field is currently lacking studies that evaluate the influence of polyamines on CAFs within the TME[162]. Likewise, although important studies in the 1990s implicated polyamine biosynthesis in tumour invasion and angiogenesis, little has been reported in the last decade (last reviewed by Soda[163]). Elevated polyamine levels promote the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases in the vascular endothelium surrounding tumours[164]. Additionally, polyamines promote the hypoxia-induced apoptosis of endothelial cells influencing hypoxia-driven neovascularization[165]. These data indicate a potential role for polyamines in angiogenesis and metastasis and warrant further investigation.

Data indicating upregulation of polyamine metabolism in hypoxic TMEs, which are inherently immunosuppressed and provide the opportunity for tumour cell immune escape and tolerance, support the potential for PBT in targeting the heterogeneity of tumours. Finally, the alkaline nature of polyamines may play a role in pH buffering and acidosis in the TME, a field with strong ties to tumour immunogenicity. New knowledge regarding transporter-mediated sequestration of polyamines into vesicles also indicate roles in pH buffering of lysosomes[17], and the subcellular localization of polyamines likely has important functional implications in all cell types, suggesting the need for improved methods for determining polyamine distribution. Considering these multifaceted, context-specific effects of polyamines, cancer model systems that adequately recapitulate the TME will be valuable tools in fully understanding and verifying the roles of polyamines within the TME and the potential utility of therapeutically modulating polyamines at the organismal level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Work in the Casero and Stewart laboratory is supported by grants from the US National Institutes of Health (CA204345 and CA235863), the Samuel Waxman Cancer Research Foundation, the University of Pennsylvania Orphan Disease Center Million Dollar Bike Ride (MDBR-20–135-SRS), the Chan Zuckerberg Initiative and a research contract with Panbela Therapeutics Inc.

Glossary

Azoxymethane–dextran sodium sulfate model
A common murine model of inflammation-associated colorectal cancer that incorporates chemical initiation of DNA adducts combined with induction of colitis

Biofilm
A structure formed by a community of the microbiota that adheres to and lines a surface such as the colonic lumen

M1 macrophage
A pro-inflammatory type of macrophage that mediates pathogen resistance but can also exacerbate inflammatory conditions and cause tissue damage

M2 macrophages
Anti-inflammatory macrophage population characterized by expression of arginase 1 (ARG1) and associated with tissue repair and immunosuppressive microenvironments

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous population of immature myeloid cells that have immunosuppressive function and undergo systemic expansion in association with cancer

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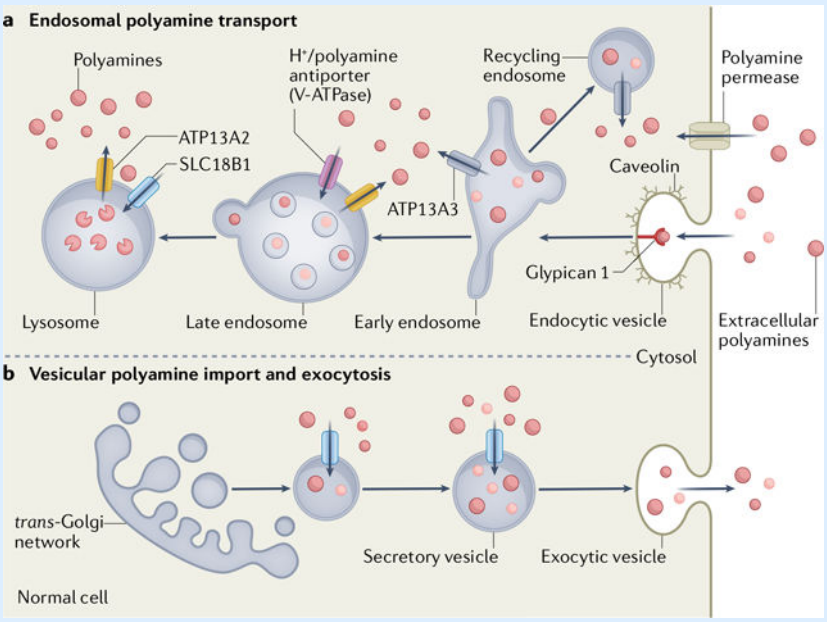
Box 1.

Polyamine uptake and intracellular polyamine transport

Current models of mammalian polyamine uptake propose that polyamines enter cells through (1) polyamine permeases followed by transport of free cytosolic polyamines into polyamine-sequestering vesicles (PSVs) or (2) receptor-mediated endocytosis, likely involving glypican 1 in membrane regions enriched in caveolin 1 (REF. [9]). All models agree that polyamines exist in PSVs, synonymous with multivesicular bodies, late endosomes and lysosomes, from which they can be released into the cytosol. Recent studies have suggested mechanistic roles for the P5B-type ATPases ATP13A2 (also known as PARK9) and ATP13A3 in this PSV escape[12,16,17]. ATP13A2 localizes to late endolysosomes, where it hydrolyses ATP to facilitate the transfer of polyamines into the cytosol, with greatest substrate specificity for spermine, followed by N^1-acetylspermine and spermidine[17,188]. ATP13A2 function influences total intracellular polyamine concentration by promoting both polyamine endocytosis and polyamine release into the cytosol, consistent with the receptor-mediated endocytosis model. ATP13A2-mediated export of vesicular polyamines is important in maintaining lysosomal membrane integrity as well as mitochondrial function, and spermine transported by ATP13A2 may be redistributed to the mitochondria[17,18]. A second P5B-ATPase, ATP13A3, similarly transports vesicular polyamines, but with greater activity for putrescine and confinement to the early and recycling endosomes[16]. Roles for ATP13A3 in polyamine transport and in predicting response to polyamine-targeting therapies were demonstrated in pancreatic cancer cells[189]. The gene encoding ATP13A3 is mutated in the polyamine transport-deficient CHO-MG cell line, where re-expression of wild type ATP13A3 rescued putrescine uptake[16] (see the figure, part **a**). SLC18B1 (also known as VPAT) is a vacuolar H^+-ATPase (V-ATPase) that enables the storage and release of spermidine and spermine in polyamine-secreting cells[12,190]. SLC18B1-mediated accumulation and exocytosis of polyamines has been observed in synaptic vesicles of neurons, microvesicles of astrocytes and secretory granules of mast cells12,191, the last of which is a component of the innate immune response that contributes to antitumour immunity through modulation of the tumour microenvironment[192]. Notably, SLC18B1 expression is greatly enriched in lysosomal vesicles of inflammation-activated macrophages, suggesting a role for lysosomal polyamine transport in the anti-inflammatory, immunosuppressive effects of polyamines[193]. SLC18B1 may therefore serve as the lysosomal polyamine importer suggested by Poulin et al.[9]. In other cell types, SLC18B1 localizes with the plasma membrane and may contribute to the extrusion process[12] (see the figure, part **b**).



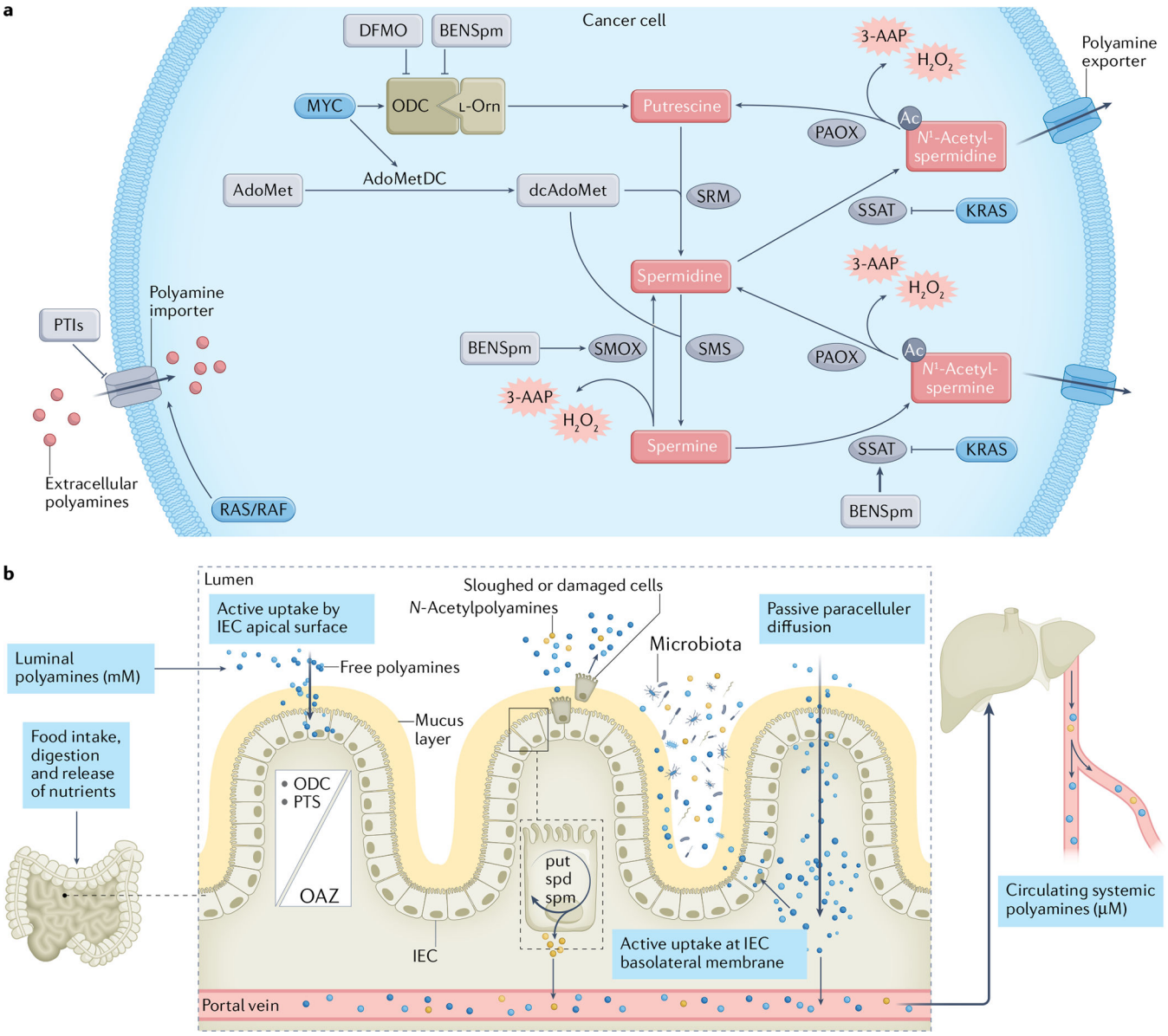


Fig. 1 |. Oncogenic regulation of polyamine metabolism and uptake and sources of extracellular polyamines in the TME.

The elevated intracellular polyamine pools required of cancer cells are maintained by oncogenes, including *MYC, KRAS* and *BRAF,* through increasing biosynthesis and uptake and decreasing catabolism. **a** | Putrescine is synthesized by ornithine decarboxylase (ODC), a rate-limiting enzyme inhibited by α-difluoromethylornithine (DFMO)[19]. *S*-Adenosylmethionine decarboxylase (AdoMetDC) produces the aminopropyl group necessary for spermidine synthase (SRM) and spermine synthase (SRS) activities[19]. Spermidine/spermine N^1-acetyltransferase (SSAT) acetylates the N^1 position of spermidine or spermine[19], allowing either export or oxidative back-conversion by peroxisomal acetylpolyamine oxidase (PAOX). Alternatively, spermine can be directly catabolized to spermidine by spermine oxidase (SMOX). By-products of PAOX and SMOX activity, including H_2O_2, 3-aminopropanal (3-AP) and 3-acetamidopropanal (3-AAP), can result

in oxidative stress[68,181]. SSAT and SMOX are induced by polyamine analogues such as N^1,N^{11}-bis(ethyl) norspermine (BENSpm)[118]. Polyamine uptake can be blocked by polyamine transport inhibitors (PTIs). **b** | Extracellular polyamines originate from the diet, microbiota, and sloughed or damaged cells. Most luminal polyamines passively diffuse into the circulation through the proximal portion of the small intestine, while some are actively transported into intestinal epithelial cells (IECs), where they may be interconverted via the polyamine metabolic enzymes or excreted as acetylated polyamines. Active import occurs at both apical and basolateral IEC membranes via the polyamine transport system (PTS)[49,182]. A decreasing expression gradient of antizyme (OAZ), a regulator of both ODC activity and polyamine transport[19], exists in enterocytes along the crypt–villus axis and correlates with an inverse gradient in ODC activity, suggesting a similar gradient of polyamine uptake[182]. Polyamines and their metabolites entering the circulation can be used by cells throughout the body, thereby affecting tumour microenvironments (TMEs) at distant sites. L-Orn, L-ornithine; dcAdoMet, decarboxylated *S*-adenosylmethionine; put, putrescine; spd, spermidine; spm, spermine.

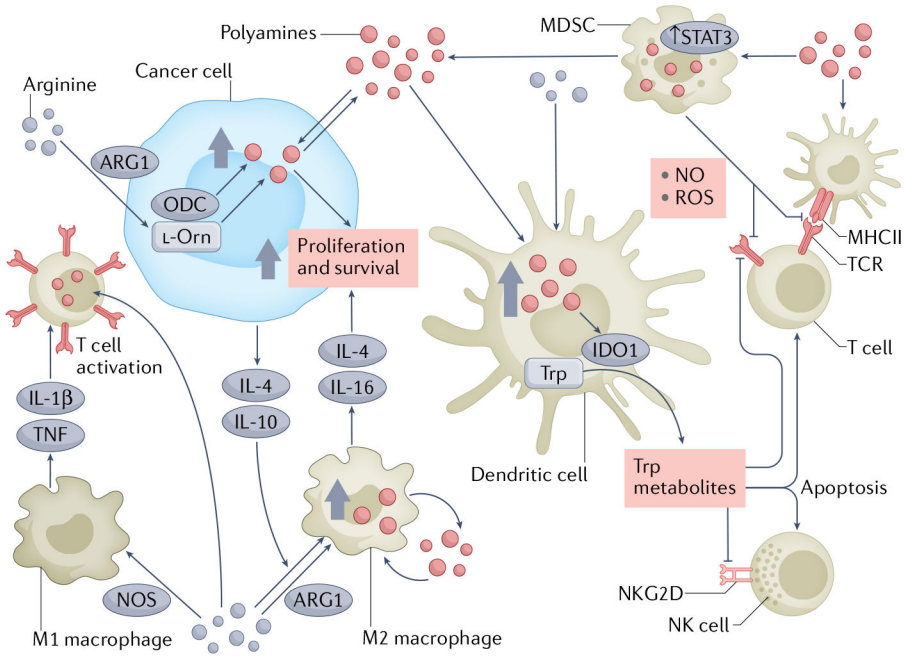


Fig. 2 |. Influence of polyamines and their modulation on immune cells in the TME.
Tumour cells maintain elevated polyamine levels through uptake of extracellular polyamines and arginine. Arginine is converted to ornithine by arginase 1 (ARG1) and results in upregulation of ornithine decarboxylase (ODC) and polyamine biosynthesis. The increased intracellular polyamine pool promotes proliferation and survival of tumour cells. Macrophage polarization is mediated by arginine metabolism: conversion of arginine into nitric oxide by nitric oxide synthase (NOS) promotes a proimmune, antitumour M1 phenotype. M1 macrophages release IL-1β and TNF to promote the proliferation and survival of T cells. The cytokines IL-4 and IL-10 released by tumour cells promote M2 polarization by upregulation of ARG1 (REF. [183]). M2 macrophages lack the ability of M1 macrophages to make nitric oxide and alternatively use upregulated ARG1 to convert arginine into ornithine[86,184,185]. M2 macrophages therefore compete with effector T cells for the arginine and glutamine required for T cell function while also producing the immunosuppressive cytokines IL-4 and IL-16 (REFS[76,85,186]). Arginine and polyamines in the tumour microenvironment (TME) can be taken up by dendritic cells to increase intracellular polyamine content. This induces indoleamine 2,3-dioxygenase 1 (IDO1) expression and contributes to an immunosuppressive phenotype[96]. IDO1 metabolizes tryptophan (Trp), the metabolites of which inhibit receptor activation and increase apoptosis in T cells and natural killer (NK) cells. Increased polyamine content activates STAT3 in myeloid-derived suppressor cells (MDSCs) and promotes their survival[93]. MDSCs produce nitric oxide and extreme levels of reactive oxygen species (ROS), leading to disruption of the interaction between the T cell receptor (TCR) and major histocompatibility complex-peptide complex and reducing the success of antigen presentation for the effector function of T cells[187]. MDSCs also export polyamines to provide dendritic cells with additional polyamines to exacerbate IDO1 expression[96]. MHCII, major histocompatibility complex class II; L-Orn, L-ornithine.

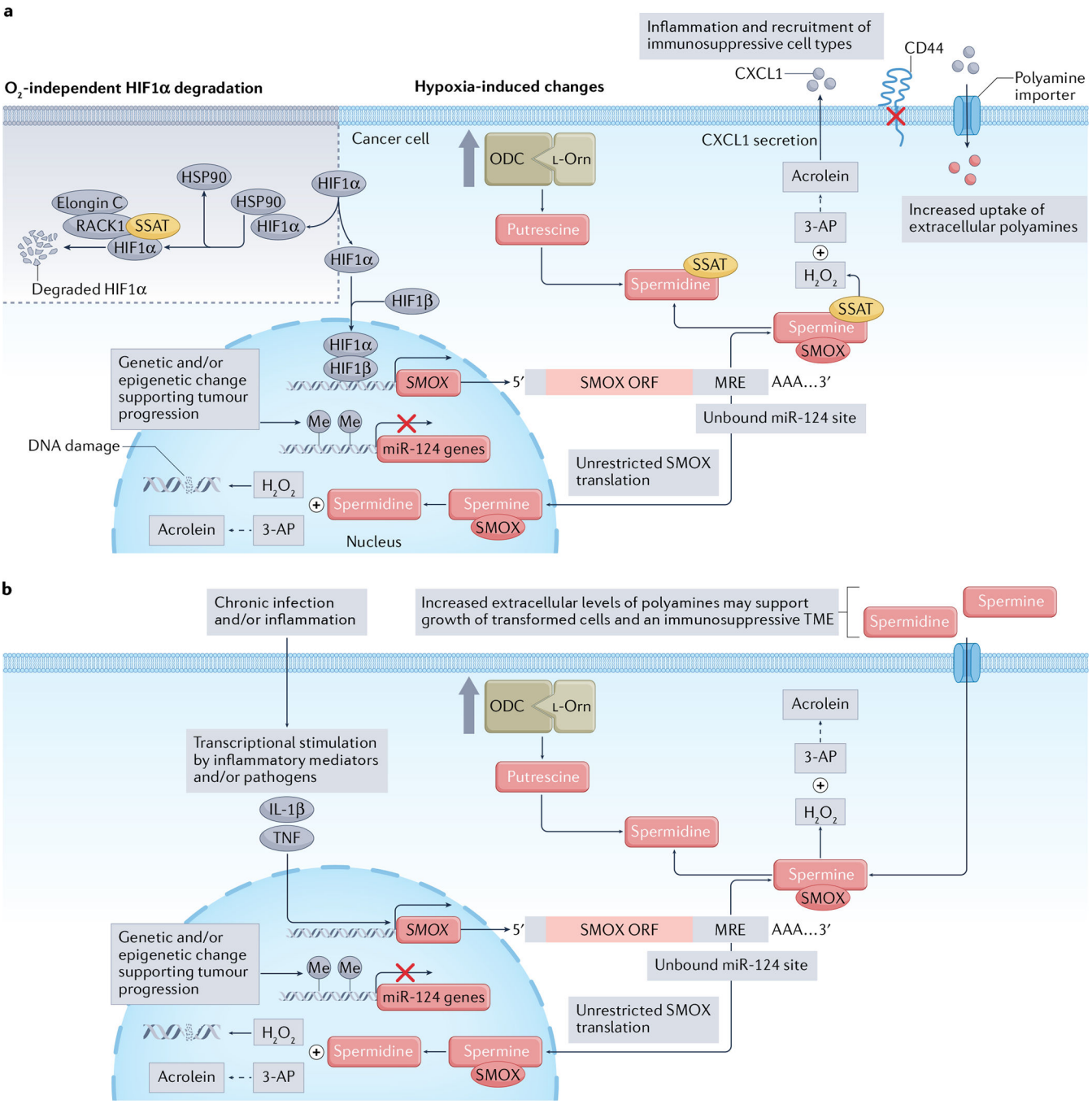


Fig. 3 |. Hypoxic and chronic infection/inflammatory microenvironments promote carcinogenic polyamine metabolism.

a | Hypoxic conditions stimulate both polyamine uptake and ornithine decarboxylase (ODC)-mediated polyamine biosynthesis, dramatically increasing tumour cell putrescine and spermidine levels[98]. Extracellular spermine augments the hypoxia-initiated reduction in CD44 cell adhesion molecule expression, facilitating tumour cell migration, invasion and metastases[99]. The polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase (SSAT) regulates the degradation of the master transcription factor hypoxia-inducible

factor 1α (HIF1α) under aerobic conditions by stabilizing its interaction with RACK1 (REF. [100]). HIF1α also directly stimulates the transcription of spermine oxidase (SMOX), a nuclear and cytosolic enzyme capable of generating DNA-damaging reactive oxygen species[103]. Acrolein originating from the SMOX reaction may facilitate cell migration by producing the pro-inflammatory chemokine CXC motif ligand 1 (CXCL1)[104], which is recognized by CXCR2-expressing tumour-associated neutrophils, myeloid-derived suppressor cells and tumour cells[105]. SMOX is negatively regulated by miR-124 (REF. [106]), expression of which is reduced in hypoxic tissues and is negatively correlated with a hypoxic gene signature[107,108]. **b** | Exposure to chronic infection and inflammation induces changes in epithelial cell polyamine metabolism, particularly through inducing SMOX and its production of reactive oxygen species, resulting in DNA damage and epigenetic changes leading to neoplasia. Enhanced methylation of SMOX-targeting miR-124 genes is observed in patients at heightened risk of *Helicobacter pylori*-associated gastric cancer development[106]. Immune and epithelial cell production of inflammatory cytokines in response to infection further stimulates polyamine metabolism[68]. Extracellular polyamines may provide anti-inflammatory effects but at the potential risk of creating an immunosuppressive microenvironment conducive to selective outgrowth of transformed cells. 3-AP, 3-aminopropanal; MRE, microRNA-recognition element; ORF, open reading frame; L-Orn, L-ornithine.

Table 1 |

Compounds in development that affect polyamine metabolism, the TME and immune response (structures are provided in Supplementary Table 2)

Drug	Target	Status
DFMO	ODC	Approved for treatment of human African trypanosomiasis and hirsutism; multiple ongoing cancer clinical trials, including chemoprevention trials[32,123,125,166–170]
BENSpm	ODC. AdoMetDC, SSAT, SMOX	Phases 1 and 2 completed. Formulated into nanoparticles[171–175]
PG-11047	ODC, AdoMetDC, SSAT, SMOX	Phases 1, 1b and 2 completed. Formulated into nanopartides[102,176–178]
SBP-101	ODC, AdoMetDC, SSAT, SMOX?[a]	Preclinical[160], phase 1 completed[159], ongoing phase 1a/1b (NCT03412799)
AMXT 1501	Polyamine transport	Preclinical use[134,135,138]; ongoing phase 1 trial in combination with DFMO (NCT03536728)
MeN44Nap44NMe (AP)	Polyamine transport	Preclinical use[22,179]
Trimer44NMe	Polyamine transport	Preclinical use[93,136,137]
MDL 72527	SMOX, PAOX	Preclinical use[180]
2,11-Met_2-Spm	SMOX PAOX?	Preclinical use[119]

AdoMetDC, *S*-adenosylmethionine decarboxylase; BENSpm, N^1,N^{11}-bis(ethyl)norspermine; DMFO, α-difluoromethylornithine; 2,11-Met2-Spm, 1,12-diamino-2,11-bis(methylidene)-4,9-diazadodecane; ODC, ornithine decarboxylase; PAOX, acetylpolyamine oxidase; SMOX, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase; TME, tumour microenvironment.

[a]Target data for SBP-101 are preliminary from the Casero and Stewart laboratory.